The functionality of tempeh addition to beef patties

By

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Abstract

Red meat has been consumed for thousands of years by humans and has played an important part in human evolution. It is a nutrient rich food which is high in protein, minerals, such as iron, zinc, selenium, and many vitamins. However in recent years there has been negative consumer reaction to red meat. This has partially been due to its saturated fat content but also due to the causal link between red meat consumption and the incidence of colorectal cancer.

Oxidative stress caused by excess free radical generation *in vivo* has been linked to the pathology of many diseases including cancers. These effects can be mitigated by consumption of dietary antioxidants, compounds which have increasingly gained consumer attention for their health benefits.

The stomach is a bioreactor where both pro-oxidative and anti-oxidative compounds from the diet interact under low pH and at a relatively high temperature of 37°C. Beef is susceptible to lipid oxidation as it contains both catalyst (free and haem iron) and substrate (lipid) of lipid peroxidation. Consumption of beef with an antioxidant was hypothesised to limit lipid peroxidation in a model stomach system.

Tempeh is a traditional fermented soy product which has antioxidant properties and is commonly consumed in vegetarian diets for nutritional benefits and meat like flavour. Tempeh could be successfully incorporated into a beef patty to provide a source of antioxidant to limit *in vivo* lipid oxidation. This research aimed to determine the appropriate legume for tempeh production, determine changes that occur in beef patties with tempeh addition and changes in model stomach oxidative processes which occur during consumption of tempeh with beef.

During the initial phase of testing soy was chosen as the legume to produce tempeh from. It had acceptable antioxidant properties, was much less labour intensive to produce and more visually appealing than azuki tempeh.

Addition of tempeh to beef patties significantly ($p < 0.05$) increased water and carbohydrate contents; significantly ($p < 0.05$) decreased protein content and had no effect on fat content.

Tempeh containing patties had an improved fatty acid profile compared to control patties as it contributed a higher level of unsaturated fatty acids. Patties with tempeh retained a lighter, redder colour for longer time during stimulated retail display than the control and were slower to brown as measured by 630-580 and 630/580 nm wavelength ratios. Tempeh containing

patties had a higher PUFA content but shelf life was more limited than the control as measured by TBARS. Tempeh containing patties were softer, less cohesive and less chewy than the control patties. Focus group discussions suggested that there was a market for the product if consumers were informed of tempehs health benefits. Overall, the experiments including a pilot and full consumer sensory studies showed that inclusion of 10% tempeh was the most acceptable level of addition. There were no significant ($p > 0.05$) differences between control and 10% tempeh patties for overall acceptability or acceptance of flavour. However, 10% tempeh patties were found to be more tender and juicier than the control ($P < 0.05$).

The *in vitro* digestion results showed no significant difference between control and 10% tempeh patties for TBARS. As there is an apparent antioxidant effect in tempeh which is negated by the high PUFA content a defatted tempeh product may be more successful.

Overall there is market potential for a novel beef patty product incorporating tempeh which has several benefits compared to a conventional control patty.

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List of abbreviations and symbols

Abbreviations:

25-OHD 25-hydroxyvitamin D_3

5DT 5-(δ-tocopheroxy) - δ-tocopherol

AAPH 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride

AUC Area under the curve

BHT Butylated hydroxytoluene

CLA conjugated linoleic acid

CVD cardiovascular disease

DPA Docosapentaenioc acid

$DPPH^{\bullet}$ 1, 1-diphenyl-2-picryl-hydrazyl radical

DDH_20 Distilled de-ionised water

DiMeIQx 2-amino-3, 4, 8-trimethylimidazo (4, 5-f) quinoxaline

ET Electron transfer

FAME fatty acid methyl esters

FC Folin-Ciocalteu reagent

FRS Free radical scavenger

HA heterocyclic amine

HAA 3-hydroxyanthranilic acid

HAT Hydrogen atom transfer

HGF human gastric fluid

HNE 4-hydroxynonenal

IQx 2-amino-3-methyl imidazo (4, 5-f) quinoxaline

LA-OH linoleic acid hydroxyl

LA-OOH linoleic acid hydroperoxide

LDL low density lipoprotein

LOOH lipid hydroperoxide

MDA malondialdehyde

MeIQx 2-amino-3, 8-dimethylimidazo (4,5-f) quinoxaline

PhIP 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine

ORAC Oxygen radical absorbance capacity

NOC n-nitrosation compound

P:S ratio Polyunsaturated: Saturated ratio

PBS Phosphate buffered saline

PUFA polyunsaturated fatty acid

RDI recommended daily intake

SFA saturated fatty acid

SGF simulated gastric fluid

SOD superoxide dismutase

ST semitendonosus

TBA thiobarbituric acid

TBARS thiobarbituric acid reactive substances

TCA Trichloroacetic acid

TPC Total phenolic content

Symbols

$^3\Sigma g$ ground or triplet state oxygen

ω-3 omega 3 fatty acid

ω-6 omega 6 fatty acid

18:2 linoleic acid

20:4 arachidonic acid

22:6 docosapentanoic acid

E molar extinction co-efficient of malondialdehyde

$f0$ initial fluorescence reading at time 0

fn / $f0$ fluorescence at time n

GC-FID gas chromatograph with flame ionisation detector

$HO_2^{\bullet}$ perhydroxyl radical

H_2O_2 hydrogen peroxide

$L^{\bullet}$ alkyl radical

LH hydrogen containing lipid molecule

$LO^{\bullet}$ alkoxyl radical

$LOO^{\bullet}$ peroxyl radical

LOOH lipid hydroperoxide

Mb Fe(IV)=O ferryl myoglobin

$^{\bullet}$MbFe(IV)=O perferryl myoglobin

O_2^{-} superoxide anion

$O_2^{\bullet-}$ superoxide anion radical

$OH^{\bullet}$ Hydroxyl radical

NADPH nicotinamide adenine dinucleotide phosphate

$R^{\bullet}$ radical species

Chapter 1: Introduction

Humans have consumed red meat for thousands of years and in fact meat played an important role in human evolution (Milton, 2003). Compared to plant foods red meat is rich in protein, fat, B vitamins and minerals such as iron, zinc and selenium (Higgs, 2000).

Over the past decades consumers have become increasingly concerned about the negative aspects of red meat consumption, which has mainly focused on the total and saturated fat contents. In addition, epidemiologists have found causal correlations between red meat consumption and incidence of cancer, particularly colorectal cancer.

Concurrently consumers have also become aware of the importance of dietary antioxidants and there has been an extensive research on the benefits and the dietary requirements of antioxidants. The effects of free radicals which can cause oxidation *in vivo* and are involved in many pathologies including carcinogenesis can be mitigated by antioxidants (Kanner, 2007).

Red meat causes oxidation *in vivo* during digestion. Meat contains haem and free iron as well as fat which cause lipid peroxidation resulting in the generation of free radicals.

The stomach can be considered a bioreactor where a variety of reactions occur involving free radicals and antioxidants from partially digested food (Gorelik, Lapidot, Shaham, Granit, Ligumsky, Kohen et al, 2005; Lapidot, Granit & Kanner, 2005a; Gorelik, Ligumsky, Kohen & Kanner, 2008b). The stomach mixes its contents at a temperature of 37°C and under conditions of low pH and presence of dissolved oxygen (Kanner & Lapidot, 2001; Lapidot, Granit, & Kanner, 2005b). Recently several studies have focussed on oxidation and antioxidative effects occurring in model stomach systems during the digestion of food. There have been several recent studies by Kanner and co-workers focussed on oxidation occurring after consumption of meat and the effects of dietary antioxidants on this process (Kanner & Lapidot, 2001; Gorelik et al., 2005; Gorelik , Kohen, Ligumsky & Kanner, 2007; Gorelik , Ligumsky, Kohen & Kanner, 2008a; Gorelik et al., 2008b; Lapidot et al., 2005a; Lapidot et al., 2005b). These studies mainly focus on red wine consumption with a meal as red wine is known to contain dietary polyphenols. However, not everyone can drink wine with their meal due to price, not

drinking alcohol or other reasons. The inclusion of an antioxidant source into a meat product would conveniently provide antioxidant in the meal.

The processed meat industry commonly uses non meat extenders to decrease costs, and to bind water to reduce cooking losses. Predominantly these extenders have been soy that has been processed into textured soy protein, soy flour or soy protein isolate (Singh, Kumar, Sabapathy, & Bawa, 2008). A beef patty extended with a vegetal antioxidant provides an opportunity to produce a novel processed meat product conveniently including a healthier replacement for some of the pro-oxidative meat which also provides antioxidant to limit free radical generation.
Legumes are widely consumed podded plants and soybean is a legume containing bioactive compounds (Messina, 1999).
Tempeh is a traditional fermented soy product originating from Indonesia, although consumption has now spread worldwide. Traditionally it is made from dehulled soybeans fermented in solid state with *Rhizopus oligosporus,* a mould which knits together the individual beans by growing its hyphae into the cotyledon of the soybeans to produce a whitish yellow cake. More recently, the production of tempeh from other substrates has been investigated as a way to improve the digestibility of and use of substrates such as chickpea, faba bean, oat, green pea, broadbean and grasspea (Angulo-Bejarano, Verdugo-Montoya, Cuevas-Rodriguez, Milan-Carillo, Mora-Escobedo, Lopez-Valenzuela et al, 2008; Berghofer, Grzeskowiak, Mundigler, Sentall, & Walcak, 1998; Nassar, Mubarak & El-Beltagy, 2008; Starzynska-Janiszewska, Stodolak & Jamroz, 2008. Tempeh has recently become popular amongst vegetarians for the meat like flavour profile it provides despite not being a meat product. For a plant food it is rich in protein and contains B vitamins normally lacking in vegetarian diets including vitamin B_{12}. It also has a flavour described as “nutty” when raw or “mushroom” when cooked which is savoury and considered close to that of meat (Hutkins, 2006). The meat like flavour is due to the Maillard reaction during cooking. Tempeh has been reported to have antioxidant activity and to contain antioxidants such as isoflavones and 3-hydroxy anthranilic acid (Wang & Murphy, 1994; Hoppe, Jha & Egge 1997; Esaki, Onozaki, Kawakishi & Osawa, 1996). As a food which has been used to replace meat in vegetarian diets which also has antioxidant activity it has the potential to be used as a meat extender with antioxidant benefits in a novel food product. Therefore, the present work investigates the antioxidant activity of

several legumes through the production of tempeh and examines the biochemical and sensory effects of addition of tempeh to beef patties.

Chapter 2: Literature review

2.1 Role of meat in the diet

Meat is a food which has been consumed by humans for thousands of years. The muscles of animals have many important nutrients however there are also negative aspects of excessive meat consumption. Nowadays much more meat is being consumed around the world due to developments in agriculture and modern trade. Larger amounts of meat in the diet may be contributing to negative health effects in humans. As part of a balanced diet, meat is a good source of certain macro and micronutrients which are present in lower amounts in other parts of the diet.

2.2 Important nutrients in meat

2.2.1 Protein and amino acids

The protein content and quality of meat is very high (Foegeding, Lanier, & Hultin, 1996). Meat protein content on a wet basis is often around 20%, although for beef this is slightly higher (Lofgren, 2005). However meat is often cooked before consumption and the subsequent water loss raises the value, thus although fresh meat contains around 20% protein, the cooking often raises this to around 30% (Lofgren, 2005). Beef is an excellent source of highly digestible protein with the total protein levels varying depending on meat cut from around 22.4-28.4% (Lofgren, 2005). Meat is considered to be a nutritionally important source of amino acids (Lawrie, 1998), and as a muscle food the ratios and types of amino acids are close to those needed for human tissue growth and maintenance (Foegeding et al., 1996). All the essential amino acids are provided by meat and the predominant amino acids are glutamic acid/glutamine (16.5%) followed by arginine, alanine and aspartic acid (Williams, 2007). According to Bhutta (2006) meat has a true digestibility of 97% whilst plant foods such as whole wheat and beans have values of 86% and 78%, respectively. Meat provides around 60% of the protein intake for developed nations and around 15% in poorer countries (Higgs & Pratt, 2003).

2.2.2 Fat

Meat provides both polyunsaturated and saturated fat, however it is the levels of saturated fat which gains the most consumer attention. Ruminant meat contains a lower ratio of polyunsaturated fatty acids (PUFA): saturated fatty acid (P: S) ratio because unsaturated fat is hydrogenated in the rumen (Enser, Hallett, Hewett, Fursey, Wood, & Harrington, 1998). For example, P: S ratios of 0.11, 0.15 and 0.68 for beef, lamb and pork, respectively have been reported (Enser, Hallett, Hewett, Fursey, & Wood, 1996). However, according to Enser et al. (1996), about 16% of the polyunsaturated fatty acids in the U.K were provided by meat. This indicated that whilst meat may contain unfavourable P: S ratios it is still a good source of PUFAs. When fat contents are compared, it is useful to compare the amounts of omega 3 (ω-3) and omega 6 (ω-6) fatty acids because high ratios of ω-6: ω-3 fatty acids are associated with chronic diseases such as cancer and heart disease (Fernandez-Gines, Fernandez-Lopez, Sayas-Barberra, & Perez-Alvarez, 2005). Ratios of ω-6: ω-3 fatty acids for UK beef, lamb and pork were found to be 2.1, 1.3 and 7.2, respectively (Enser et al., 1996). Despite this meat is still regarded as an important contributor of ω-3 fatty acids to the diet. It was found that meat, poultry and game contributed to 42% of the ω-3 PUFA intake in an Australian diet survey, which was slightly lower than the 48.0% provided by fish and seafood (Howe, Meyer, Record, & Baghurst, 2006). It is worth mentioning, that fish contains much higher levels of ω-3 per mass unit (Williams, 2007). Furthermore, docosopentaenoic acid (DPA) which is an important ω-3 fatty acid is found at higher concentrations in red meat compared to fish (Howe et al., 2006).

Meat contains conjugated linoleic acid (CLA) which is known to have health benefits (Arihara, 2006). It is most commonly found in ruminants as their rumen microflora use an isomerase to convert linoleic acid to CLA (Arihara, 2006). There is 3-8 mg of CLA per gram in beef fat and the most common isomer is octadeca-*c*9 *t*-11 dienoic acid which has anticarcinogenic activity (Arihara, 2006). The fat content available in meat has been reduced in the past thirty years as a result of selective breeding practices and changes in animal diet have produced leaner animals. Leaner production is favoured by carcass classification systems and modern butchery methods including removing inter-muscular fat and having closer fat trim levels on retail meat, all which impact on the total intake of fat from red meats (Higgs, 2000; Lofgren, 2005).

2.2.3 Vitamins

Meat is a rich source of all B vitamins except folic acid (Speedy, 2003). Murphy and Allen (2003) found that meat (per weight or per unit of energy) is often more abundant than plant foods in six micronutrients (iron, zinc, vitamin B_{12}, riboflavin, vitamin A and calcium). Ruminant meat is abundant in vitamin B_{12} which is in a bioavailable form for humans (Ortigues-Marty et al., 2006). Raw beef contains on average 13.86 ng/g of vitamin B_{12} (Ortigues-Marty et al., 2006). A serving of 100 grams of lean red meat contains more than 25% of the recommended daily intake (RDI) of niacin, vitamin B_6 and vitamin B_{12} (Williams, 2007). The same serving size will also provide more than 10% of the RDI of pantothenic acid and riboflavin (Williams, 2007). Beef contains 0.52 mg/100g of vitamin B_6 which is more bioavailable than from plants because some vegetables contain glycosides which impair vitamin B_6 absorption (Mulvihill, 2004). Meat contains around 5-7 mg of niacin and meat is regarded as the richest food source of this nutrient (Mulvihill, 2004).

Cooked New Zealand beef contains 0.118-0.161 µg/100g of vitamin D_3 and 0.35-0.73 µg/100g of 25-hydroxyvitamin D_3 (25OHD) (calcidiol) depending on the meat cut (Purchas, Zou, Pearce, & Jackson, 2007). Meat is arguably a significant source of vitamin D despite the fact it contributes only a small amount, due to the high biological activity of 25OHD in meat (Purchas et al., 2007).

Meat also contains a few antioxidants such as carnosine, glutathione and co-enzyme Q_{10}. The levels of taurine, carnosine, co-enzyme $Q_{10,}$ and creatine in beef semitendinosous muscle were found to be 38.6, 452.6, 2.18 and 401.8 mg/100 g, respectively (Purchas, Rutherfurd, Pearce, Vather & Wilkinson, 2004). Taurine is considered to be important in numerous physiological and pharmacological cell functions (Redmond, Stapleton, Neary, & Bouchier-Hayes, 1998). Meat is an important source of the antioxidant glutathione found in muscle tissue, which protects against pathological and toxicological reactions and maintains ascorbate in its reduced form (Higgs, 2000). Glutathione is a co-enzyme for other enzymes which transport electrons and is a strong detoxifying agent for toxins such as heavy metals and contaminants from cigarette smoke due to its chelating ability (Liu & Eady, 2005). Health and aging in human beings has been linked to the concentration of this antioxidant in the human body (Liu & Eady, 2005), although it is unclear the form it has after being digested and absorbed into the body as it is quite unstable.

2.2.4 Minerals

In addition to its vitamin content, meat is also an important source of several minerals.
Meat is particularly rich in zinc and iron (Speedy, 2003). In beef there is around 291 mg/100g of zinc (Hambidge & Krebs, 2007). Around 20-40% of zinc is readily absorbed from meat and zinc bioavailability is enhanced when consumed with animal protein (Higgs, 2000), as in the case of meat. Plant foods contain minerals as well as the absorption inhibitors such as phytate and oxalate and so less zinc is absorbed when only plant foods are consumed (Higgs, 2000). Inhibitors form ligands with zinc and iron making them less bioavailable (Biesalski, 2005).

The high iron content in meat is improved by the fact that 50-60% of meat's iron is in the haem form, which is absorbed more efficiently than non-haem iron from plant sources (Higgs, 2000). The superior bioavailability of haem iron consumed with meat was demonstrated experimentally (Hurrell, Reddy, Juillerat & Cook, 2006; Layrisse, Martinez-Torres & Roche, 1968; Martinez-Torres & Layrisse, 1970; Martinez-Torres & Layrisse, 1971). In fact Johnson and Walker (1992) stated that diets containing beef can double the amount of iron being absorbed. Plant foods can contain compounds which inhibit absorption of iron such as phytates and those which promote iron absorption such as ascorbic acid (Samman, 2007). Unlike plant foods, meat does not contain absorption inhibitors such as phytates which decrease iron absorption, in fact the consumption of meat with vegetables increases the amount of iron absorbed from vegetables (Higgs, 2000). Therefore, the choice of diet has a large impact on the iron status of the individual as the most frequent causes of iron deficiency are diets with low haem and low total iron intake combined with a high intake of inhibitors (Samman, 2007). Iron is essential for transport of oxygen through the body (through haemoglobin), oxygen storage in muscles (through myoglobin) and as a co-factor for enzymes involvement in metabolic pathways (Samman, 2007).

It has been speculated that there is a "meat factor" which enhances the ability to absorb iron from a meal (Zhang, Carpenter & Mahoney, 1990; Hurrel et al, 2006; Layrisse et al., 1968; Samman, 2007). There are three proposed mechanisms to explain this phenomenon. Firstly, it is hypothesised that iron is solubilised and that makes it more readily available for uptake by forming complexes with the amino acids, polypeptides or proteins in the meat (Zhang et al.,

1990). Secondly, iron complexation is also hypothesised to occur by the secretion of gastrin or other gastric factors (not including gastric acid) stimulated by meat consumption (Zhang et al., 1990) which can maintain the solubility of iron. Thirdly, it is proposed that meat consumption stimulates secretion of gastric acid solubilising more iron for absorption.

The hypothesis for protein contribution to the "meat factor" is supported by the work of Martinez-Torres and Layrisse (1970) which found that iron absorption from black beans increased two fold in the presence of both fish and a mixture of amino acids representative of 100 g of fish. More recently, Hurrel et al. (2006) reported that consuming a meal with beef in place of egg white increased iron absorption 2-3 fold. A similar effect was found when beef protein extract and haem free beef were consumed instead of egg white or beef. This suggests that it is the beef protein not the beef haem that causes the increased iron absorption (Hurrel et al, 2006).

Haem

The iron in the haem is held within a porphyrin ring which is composed of four linked pyrrole rings (Schwartz, von Elbe & Giusti, 2008). The iron has six co-ordination sites, four bonded to the nitrogen atoms of the pyrrole rings, one bound to a histidine from a globin molecule and one site available to bind compounds such as, oxygen, hydroxyl group, water molecule, carbon dioxide and nitric oxide which donate electronegative atoms (Schwartz et al., 2008). Iron in the ferrous (2+ oxidation) state can bind oxygen forming the pigment oxymoglobin which gives the characteristic colour of fresh red meat. Oxidation caused by heating or the presence of oxidants causes the oxidation state to increase and the iron to be in the ferric state (3+) and the pigment in this state is known as metmyoglobin. The globin is a single chain globular protein containing 153 amino acids and contains the haem porphyrin within a hydrophobic cavity (Schwartz et al., 2008; von Elbe & Schwartz, 1996). This complex is called myoglobin and stores oxygen within the muscle tissue to be used later by metabolism (Schwartz et al., 2008). Four of these molecules linked in a quaternary structure called haemoglobin are used to transport oxygen throughout the body via the circulatory system.

Bjorn-Rasmussen, Hallberg, Isaksson, and Arvidsson (1974) reported that the average daily absorbed haem and non-haem iron from radioisotope labelled mixed meals were 37.3 ± 3.8% and 5.3 ± 1.8%, respectively. The subjects were thirty two Swedish male soldiers consuming meals based on their normal diet which consisted of 10.6% haem containing and 89.4% non-haem contain foods, respectively (Bjorn-Rasmussen et al., 1974). As mentioned before the nutritional advantage of meat is that it contains the highly bioavailable haem iron as well as non haem iron. Bioavailable iron is readily absorbed by mucosal cells in the upper duodenum and utilised by the body. In order to be available, the iron must be soluble which facilitates the crossing of the cell barrier (Neale, 1992). Haem iron, unlike non-haem iron, being in the centre of prosthetic group is protected from interactions with other compounds and can be absorbed in its intact form which explains its high bioavailability (Singh et al., 2006). On the other hand, non-haem iron can interact with other compounds from the meal which can affect the oxidation state, solubility and the amount absorbed by the specific transporters on the surface of the mucosal cells.

Selenium

On average meat contains 10 μg of selenium per 100 grams (Higgs & Pratt, 2003) and it has been suggested that around 17% of selenium in the American diet is supplied solely by beef (Biesalski, 2005). One of the important biochemical functions for selenium is being the active site of the important enzyme glutathione peroxidase which is involved in detoxifying and metabolising oxygen (Biesalski, 2005). Selenium may protect against chronic diseases due to its antioxidant activity (Biesalski, 2005).

2.3. Negative aspects of meat consumption

The ω-6/ω-3 ratio of traditional meat products (e.g. sausages, salamis) is higher than 15 (Reglero et al., 2008; Simopoulos, 2008) and higher values are associated with increased risk of cardiovascular disease. Furthermore, there has been a debate in recent years as to whether there is a link between red meat consumption and cancer, especially colorectal cancer (Alaejos, Gonzalez & Afonso, 2008; Armstrong & Doll, 1975; Baghurst, 2007; Demeyer, Honikel & De

Smet, 2008; Giovannucci, Rimm, Stampfer, Colditz, Ascherio & Willett, 1994; Sesink, Termont, Kleibucker & Van der Meer, 2000; Sesink, Termont, Kleibucker, & Van der Meer, 1999). Certainly some compounds produced during red meat cooking are probable or possible carcinogens such as heterocyclic amines (HAs) and polyaromatic hydrocarbons (PAHs) (Alaejos et al., 2008; Baghurst, 2007; Jagerstad, Skog, Arvidsson, & Solyakov, 1998). There is also the issue of pro-oxidative activity of red meat which is outlined in Section 2.4.4.

A link between mortality from the five most common cancers (lung, colorectal, prostate, stomach and breast) in the United Kingdom and meat consumption was investigated by Key, Davey and Appleby (1999a). There was no significant difference between meat eaters and vegetarians for any of the five cancers. However, Key et al. (1999b) analysed results from five previous cohort studies and found that death rate ratios for ischaemic heart disease in the age groups >65, 65-79 and 80-89 years were 45 % ($P<0.001$), 31% ($P<0.001$) and 8% (NS) lower for vegetarians than non vegetarians.

Key et al. (1999a) found that vegetarians were 24% less likely to die from ischemic heart disease than meat eaters. Armstrong and Doll (1975) reported a strong link between meat consumption and the incidence of colorectal cancer and since then colorectal cancer has been widely suggested to be linked to red meat consumption. Interestingly of the 23 countries studied, New Zealand had the highest meat consumption per capita and colon cancer incidence in women (~ 41 /per 100 000). Since then, several epidemiological studies showed evidence that suggests a link but does not explain how meat contributes to colorectal cancer although there are several theories (Higgs, 2000).

According to Higgs (2000) there are four components of meat which may contribute to development of cancer; (1) heterocyclic amines, (2) fat, (3) n-nitrosation products and (4) iron.

2.3.1 Heterocyclic amines

Heterocyclic amines (HAs) are considered to be carcinogenic. Polar HAs are formed during cooking from the reactions of creatine, carbohydrates and amino acids (Murkovic, 2004) all of which are present in meat. Low concentrations of HAs are formed during the boiling of meat but the concentration increases significantly when the temperature is raised above 150°C (Murkovic, 2004) which is commonly encountered during frying, grilling, baking and roasting.

Johansson, Fredholm, Bjerne and Jagerstad (1995) investigated the amounts of the HAs, 2-amino-3,8-dimethylimidazo(4,5-f)- Quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo(4,5-f)quinoxaline (DiMeIQx) and 2-amino-l-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), production during frying of burger patties at 165°C and 200°C. The amounts of MeIQx, DiMeIQx and PhIP ranged from 0.2-1.6 ng/g, undetectable-0.4 ng/g, and 0.08-1.5 ng/g respectively (Johansson et al., 1995). Increasing the frying temperature increased the amounts of MeIQx, DiMeIQx and PhIP (Johansson et al., 1995).

An increased risk of colon cancer from red meat consumption mainly was linked to well done and very well done cooked meat (Sinha & Rothman, 1999). These authors suggested that some of the increased risk could be explained by cooking practices, thus red meat cooked at lower temperatures may not be as harmful. Kampman, Slattery, Bigler, Leppert, Samowitz, and Caan (1999) found no association between total red meat consumption and colon cancer, however the doneness of the meat was related to increased risk of the cancer. Interestingly, an association between colorectal cancer risk and HAs is most strongly associated with MeIQx and is only observed in men (Alaejos et al., 2008). Cooking chicken produces higher levels of PhIP than cooking red meat (Sinha et al., 1995). Cooking meat together with food containing phenolic antioxidants, antioxidant vitamins and carotenoids are suggested for reducing HA formation (Alaejos et al., 2008).

2.3.2 Fat

It is hypothesised that high saturated fat contents such as those found in meat can increase intestinal excretion of bile acids and fatty acids (Sesink et al., 2000) or stimulate the production of diacylglycerol which is a potential mitogen (Sesink et al., 1999). Bile acids are converted to secondary bile acids by colonic microflora and one of these secondary bile acids, deoxycholic acid, is known to be linked to colon cancer (Bingham, 2006). Bile secretion during digestion is increased by both animal and vegetable fats however epidemiological evidence indicates that animal fat specifically from red meat increases the risk of colon cancer (Giovannucci & Goldin, 1997). However Giovannucci et al. (1994) found a significant correlation between red meat and colon cancer but not between animal fat and colon cancer even though there was a correlation between red meat and animal fat. The epidemiological evidence at present shows only a very weak association between colorectal cancer and high fat diets (Bingham, 2006). This implies

that it is unlikely that a fat induced mechanism is responsible for an apparent link between red meat and cancer.

2.3.3 N-nitroso compounds

The formation of n-nitrosation compounds (NOCs) which include known carcinogens are another proposed mechanism linking red meat consumption to cancer (Bingham, 2002). These compounds can be synthesised *in vivo* in the colon by reaction of nitrite (produced from nitrates anaerobically) with amines and amides from bacterial de-carboxylation of amino acids (Baghurst, 2007). The link to cancer was proposed because alkylative mutations are related to bowel cancer and NOCs are alkylators (Bingham, 2002). High meat diets produce an increase in colonic levels of ammonia which promotes carcinoma formation in rodents (Bingham, 2002). They can also be produced when amides and amines from bacterial decarboxylation of amino acids are n-nitrosated by a nitrosating agent (Bingham, 2002). A parallel increase in faecal NOC levels was observed when there was an increase in the amount of protein consumed (Bingham, 2002). Demeyer et al. (2008) concluded that haem and endogenous n-Nitrosation are the most likely explanations of a link between processed meat and cancer.

Care should be exercised when interpreting the results from studies that combine processed meat and red meat in the same data set. This is because processed meat often contains many non-meat components and higher levels of salt and saturated fat which are risk factors for diseases themselves.

2.3.4 Catalytic effects of iron

The presence of iron may not be entirely positive as this metal can act as a pro-oxidant. It has been hypothesised that free iron participating in Fenton reaction produces hydroxyl radicals and that these radicals are responsible for a link between red meat and cancer (Bingham, 2002). In addition, haem can be linked to the production of heterocyclic amines (Jagerstad et al., 1998), the proposed saturated fatty acid mechanism (Sesink et al., 2000; Sawa, Akaike, Kida,

Fukishima, Takagi & Maeda, 1998) and production of n-nitrosation products (Cross, Pollock, & Bingham, 2003).

Glei et al. (2002) modelled the the role of iron in carcinogenesis by adding ferric nitrilotriacetate (Fe-NTA) to an HT29 clone 19A human tumour cell line. The addition of 500 and 1000 μM Fe-NTA significantly ($p < 0.001$) caused DNA strand breaks after 15 minutes incubation. DNA bases also were oxidised after the addition of 250-1000 μM Fe-NTA and more damage to the DNA at 1000 μM Fe-NTA was due to oxidised bases than strand breaks (Glei et al., 2002). Addition of H_2O_2 to cells pre-treated with Fe-NTA produced a significant ($p < 0.01$) increase in DNA damage (Glei et al., 2002) which demonstrates the ability of a Fenton like mechanism to promote *in vitro* carcinogenesis. Sesink et al. (1999) suggested that haem iron but not iron alone caused detrimental intestinal effects as the constituents of haem protoporphyrin and inorganic iron did not cause a cytotoxic effect or increased epithelial proliferation on their own.

Red meat was proven to have a correlation with colon cancer, however fish intake was not associated with risk and a slight negative association was observed with poultry consumption (Giovannucci et al., 1994). The difference between meat types and risk of colon cancer could be due to the high levels of haem in red meat.

The catalytic activity of haem iron may be linked to other proposed mechanisms of colon cancer formation. Sesink et al. (2000) found that fat alone did not affect the levels of cations found in faeces, however haem increased faecal cation concentrations in low, medium and high fat diets which demonstrates that haem in the presence of fat impairs absorption of cations by epithelial damage. There was a significant interaction ($p < 0.001$) between haem and fat on faecal cation concentration (Sesink et al., 2000) which provides evidence for a haem induced lipid oxidation mechanism.

Formation of the HAs; 2-amino-3-methyl imidazo (4,5-f) quinoxaline (IQx), MeIQx and DiMeIQx were increased two fold after addition of iron (Fe^{2+} and Fe^{3+}) to a model system containing liquid creatine, glycine and glucose (Jagerstad et al., 1998). Thus iron induced oxidation may produce carcinogenic HAs in addition to other radical species.

To understand the damage which oxidation can do to the body it is first important to explain oxidation and the oxidation which occurs in meat both *in vivo* and *in vitro*.

2.4 Oxidation

Oxidation reactions are major degradative reactions affecting organic and inorganic systems. Oxygen first appeared in the atmosphere in significant amounts more than 2 x 10^9 years ago (Halliwell & Gutteridge, 1989) and this compound has a toxic effect which varies greatly amongst organisms. This varies from strict anaerobes which can only tolerate virtually anaerobic conditions to plants and animals which suffer toxic effects when exposed to oxygen at levels above atmospheric concentration (Halliwell & Gutteridge, 1989). Detrimental effects of oxygen can be attributed to free radicals which are defined by Halliwell and Gutteridge (1989) to be any species containing one or more unpaired electrons in its molecular orbitals capable of existing independently.

2.4.1 Detrimental effects of lipid peroxidation

Lipid peroxidation detrimentally affects the nutritional and organoleptic properties of foods and decreases its shelf life. Oxidative processes in foods affect lipids, carbohydrates, pigments, proteins, vitamins and the quality overall (Kanner, 1994) and lipid peroxidation is a significant cause of quality loss in meat (Gray, Gomaa & Buckley, 1996). The compounds generated from this process include toxic compounds and volatile compounds imparting an "off" flavour.

Two basic mechanisms are responsible for non enzymatic lipid oxidation; autoxidation (abstraction) and 'ene' addition (Parkin & Damodaran, 2003). Autoxidation proceeds by the three general steps of initiation, propagation and termination. During the 'ene' addition reaction a highly electrophilic $^1O_2$ is added at the double bond of a fatty acid where there is high electron density (Parkin & Damodaran, 2003). Once hydroperoxides are formed by the addition of oxygen the fatty acids can undergo further reactions (Parkin & Damodaran, 2003).

During the initiation step, hydrogen is abstracted from a fatty acid to form the alkyl ($L^{\bullet}$) radical (McClements & Decker, 2008; Etsuo, 2009). This free radical is then stabilised by delocalisation over the double bond(s) which results in double bond shifting, and the formation of conjugated double bonds for polyunsaturated fatty acids (McClements & Decker, 2008). The shifting of double bonds produces fatty acids in the *cis* or *trans* configuration with the more stable *trans* form predominating (McClements & Decker, 2008; Etsuo, 2009).

Once the reaction is initiated the next step of propagation occurs. This step involves the addition of oxygen to form hydroperoxides and produce more radicals (Parkin and Damodaran, 2003). The free radicals on triplet oxygen will not directly abstract a hydrogen atom as they are low energy (McClements & Decker, 2008). However triplet oxygen contains two electrons with the same spin direction which cannot share the same spin orbital, making it biradical (McClements & Decker, 2008). The two free radicals can react with the alkyl radical, with one binding to the radical forming a covalent bond, whilst the other radical is free (McClements & Decker, 2008). This produces the peroxyl radical ($LOO^{\bullet}$), which have higher energy allowing them to abstract a hydrogen atom from another molecule (McClements & Decker, 2008; Etsuo, 2009), a fatty acid in the case of autoxidation. Unsaturated fatty acids are vulnerable to peroxyl radical attack as they contain a weak carbon-hydrogen covalent bond (Pratt, Talman & Porter, 2011; Catala, 2009). The addition of a hydrogen atom to the peroxyl radical produces a lipid hydroperoxide (LOOH) and the abstraction from the fatty acid produces a new alkoxyl radical on the molecule (McClements & Decker, 2008; Catala, 2009). The reaction may be summarised according to the following equations (Carlsen, Moller & Skibsted, 2005):

$$LH + R^{\bullet} \rightarrow RH + L^{\bullet}$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$$

Where $R^{\bullet}$ is a radical species and LH is a hydrogen-containing lipid molecule.

2.4.2 Catalysts of lipid peroxidation

When oxygen is in the ground or triplet state ($^3\Sigma g$), it contains two unpaired electrons in its π orbitals and can not react directly with unsaturated fatty acids which are in the singlet state (Halliwell & Gutteridge, 1989; Gray et al., 1996; Kanner, 1994). This barrier does not exist for transition metals (Kanner, 1994) which have unpaired electrons, or singlet forms of oxygen such as the superoxide radical which has an extra electron in the π - orbital (Halliwell & Gutteridge, 1989). The process of lipid peroxidation can be facilitated by factors such as;

increased number of unsaturated double bonds, increased concentration of catalysts, temperature, presence of enzymes, UV light, transition metals and presence of oxygen.

2.4.2.1 Transition metals

The elements of the d block of the periodic table contain unpaired electrons which give them a radical nature due to their variable oxidation state as these elements can donate or gain an electron (Halliwell & Gutteridge, 1989). Transition metals are ideal for catalysing lipid oxidation as they contain labile d electrons (Kanner, Hazan & Doll, 1988). Transition metals may abstract a proton from a molecule such as a PUFA, lowering its oxidation state and producing a radical. A transition metal which occurs in meat and causes oxidative reactions is iron. Iron is known to catalyse the Fenton reaction, causing oxidative changes.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

The reaction of the ferrous ion with hydrogen peroxide (H_2O_2) generates a hydroxyl radical ($OH^{\bullet}$) which can initiate further oxidation.

Further intermediate reactions which may occur from the mix of iron and hydrogen peroxide in Fenton reaction were outlined by Halliwell and Gutteridge (1989):

$$OH^{\bullet} + H_2O_2 \rightarrow H_2O + H^{+} + O_2^{\bullet -}$$

$$O_2^{\bullet -} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

$$OH^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + OH^{-}$$

Hydrogen peroxide is present in all aerobic cells at low concentrations and is generated as a metabolite through several biochemical pathways (Kanner, 1994). It can be generated by mitochondria, microsomes, peroxisomes and cytosolic enzymes when a substrate is present (Kanner, 1994). Specifically it can be generated from superoxide ($O_2^{\bullet -}$) produced in these systems through oxidation catalysed by superoxide dismutase (SOD) or non-enzymic

dismutation of $O_2^{\bullet-}$ produced during autoxidation of oxymyoglobin (Kanner, 1994; Gatellier, Anton & Renerre, 1995). In meat the majority of H_2O_2 is generated by non-enzymatic reactions (Harel & Kanner, 1985a; Harel & Kanner, 1985b).

Another important reaction of iron is the lipid oxidation catalysed by the redox cycling of superoxide anion, thiols, ascorbic acid, reduced nicotinamide adenine dinucleotide phosphate (NADPH), cysteine or glutathione known as the Haber-Weiss reaction (Kanner, 1994; McClements & Decker, 2008). The overall reaction according to McClements and Decker (2008) can be summarised as shown below:

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

The reduction of oxygen produces species such as hydrogen peroxide, superoxide anion radical ($O_2^{\bullet-}$) and perhydroxyl radical ($HO_2^{\bullet}$) which directly and indirectly participate in meat oxidative reactions (Kanner, 1994).

2.4.2.2 Myoglobin

Myoglobin as mentioned before contains an iron atom in its porphyrin ring and this iron can change in oxidation state during reactions with other molecules. The lowest iron oxidation state is the ferrous form (Fe^{2+}) with water bound to the iron nucleus in the case of deoxymoglobin or oxygen bound to the iron in the case of oxymyoglobin. There is also metmyoglobin (Fe^{3+}), ferrylmyoglobin (Fe^{4+}), and the short lived perferryl radical that can exist at different stages of oxidation. Myoglobin contains iron in variable valencies and may act as either an antioxidant (Lapidot et al., 2005a; Alayash, Patel, & Cashon, 2001) or pro-oxidant (Baron, Skibsted & Andersen, 1997; Lapidot et al., 2005a) depending on the presence of reducing compounds at certain concentrations.

As mentioned above lipid hydroperoxides are generated during lipid peroxidation and the presence of myoglobin catalyses the breakdown of lipid hydroperoxides (Baron et al., 1997;

Reeder & Wilson, 1988; McClements & Decker, 2008). Neither metmyoglobin nor H_2O_2, alone can initiate lipid peroxidation, but the presence of both compounds can act as a catalyst for the initiation of lipid oxidation (Kanner & Harel, 1985). Four mechanisms have been proposed for myoglobin induced lipid oxidation (see below). These are; (1) a Fenton-like mechanism, (2) an Fe(III)/Fe(IV) mechanism, (3) a pseudoperoxidase mechanism and (4) an Fe(II)/Fe(IV) mechanism (Carlsen et al., 2005).

(1) A Fenton like mechanism is similar to the reaction described previously under the reactions of transition metals. However in addition to the equation stated above, haem Fe^{3+} catalysed oxidation of H_2O_2 can be involved (Carlsen et al., 2005).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

$$Fe^{3+} + H_2O_2 \rightarrow HO_2^{\bullet} + H^{+}$$

(2) The haem(III)/haem (IV) reaction can be summarised by the following equation (Carlsen et al., 2005).

$$\text{Mb Fe (III)} + LOOH \rightarrow \text{Mb Fe(IV)=O} + LO^{\bullet} + H^{+}$$

$$\text{Mb Fe(IV)=O} + LOOH \rightarrow \text{Mb Fe (III)} + LOO^{\bullet} + H_2O$$

The strong oxidising power of ferrylmyoglobin (Mb Fe(IV)=O) facilitates the second part of the reaction where Fe(III) is regenerated (Carlsen et al., 2005).

(3) The pseudoperoxidase mechanism involves the oxidation of metmyoglobin by hydrogen peroxide to the perferryl radical (equation) then removal of an electron to form ferryl myoglobin followed by reduction to metmyoglobin. Thus, two electrons are transferred from metmyoglobin and are regenerated after receiving two electrons from donors (Carlsen et al., 2005).

$$\text{MbFe(III)} + H_2O_2 \rightarrow {}^{\bullet}\text{MbFe(IV)=O} + H_2O$$

Peroxidases catalyse the reduction of hydroperoxides by reducing substrates as shown below (Kröger-Ohlsen, Carlsen, Andersen & Skibsted, 2002):

$$H_2O_2 + 2\ LH \rightarrow 2\ L^{\bullet} + 2\ H_2O$$

(4) A mechanism of catalysis does not seem to occur for the proposed Fe(II)/Fe(IV) pathway because the Fe(II) is not regenerated when Fe(III) is the final product (Carlsen et al., 2005). Hydrogen peroxide can generate ferryl myoglobin from myoglobin by accepting two electrons (Reeder & Wilson, 2005):

$$\text{Mb- Fe(II)} + H_2O_2 \rightarrow {}^{\bullet}\text{Mb Fe(IV)-}O^{2-} + H_2O$$

The ferryl myoglobin is either autoreduced to ferric haem or reacts with ferrous haem as shown below (Reeder & Wilson, 2005).

$${}^{\bullet}\text{Mb Fe(IV)-}O^{2-} + \text{Mb-Fe(II)} + 2H^{+} \rightarrow 2\text{Mb-Fe(III)} + H_2O$$

However when Fe (III) is produced from the reactions of Fe(II) and peroxides, it does produce a catalytic effect as demonstrated in the Fe(III)/Fe(IV) and pseudoperoxidase mechanisms (Carlsen et al., 2005). However from a health perspective when cooked meat is consumed the pigment is in the metmyoglobin form.

Although there are many theories, backed by evidence, explaining the progress of oxidative processes in iron reactions, it is likely that more than one mechanism is involved in myoglobin/hydroperoxide reactions due to the complexity of the products formed (Maiorino, Urisni & Cadenas, 1994).

Ferryl myoglobin has two electrophilic centres; a protein radical and an oxoferryl complex (Giulivi & Cadenas, 1998). The amino acid radical is probably located close to the haem on an aromatic amino acid (Giulivi & Cadenas, 1998). Ferryl haem (Fe (IV) =O) can abstract a proton by its oxoferryl complex to initiate lipid peroxidation (Reeder, Svistunenko, Cooper & Wilson, 2004; Maiorino et al., 1994) as shown below with a reduction in the oxidation state:

$$P\text{-}Fe(IV)\text{-}OH + LH \rightarrow P\text{-}Fe(III)\text{-}H_2O + L^{\bullet}$$

or hydrogen abstraction followed by a redox reaction with a lipid hydroperoxide (Kanner & Harel, 1985)

$$P^{\bullet+}Fe(IV)=O + LH \rightarrow P\text{-}Fe(IV)=O + L^{\bullet} + H^{+}$$

The alkoxyl radical is produced in the above reaction and generates the peroxyl radical by reaction with molecular oxygen as shown below (Reeder & Wilson, 2005).

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$$

$$P\text{-}Fe(IV)=O + LOOH \rightarrow P\text{-}Fe(III)LOO^{\bullet} + OH^{-}$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$$

2.4.2.3 Effect of pH

At acidic pH myoglobin has increased pro-oxidative and pseudoperoxidative activity (Kanner & Lapidot, 2001; Reeder & Wilson, 2005) and a much higher lipid peroxidation rate (approximately 7 x 10^4 times) has been observed at pH 3.0 compared with at pH 7.0 (Lapidot et al., 2005b). Reeder and Wilson (2001) hypothesised that at low pH the protonation of the ferryl oxygen compound activates the oxoferryl complex converting it to Fe^{4+}-OH^{-} which is unstable and has a radical like nature. By the abstraction of an electron from either the porphyrin or protein producing a radical the ferric species is regenerated via auto-reduction (Reeder & Wilson, 2001). Myoglobin induced lipid oxidation at reduced pH is of interest as there are some *in vivo* oxidative reactions which occur under acidic conditions (Reeder & Wilson, 2001; Reeder & Wilson, 2005) and because this is the pH environment of the stomach, where consumed food often contains myoglobin as well as lipids.

The impact of myoglobin induced lipid oxidation on meat quality (Kanner, 1994) and human health (Reeder & Wilson, 1998; 2001; 2005; Reeder et al., 2004) have been investigated extensively.

2.4.3 Mechanisms of antioxidant action:

Lipid peroxidation can be prevented by addition of antioxidants. Antioxidants prevent lipid peroxidation by many mechanisms including metal chelation, free radical scavenging, oxygen scavenging, proton donation and stabilisation of radicals by donating electrons.

Different antioxidants can slow lipid oxidation by scavenging free radicals at initiation, propagation and β-scission stages. The β-scission reaction produces low molecular weight volatile compounds from the decomposition of fatty acids (McClements & Decker, 2008). Free radical scavengers (FRS) donate protons and react faster with free radicals than unsaturated fatty acids. The transfer of this proton is more energetically favourable if the bond energy is weak. An effective FRS will form a low energy radical by resonance stabilization or sharing electrons because a high energy radical is more likely to oxidise unsaturated fatty acids. Furthermore, it is expected that FRS will not react with oxygen to produce hydroperoxides, which would otherwise propagate the reaction. A free radical scavenger can terminate reactions by reacting with either a radical or another FRS to form non radical products. Phenolic compounds, which widely occur in plants, can donate the hydrogen of their hydroxyl group and delocalise the energy from the subsequent radical through its ring structure (McClements & Decker, 2008). Metal chelators, which often contain many carboxylic or phosphate groups such as phytate (McClements & Decker, 2008), increase the activation energy of reactions by binding metal ions which can prevent the reaction or slow its rate (Jadhav, Nimbalkar, Kulkarni & Madhavi, 1996).

2.4.4 The stomach as a bioreactor

The stomach is regarded as a perfect bioreactor where the interaction of many food components, such as lipids, proteins and carbohydrates at different stages in breakdown in the presence of enzymes are constantly being mixed at a temperature of 37°C and under an acidic environment. Under these conditions several different reactions could occur which lead to increased lipid oxidation especially in lipid rich foods (Kanner & Lapidot, 2001).

Lipid hydroperoxides which catalyse myoglobin oxidation in foods can also be generated during digestion, particularly in gastric fluid which has a low pH and contains absorbed oxygen (Kanner & Lapidot, 2001). These authors and their co-workers have studied extensively the

oxidative reactions in relation to meat and lipid consumption using human gastric fluid (HGF) (Kanner & Lapidot, 2001; Gorelik et al., 2005; Lapidot et al., 2005b), simulated gastric fluid (SGF) (Lapidot et al., 2005a; Gorelik et al., 2005; Gorelik et al., 2007; Lapidot et al., 2005b) and in rats (Gorelik, Ligumsky, Kohen & Kanner, 2008b). SGF is a model gastric fluid containing in these studies a low (0.2%) salt concentration, 0.32% pepsin, and a low pH to mimic gastric fluid during *in vitro* digestion (Gorelik et al., 2005; Kanner & Lapidot, 2001; Lapidot et al., 2005b; Lapidot et al., 2005a). Furthermore, these authors investigated the effects of several dietary antioxidants on these processes. The oxidation of food is greatly enhanced in the presence of SGF or HGF (Kanner & Lapidot, 2001) (see Table 1 for a summary of these studies). These model systems proved to be ideal for studying the generation of radicals *in vitro* and can be used to investigate the health benefits of antioxidants when they are in the digestive environment. Radical generation in the stomach was found to co-oxidise vitamins (Gorelik et al., 2005) and generates harmful compounds including malondialdehyde (MDA), 4-hydroxynonenal (HNE), hydroperoxides, F-2 isoprostanes, reactive oxygen species (Sawa et al., 1998; Gorelik et al., 2008a). See Table 2 for a summary of radical species which can be formed during lipid peroxidation.

Gorelik et al. (2008b)	Rat stomach *in vivo* digestion	Rats consuming turkey had a reduction in MDA Rats consuming turkey had a 50% increase (above baseline) in plasma MDA	Rats consuming turkey with red wine concentrate had a reduction in MDA threefold higher Rats consuming turkey with red wine concentrate had a 34% decrease (below baseline) in plasma MDA
Lapidot et al. (2005a)	Digestion in SGF	At low concentrations of ascorbic acid free iron catalysed lipid peroxidation	At higher ascorbic acid levels free iron induced lipid oxidation was inhibited Ascorbic acid had an antioxidative effect on myoglobin induced lipid peroxidation. Catechin at 25 and 250-500 µm inhibited lipid peroxidation in a system containing metMb/iron/AA and a iron AA system respectively
Lapidot et al. (2005b)	SGF	Metmyoglobin at low concentration (1:30) increased amount of hydroperoxides	Catechin at 100 µm or 250 µm produces an antioxidant effect in the presence of low (5 µm or 10 µm respectively) levels of metmyoglobin. Metmyoglobin at high concentration (1:3) acted antioxidatively and decomposed hydroperoxides

Author(s)	Test Conditions	Main Findings	Antioxidant effects
Kanner and Lapidot (2001)	Digestion in SGF and HGF	In HGF metmyoglobin catalysed lipid peroxidation increasing LOOHs. In SGF metmyoglobin increased LOOH level.	Red wine polyphenols in HGF reduced LOOH level.
Gorelik et al. (2005)	Digestion in SGF and HGF	In HGF and SGF myoglobin catalysed lipid peroxidation and co-oxidised vitamin E	In HGF and SGF 2.5 mM catechin equivalent inhibited lipid oxidation and inhibited vitamin co-oxidation
Gorelik et al. (2008a)	Human plasma	Consuming turkey raised plasma MDA of subjects	Consuming red wine with turkey lowered plasma MDA of subjects. Consuming turkey pre-soaked in wine and consuming red wine lowered plasma MDA further
Gorelik et al. (2007)	SGF	Digestion generated LOOH and increased TBARS	Addition of saliva lowered LOOH and decreased TBARS

Table 1: Summary of studies of myoglobin and iron induced lipid peroxidation under model digestive conditions

2.4.5 Free Radicals

The reaction of lipid hydroperoxides with haem generates peroxyl radicals which cause DNA breakages which are harmful to cell function and survival (Sawa et al., 1998). Peroxyl radicals can generate the superoxide and hydroxyl radicals which can oxidise DNA bases (Sawa et al., 1998) and cause loss of purine and pyrimidine bases (Kanazawa, Sawa, Akaike & Maeda, 2002). Damage to DNA results in mutations which have been suspected to cause carcinogenesis and tumour development (Kanazawa et al., 2002).

Table 2: Summary of selected radical species that can be formed during lipid peroxidation including those catalysed by myoglobin and routes of formation

Radical name	Abbreviation	Route(s) of formation	Reference
Superoxide	$O_2^{\cdot}$	$OH^{\cdot} + H_2O_2 \rightarrow H_2O + H^+ + O_2^{\cdot}$	Halliwell and Gutteridge, 1989
Alkyl	$L^{\cdot}$	$LH + R^{\cdot} \rightarrow RH + L^{\cdot}$ $LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$ P-Fe(IV) -OH + LH→ P-Fe(III) - $H_2O + L^{\cdot}$	Carlsen et al., 2005; Reeder et al., 2004; Maiorino et al, 1994
Peroxyl	$LOO^{\cdot}$	$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$ Mb Fe(IV)=O + LOOH→ Mb Fe (III) +$LOO^{\cdot}$ +H_2O	Reeder and Wilson, 2005; Carlsen et al., 2005
Hydroxyl	$OH^{\cdot}$	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^-$	McClements and Decker, 2008
Alkoxyl	$LO^{\cdot}$	Mb Fe (III) + LOOH→Mb Fe(IV)=O + $LO^{\cdot} + H^+$	Carlsen et al., 2005
Perhydroxyl	$HO_2^{\cdot}$		Harel and Kanner, 1985b
Perferrylmyoglobin	.MbFe(IV)=O	MbFe(III) + $H_2O_2 \rightarrow$ .MbFe(IV)=O + H_2O	Carlsen et al., 2005

2.4.6 Aldehydes and their degradation products

Aldehydes, formed from lipid hydroperoxides which breakdown in the stomach, are toxic products of gastric lipid peroxidation (Kanazawa & Ashida, 1998b). Linoleic acid hydroperoxide (LA-OOH) was observed to decompose into linoleic acid hydroxyls (LA-OH), epoxyketones and aldehydes (Kanazawa & Ashida, 1998a, Kanazawa & Ashida, 1998b). Esterbaur, Schaur and Zollner (1991) stated that the process leading to formation of aldehydes is probably β cleavage of lipid hydroperoxides, more specifically the lipid alkoxyl radicals.

During oxidation of fatty acids a potentially carcinogenic dialdehyde called malondialdehyde (MDA) can be formed (Grotto et al., 2009; McClements & Decker, 2008). The thiobarbituric reactive substances (TBARS) assay measures MDA (amongst other compounds) and is used as an index of lipid peroxidation which will be discussed further (Chapter 3) (McClements & Decker, 2008). Malondialdehyde is formed from the oxidation of PUFAs with more than two methylene interrupted double bonds, principally arachidonic acid (20:4) and docosahexaenoic acid (22:6) (Esterbauer, Schaur & Zollner, 1991). Arachidonic acid could be degraded to hydroperoxides followed by β cleavage of the fatty acid chain to produce a hydroperoxyaldehyde (Esterbauer et al., 1991). This compound can then form MDA either by β scission or by way of an acrolein radical which can react with a hydroxyl radical to generate MDA in the enol form (Esterbauer et al., 1991). Acrolein is a highly cytotoxic 2-alkenal that is structurally similar to MDA (Esterbauer et al., 1991).
The compound 4-hydroxy nonenal (HNE) can be formed from ω-6 PUFAs such as 20:4 (arachidonic acid) and 18:2 (linoleic acid) (Esterbauer et al., 1991).
HNE is considered to be more toxic than MDA (Halliwell and Gutteridge, 1989). At levels above 100 μM HNE is cytotoxic leading to rapid cell death and at 1-20 μM it inhibits DNA and protein synthesis (Esterbauer et al., 1991).

During lipid peroxidation protein molecules can be attacked and damaged by peroxyl and alkoxyl radicals, and aldehydes can react with protein –SH groups (Halliwell & Gutteridge, 1989). Amino groups on proteins can be attacked by dialdehydes such as MDA which forms intramolecular cross-links and cross-links between different protein molecules (Halliwell &

Gutteridge, 1989). Also, during lipid peroxidation enzymes which require –SH or $–NH_2$ groups are normally inhibited which affects normal cellular function (Halliwell & Gutteridge, 1989).

Although the stomach as a bioreactor can produce detrimental products of lipid peroxidation there are options for mitigating the damage. The use of antioxidants in meat products inhibits lipid oxidation during storage and the consumption of antioxidants with a meal containing catalysts for lipids oxidation can minimise the damage caused by oxidative processes. The consumption of catechin (Kanner & Lapidot, 2001; Gorelik et al., 2005; Lapidot et al., 2005a; Lapidot et al., 2005b), quercetin (Lapidot et al., 2005b), ascorbic acid (Gorelik et al., 2005, Lapidot et al., 2005a) and red wine polyphenols (Kanner and Lapidot, 2001; Gorelik et al., 2005, Lapidot et al., 2005a, Gorelik et al., 2008b) together with meat has been studied to observe if dietary antioxidants can limit the myoglobin induced lipid peroxidation.

Catechin was able to prevent metmyoglobin catalysed β-carotene oxidation (Kanner & Lapidot, 2001), inhibited lipid oxidation in an iron-ascorbic acid system (Lapidot et al., 2005a) and had an anti-oxidative effect on metmyoglobin catalysed linoleate peroxidation (Lapidot et al., 2005b). Red wine polyphenols in simulated gastric fluid (SGF) were able to inhibit lipid peroxidation at 1.6 mM and abolish the breakdown of hydroperoxides at 4.1 mM (Lapidot et al., 2005a). Red wine polyphenols also exhibited good ability in reducing the amount of MDA produced *in vivo* after eating meat. Gorelik et al. (2008b) observed that rats which ate a meal of turkey initially had an increase in hydroperoxides in the stomach followed by a reduction in hydroperoxides 90 minutes after the consumption of the meal. The same trend was observed for rats consuming turkey with red wine polyphenols, however, more than 3 fold reduction in hydroperoxides was found. The inclusion of turkey meat in the rat's diet increased the plasma MDA level by 50%, whereas red wine polyphenols decreased the plasma MDA by 34% from the basal level 90 minutes after consumption. In humans, the consumption of red wine with turkey meat decreased plasma MDA concentrations by 63%, however the most dramatic effect was observed in volunteers which consumed turkey pre-soaked in wine which inhibited the increase of plasma MDA (Gorelik et al., 2005). Malondialdehyde is considered to be a marker of oxidative stress (Chole, Patil, Basak, Palandurkar & Bhowate, 2010) and can be detected with the TBARS assay which is a useful system for detecting the cytotoxic potential of a meal

containing myoglobin and lipids (Gorelik et al., 2005; Kanner & Lapidot, 2001; Lapidot et al., 2005a; Lapidot et al., 2005b).

The results reported above suggest that consuming dietary antioxidants with meat has the health benefit of ameliorating the effects of gastric environment promoting lipid peroxidation. However there is a need to investigate a variety of dietary sources which can be consumed with meat as not all consumers drink red wine. As demonstrated by the work of Gorelik et al. (2008a), the incorporation of the dietary antioxidant into the meat prior to cooking provides health benefits in terms of reducing the oxidative processes as well as convenience.

2.5 Meat Extenders

Processed meat offers the opportunity to incorporate an ingredient with a health benefit into a meat product and the use of meat as a vehicle for health promoting compounds have been reviewed by Fernandez-Gines et al. (2005). Several food compounds such as vegetable oils, natural antioxidant extracts, fish oils, fibre and soy (in protein isolate and oil forms) have been used to produce functional meat products (Fernandez-Gines et al., 2005). Processors normally add extenders to meat to reduce cost as well as to reduce cooking losses (to retain a juicy product); to improve nutritional composition, for emulsification; to modify texture; to increase shelf life; and to improve colour stability (Mills, 2004). Ingredients which have been used for meat extension include flour, dextrose, cellulose, proteins from plants, milk or animal products and hydrocolloids (Mills, 2004). More in depth information on this topic is covered in Chapter 4. Several extenders have been investigated for effects on processed meat products including legume flours (Dzudie, Scher & Hardy, 2002; Kassama, Ngadi & Raghavan, 2003; Modi, Mahendrakar, Rao & Sachindra, 2004), sorghum (Huang, Zayas & Bowers, 1999), wheat (Mansour & Khalil, 1999: Ulu, 2004), textured whey protein (Hale, Carpenter & Walsh, 2002), whey protein concentrate (Ulu, 2004), and okara and other non flour forms of soy (Kassama et al., 2003; Katayama & Wilson, 2008; Turhan, Temiz & Sagir, 2009; Ulu, 2004). Soy in particular is commonly used as an ingredient in processed meats and deserves some attention in the following section.

2.6 Soy

Soy is commonly used as an ingredient in processed meats and is the most common plant protein used in processed meat products (Macedo-Silva, Shimokamaki, Vaz, Yamamoto & Tenuta-Filho, 2001). Soy has multi-functional properties such as being a stabiliser and emulsifier (Lusas & Riaz, 2005), ability to improve texture and water holding capacity of the final product, as well as the high nutritive value of soy protein.

2.6.1 Processed soy ingredients

Soybeans are often processed to remove "beany" flavour before addition to food products and the major types used as food ingredients are soy protein concentrates, soy flour and grits and soy protein isolates (Pearson, 1976). For meat extenders textured soy protein, spun soy protein and textured soy protein isolate are the main ingredients used (Pearson, 1976).

Soy flour is often produced from defatted soybeans which are milled through a 100 mesh sieve (0.157 mm pore size) or finer, whilst soy grits are produced from coarser particles (Singh et al., 2008; Lusas & Riaz, 1995). The de-fatting step prevents " beany" flavour developing due to the activation of lipoxygenase during processing as a result of favourable moisture, heat and time conditions (Lusas & Riaz, 1995). Soy protein concentrates are more refined than flours which contain 70% or more protein on a dry weight basis (Singh et al., 2008; Lusas & Riaz, 1995).

2.6.2 Textured soy products

Textured soy proteins are produced by extrusion (Pearson, 1976; Singh et al., 2008; Mills, 2004). The moist soy flour (or flakes) is pushed under high pressure and temperature through a die and after the release through the die produces a product with expanded open cell structure with a texture similar to meat (Pearson, 1976). Spun soy protein is made by spinning soy into fibres but is used for meat analogues rather than as a meat extender (Pearson, 1976). Textured soy protein isolate is produced by heating a slurry of soy protein isolate with a solids content of up to 35% under pressure, followed by cooling (Pearson, 1976). Hydroxides, phosphate ions

and polyvalent linking agents are then added to increase the pH which is needed to make the protein reactive enough to form filaments (Pearson, 1976). This protein is mainly used for processed meat products which lack structure, for example they give water binding and textural properties to processed poultry meat (Pearson, 1976). It is often used in hamburger patties as it imparts a desired texture and is good for water binding to retain juiciness (Pearson, 1976) as well as retaining the dimensional stability and shape of the patties and meat pieces during heating (Singh et al., 2008). These textured proteins offer the benefits of extending meat which is more expensive and incorporating the many health benefits of soy into a meat product (Singh et al., 2008). In addition, soy protein isolates at level of 1-4% in emulsified meats can produce a visually appealing product which has no "off flavours", lower cooking losses and greater yields (Singh et al., 2008). Although textured soy proteins have many desired attributes one obstacle to overcome is the decrease in flavour which caused by a flavour dilution effect in the processed product (Singh et al., 2008). This problem is not so great in hamburgers in which meat can be supplemented with soy products up to 20% without the need for a flavour enhancer (Singh et al., 2008).

2.6.3 Soy health benefits

Apart from the economical and technological benefits described above, soybean products can also provide some health benefits that are normally found in soy. For example, soy may reduce the risk of cancer, heart disease and osteoporosis; treat menopausal symptoms; and promote bone formation (Messina, 1999; Riaz, 1999; Blum, Heaton, Bowman, Hegsted & Miller, 2003). These health benefits have been linked to a class of compounds called isoflavones. They are a sub-class of flavonoids which have the structural feature of a flavone nucleus of 2 benzene rings (A and B) linked by a heterocyclic pyrane C ring (Messina, 1999). The position of the benzene B ring distinguishes between flavonoids and isoflavonoids (Messina, 1999) as the former is linked to the C ring at the 2 position and the latter is linked at the 3 position (Wang & Murphy, 1994). In soybeans and soy foods the three aglycons genistein, daidzein and glycetein exist in four forms giving 12 isoflavones in soy foods in total (Wang & Murphy, 1994). Although flavones are found in many plants, isoflavones are found in only a few because of the presence of the enzyme chalcone isomerase which converts the flavone precursor 2 (R)

naringinen to 2 hydroxydaidzein in these plants (Coward, Barnes, Setchell & Barnes, 1993). These compounds possess both antifungal and antioxidant activity (Wang & Murphy, 1994). They are called phyto-oestrogens as they possess oestrogenic activity and occur in plants (Isanga & Zhang, 2008). The isoflavones are hypothesised to act as weak oestrogens in a low oestrogen environment and weak anti-oestrogens in a high oestrogen environment (Messina, 1999). They are able to bind to oestrogen receptors and have a similar structure (Isanga & Zhang, 2008). Oestrogen which normally functions as a hormone and is produced in the ovaries declines after menopause (Riaz, 1999). Isoflavones could be used as an alternative to hormone replacement therapy in post menstrual women (Blum et al., 2003).

Concerns have been raised that consumption of the phytoestrogenic isoflavones by men has an adverse effect on male reproductive health and feminising effects; however a review of clinical evidence suggests this is not the case (Messina, 2010). Some evidence suggests that isoflavones may have a detrimental effect on prostate cancer but the majority of evidence suggests that consumption of the phytoestrogenic isoflavones reduces the risk of prostate cancer (Adlercreutz, 2002; Wuttke, Jarry & Seidlova-Wuttke, 2010). The primary isoflavones in soybeans genistein and daidzein may lower the risk of cancers of the prostate, colon, rectum, breast, lung and stomach (Riaz, 1999). Of the approximately 9000 papers published on isoflavones, over 20% have focused on cancer (Messina, 1999). Recent interest in flavonoids and isoflavones has seen the number of publications on biological activity of these compounds double from 2000-2007 compared to the number published in the preceding 60 years (Rochfort & Panozzo, 2007). There has been much research focused on a reduced incidence of breast cancer from consuming soy because of the lower rates in Asian countries where soy is often consumed (Messina, 1999). Overall there is little evidence of a reduced risk of breast cancer in post menopausal woman but some evidence of a reduced risk in pre-menopausal women (Messina, 1999; Bingham, 2002). The anti-cancer activity of isoflavones is thought to be partly due to their ability to scavenge oxidants involved in carcinogenesis (Isanga & Zhang, 2008). Soy diets were shown to lower plasma MDA levels significantly and raise total anti-oxidant capacity (Azadbakht, Kimiagar, Mehrabi, Esmaillzadeh, Hu & Willett, 2007).

Soy may reduce the risk of heart disease as it has cholesterol lowering effects. Cholesterol levels were lowered when soy protein was substituted for animal protein in the diet (Potter,

1998). The isoflavones may lower cholesterol due to their similarity to mammalian oestrogens which lower low density lipoprotein level and increase levels of high density lipoprotein cholesterol (Potter, 1998). There are two mechanisms proposed for the cholesterol lowering ability of soy. Potentially, when soy protein is consumed intestinal absorption of bile acids and cholesterol is interrupted (Potter, 1998). It is also proposed that soy protein alters the metabolism of cholesterol and/or lipoproteins in the liver (Potter, 1998). When soy is consumed, the liver's metabolism changes to use more cholesterol to produce bile acids; so the activity of cholesterol biosynthesis and LDL receptor increases (Omoni & Aluko, 2005). This increases the removal of cholesterol from the blood by the LDL receptor and consequently the blood cholesterol levels decrease (especially the LDL fraction) (Omoni & Aluko, 2005).

It has been hypothesised that soy isoflavones prevent bone resorption and stimulate bone formation which ameliorates osteoporosis (Omoni & Aluko, 2005). Dietary soy has been demonstrated to have beneficial effects on bone tissue in a rat model of oestrogen deficiency bone loss (Blum et al., 2003). The mechanism by which this occurs is different from the usual effects of oestrogen on bone thus phyto-oestrogenic isoflavones must act through a different mechanism (Blum et al., 2003).
In addition to suggested health benefits mentioned above, soy contains fibre, lecithin, saponins, Bowman-Birk inhibitors, phytosterols and ω-3 fatty acids which may act alone or in synergy to produce health benefits (Omoni & Aluko, 2005).

2.7 Tempeh

There are a variety of fermented foods and these may comprise one third of all food consumed worldwide (Nout & Keirs, 2005). In Asia, a diverse range of soybean products including soy sauce, natto, daejung (soy bean paste), miso, tofu, sufu and tempeh are regarded as a staple diet. These products are now consumed worldwide due to globalization and world mobilization.

2.7.1 Tempeh fermentation

Tempeh is a plant substrate, traditionally soybean fermented in a solid state with a species of *Rhizopus* fungi. Soybeans are soaked, dehulled, cooked and fermented with the fungal hyphae growing into the bean cotyledon and knitting it together into a sliceable mass. The soaking step hydrates the soybeans and allows the hulls to be loosened which make them easier to remove. It also leaches saponins which act as antimicrobial compounds (Nout & Kiers, 2005). This enables microbial activity and acidification by endogenous lactic acid bacteria can occur during this step which inhibits growth of pathogens (Hutkins, 2006). Soybeans contain high levels of sugars which cause flatulence such as stachyose and raffinose which are α-galactosides of sucrose (Nout & Kiers, 2005) and soaking removes these compounds (Hutkins, 2006). Glucose and fructose are released from the hydrolysis of these sugars by invertases and glucosidases and provide nutrients for the natural and added microflora (Hutkins, 2006). The cooking step destroys anti-nutritional compounds such as haemagluttinins and trypsin inhibitors (Hutkins, 2006), releases nutrients for mould growth and removes competition for mould growth (Steinkraus, 1983; Hachmeister & Fung, 1993). The fermentation is the step when biochemical changes occur which cause the largest changes in tempeh processing. The Rhizopus partially digests the soybeans and uses the digested material as nutrients for its growth. The fungal hyphae can penetrate 1mm or 25% of the cotyledon and hold it together (Hutkins, 2006).

2.7.2 Nutritional composition of tempeh

Tempeh has a high content of protein (19%) or as high as 40% of its dry mass (Hutkins, 2006; Chang, Hsu, Chou, Chen, Huang & Chung, 2009; Baumann & Bisping, 1995). Soluble nitrogen increases during the fermentation up to four fold as the protein fraction is digested by the starter culture (Hutkins, 2006; Astuti, Meliala, Dalais & Wahlqvist, 2000; Zamora & Veum, 1979). Free amino acid concentration increases up to five fold after 30 hours of fermentation (Baumann & Bisping, 1995) whilst the overall amino acid content decreases by 3.62-27.9% after 48 hours of fermentation (Astuti et al., 2000). This indicates that the microorganism liberates amino acids during digestion by breaking down proteins, and further digests amino acids which release soluble nitrogen. Despite a quarter of the soybean protein degrading to

soluble nitrogen (Hutkins, 2006), the protein concentration increases during fermentation. For example, protein concentration was increased (van der Riet, Wight, Cilliers & Datel, 1987; Nassar et al., 2008), or slightly increased by fermentation (van der Riet et al., 1987; Zamora & Veum, 1979). The net protein utilisation is improved by fermentation and this suggests that during fermentation there is a change in the pattern and/or content of amino acids (Zamora & Veum, 1979).

Lipid contents are reduced during tempeh making because the lipase enzyme hydrolyses triglycerides into free fatty acids (Astuti et al., 2000) as well as mono and diglycerides and a small amount of glycerol (Hutkins, 2006). The lipid contents decrease by 26% (Astuti et al., 2000) with most free fatty acids later oxidised by *R. oligosporus* for energy (Hutkins, 2006). Fatty acid distribution changes with an increase in linoleic and oleic acids as a result of a decrease in linolenic acid (Nout & Kiers, 2005) and palmitic and stearic acids are also produced (Nout & Rombouts, 1990).

Carbohydrates are also utilised by the starter culture, however larger losses of soluble carbohydrates are observed during processing (soaking and cooking steps) than during fermentation (van der Riet et al., 1987). Starch levels are reduced during fermentation (van der Riet et al., 1987), but an increase in glucose was observed which could be attributed to digestion of complex carbohydrates to simpler ones (Astuti et al., 2000). The enzymatic degradation of polysaccharides during fermentation produces a variety of water soluble high molecular weight polysaccharides (Nout & Kiers, 2005). Some of the degraded compounds include; cellulose, pectin and other fibre components with pentoses (xylose, arabinose) and hexoses (galactose, glucose) released (Hutkins, 2006). A loss of fibre was noticed during processing and a small loss of fibre was observed after 24 hours fermentation (van der Riet et al., 1987). In contrast non-soy tempeh (see table 3) significantly ($p < 0.05$) increased in fibre content as a result of fermentation (Nassar et al.,2008)

Table 3: Summary of studies utilising non soy ingredients for tempeh production

Authors	Fermented material	Main findings
Angulo-Bejarano et al. (2008)	Chickpea flour	Fermentation improved essential amino acid content. The nutritional indices of protein digestibility corrected amino acid score, net protein retention, *in vivo* protein digestibility, protein efficiency ratio and calculated protein efficiency ratio were improved by fermentation.
Berghofer et al. (1998)	Faba beans, oats, soybeans	Fermentation increased the antioxidative properties of the raw materials
Nassar et al. (2008)	Faba beans and Faba beans with varying percentages of lupine, chickpeas and peas included in the composition	Tempeh produced from a mixture of 25% of each legume had the highest nutritional quality. Fermentation improved nutritional properties of all tempehs by increasing fibre and protein contents and decreasing flatulence producing sugars
Starzynska-Janiszewska et al. (2008)	Grass pea (*Lathyrus sativus*, Derek and Krab cultivars)	Fermentation increased radical scavenging activity which was more pronounced in the $DPPH^{\cdot}$ assay

Tempeh is richer in some vitamins than soy and the processing and fermentation remove anti-nutritional factors which make it a more nutritious product compared with soybean. Phytic acid is reduced during processing; however larger amounts are removed during fermentation (van der Riet et al., 1987). Phytic acid is an anti-nutritional compound which strongly chelates minerals such as calcium, magnesium, iron and zinc forming insoluble complexes that hinder their absorption (Hachmeister & Fung, 1993). The amounts of trace minerals generally do not change but their solubility does (Astuti et al., 2000). Most iron in tempeh is normally bound to

protein, however as fermentation progresses and protein breaks down the free iron is released, becoming more soluble (Astuti et al., 2000). Liver iron concentrations were significantly higher in rats fed tempeh than those fed soybeans (Kasaoka, Astuti, Uehara, Suzuki & Goto, 1997). Levels of calcium, sodium, manganese and phosphorous increased but there was a noticeable decrease in potassium (van der Riet et al., 1987). These authors observed a large amount of potassium leakage during fermentation as the water released into the fermentation bag was rich in the mineral (van der Riet et al., 1987). Increases in mineral content could be due to the loss of mass which occurred during fermentation without a parallel loss of minerals (van der Riet et al., 1987).

Tempeh is one of only a few plant foods which contain vitamin B_{12} which makes it important for vegetarian diets (Hutkins, 2006). The concentration per 100 g is generally 0.1 to 0.2 μg in cooked tempeh (Hutkins, 2006). Thiamine concentrations drops during fermentation, however those of nicotinic acid and riboflavin increase (van der Riet et al., 1987; Astuti et al., 2000). During fermentation small amounts of carotenoids are formed and some strains produce β-carotene (Nout & Kiers, 2000). Tocopherols change during fermentation with levels of β, γ, and δ tocopherols increasing while α remains unchanged.

Tempeh, like soy is rich in antioxidants and the fermentation process improves the antioxidant potential. Fermentation increases the antioxidant capacity in both soy (Berghofer et al., 1998) and non soy tempeh (Starzynska-Janiszewska et al., 2008; Berghofer et al., 1998). Tempeh contains isoflavones (Wang & Murphy, 1994) and other antioxidative compounds (Hoppe et al., 1997; Esaki et al., 1996) including amino acids and peptides (Watanabe, Fujimoto, & Aoki, 2007). Hoppe et al. (1997) proposed that the antioxidant activity in tempeh is a synergistic one between tocopherols and amino acids released during fermentation. Tempeh contains higher amounts of isoflavones than other traditional oriental foods such as tofu, honzukuri miso, fermented bean curd and bean paste (Wang & Murphy, 1994). During fermentation total isoflavone levels are generally reduced (Wang & Murphy, 1994; Hutchins, Slavin & Lampe, 1995; Watanabe et al., 2007) where only 60% of the isoflavones of the original soy are available after fermentation but these isoflavones were more readily available as a result of hydrolysis of the glycosidic forms (Hutchins et al., 1995). The forms of the isoflavones are changed by fermentation to tempeh. The aglycons are the major form of isoflavones, probably due to hydrolysis of the glycosidic forms (Wang & Murphy, 1994; Hutchins et al., 1995; Watanabe et al., 2007). The glycosides daidzin and genestin decreased and there was an

increase of daidzein and genistein (aglycon isomers) of 40 and 8 times, respectively, during fermentation (Hutchins et al., 1995). Other antioxidants isolated from tempeh include 3-hydroxyanthranilic acid (HAA) (Esaki et al., 1996) and 5-(δ-tocopheroxy) - δ-tocopherol (5DT) (Hoppe et al., 1997). HAA was found in tempeh but not soybeans and had stronger antioxidant activity than genistein in a soybean oil system (Esaki et al., 1996). Different fermentation conditions and different starter cultures can produce different antioxidant compounds. Fermenting tempeh anaerobically after aerobic fermentation produced tempeh with stronger antioxidant activity (Watanabe et al., 2007). Tempeh was observed to have the best DPPH radical scavenging activity at 10 days fermentation time (Chang et al., 2009). When tempeh was fermented with *Aspergillus satoi* the daidzein and genistein were hydroxylated enzymatically at the eighth position to produce more powerful antioxidants (Esaki, Onozaki, Morimitsu, Kawakishi & Osawa 1998).

2.7.3 Health benefits of tempeh

Tempeh is rich in nutrients and has many health benefits including many of those attributed to soy. Rats which consumed soy had superoxide dismutase activity 3.6 fold lower than those that consumed tempeh (Kasaoka et al., 2007). The liver TBARS were 72% lower in rats which consumed tempeh than those which consumed soy (Kasaoka et al., 2007). There are studies supporting the potential benefits of tempeh in fighting chronic, non infectious disease, particularly cardiovascular disease and cancer (Karyadi & Lukito, 1996). Tempeh can be considered beneficial in preventing cardiovascular disease as it contains high levels of isoflavones, folate, vitamin B_{12}, dietary fibre, a beneficial fatty acid composition and a low glycaemic index (Lukito, 2001).

Tempeh can be consumed as a meat alternative because it is a protein rich plant food (Lukito, 2001) and is high in B vitamins including vitamin B_{12} which is not often found in plant foods. Tempeh is not often consumed raw and has a bland mushroom like flavour in this form (Hutkins, 2006). Cooking changes the flavour to nutty and it starts to resemble the flavour of cooked meat (Hutkins, 2006). The Maillard reaction where amino acids are heated with reducing sugars is responsible for the flavour of both meat and tempeh, thus cooking tempeh

resembles the meat cooking process (Hutkins, 2006). The lipid component of tempeh may be a precursor for this cooked flavour (Hutkins, 2006).

Tempeh has previously been incorporated into ham (Kuo, Wang, Peng & Ockerman, 1989) and a patent exists for the creation of a processed fish product containing tempeh (Györgi, 1972).

As the flavour of tempeh is similar to meat and is often used for partial or full replacement, there is potential for new products to be developed by incorporating tempeh in meat with main emphasis on health benefits. The presence of meat and tempeh both improve the bioavailability of minerals, particularly iron.

However, it is unknown how the free iron from both products will behave under digestion conditions. The addition of tempeh adds a different amino acid profile, favourable change to the fatty acid profile, a higher B vitamin content than many other extenders and increases isoflavones. As meat has been suspected to increase the likelihood of cancer particularly colorectal cancer, the antioxidants contained in tempeh may be able to limit oxidation in stomach if the tempeh and meat are incorporated together in a product. Thus addition of tempeh to a meat product may provide an antioxidant source which complements the meat flavour in a processed meat product. This theory is the basis of the research to be undertaken.

2.8 Research objectives and outline of research

It is hypothesised that the addition of tempeh will produce favourable changes in the oxidative processes of beef patties during *in vitro* digestion. This research will aim to test this hypothesis. The objectives were:

- To find the best legume for fermentation to tempeh to be incorporated into hamburger patties by measuring antioxidant properties.
- To test the changes in a variety of physical, chemical and sensory quality parameters which occur with addition of tempeh and to chose an appropriate level of tempeh to incorporate in a novel buger patty
- To test the effect on *in vitro* oxidative processes which occur with addition of a specified level of tempeh in a beef patty

The first phase of research which is outlined in Chapter 3 is concerned with testing the radical scavenging and total phenolic content of different beans to observe how these change with fermentation and to find out which will be most suitable to select for addition to a burger patty to undergo further physical, chemical and consumer tests.

The research in Chapter 4 tests the changes in a variety of physical, chemical and sensory quality parameters which occur with addition of tempeh and an appropriate level of tempeh to incorporate in a novel burger patty is determined. At this point the level of tempeh is chosen for sensory testing and as a level to test in Chapter 5.

In Chapter 5 the burger patties are subjected to *in vitro* digestion to determine their oxidative potential under conditions similar to the normal digestive environment

Chapter 3. Total phenolic content and radical scavenging properties of tempeh substrates

3.1 Introduction

Legumes are podded plants including beans and lentils and are widely consumed throughout the world (Messina, 1999). In addition to having significant amounts of protein, fibre and minerals, legumes are rich sources of antioxidants. They contain a high amount of phenolic compounds, especially those which have high anthocyanin content in their seed coats (Acar, Gokman, Pellegrini & Fogliano, 2009). A survey of the total antioxidant capacity (TAC) of over 100 foods was carried out by Wu, Beecher, Holden, Haytowitz and Prior (2004) and red kidney beans and small red beans were found to have very high TACs, especially in the hydrophilic fraction. The TAC of dry red kidney beans were 46 fold, 11 fold and 2 fold higher than tomatoes, red grapes and blueberries, respectively. Small red beans had the highest hydrophilic oxygen radical absorbance capacity (ORAC) value of all samples. Heating of the beans which occurs during tempeh processing has the potential to destroy bioactive compounds but could also form antioxidative Maillard Reaction products (Acar et al., 2009). Heating has been demonstrated to raise TAC of kidney beans, yet lower the activity in soybeans (Acar et al., 2009).

Several studies demonstrated that fermentation increases the antioxidant activity of pulses (Lee, Hung & Chou, 2008a; Lee, Yang & Mau, 2009; Hubert, Berger, Nepveu, Paul & Dayde, 2008; McCue & Shetty, 2003a; Chang et al., 2009; Lin, Wei & Chou, 2006) thus fermentation seems to be an ideal way to improve the nutritional status of pulses before consumption or incorporation into other products.

There are several different methods available for determining the antioxidant activity which measures various antioxidant parameters (Frankel & Meyer, 2000). The methods used in this chapter are total phenolic content (TPC), 1, 1-diphenyl-2-picryl-hydrazyl ($DPPH^{\bullet}$) radical scavenging and oxygen radical absorbance capacity (ORAC). Total phenolic content measures

amount of compounds which contain a phenol group, whilst DPPH and ORAC measure the ability of the compound tested to prevent oxidation by scavenging radicals.

Antioxidant assays can be categorised as operating by electron transfer (ET) which measures the reducing capacity of an antioxidant or hydrogen atom transfer (HAT) mechanisms which measure an antioxidant's ability to donate hydrogen atoms (Huang, Ou & Prior, 2005). ORAC is an HAT assay whilst the DPPH and TPC assays are both electron transfer assays (DPPH has recently been reclassified as an ET assay) (Huang et al., 2005).

The DPPH assay uses an azo (R-N=N-R) initiator to produce radicals which are reduced by the antioxidant compounds in the sample according to the reaction (Frankel & Meyer, 2000; Brand-Williams, Cuvelier & Berset, 1995):

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$$

$$DPPH^{\cdot} + R^{\cdot} \rightarrow DPPH\text{-}R$$

Technically $DPPH^{\cdot}$ can react by either a HAT or ET mechanism. However when tested in ethanol or methanol there is a rapid electron transfer reaction from phenoxide ions to DPPH and the abstraction of a H atom from the phenol group is slow and only marginally involved as these alcohol solvents are strong hydrogen bond acceptors (Huang et al., 2005; Foti, Daquino & Geraci, 2004). The phenoxide anion is a phenol group which is lacking hydrogen and therefore has a negative charge, which is able to donate electrons.

The DPPH assay is a relatively simple assay to measure antiradical activity of compounds (Brand-Williams et al., 1995). It has also been widely used in the literature available on antioxidant activity of pulses. However different antioxidant compounds react with the DPPH radical at different rates and with different stoichiometries thus it is important to measure the reaction at steady state which may be up to 24 hours after the sample is added (Brand-Williams et al., 1995). Also, when differing ratios of sample: antioxidant are tested the time response curve is not linear, complicating the analysis (Brand-Williams et al., 1995; Frankel & Meyer, 2000; Sanchez-Moreno, Larrauri & Saura-Calixto, 1998).

The ORAC assay also utilises an azo initiator 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) thermally degraded at 37°C and produces peroxyl radicals. The peroxyl radicals attack the fluorescein probe used in the assay decreasing the fluorescence. The assay utilises a trolox standard curve with the curve of trolox concentration plotted against the fluorescence remaining over the time of testing. Larger areas under the curve indicate a higher antioxidant effect as the trolox competes with the peroxyl radicals to inhibit degradation of the fluorescein probe. The area under the curve of a sample is compared to the area under the trolox standard curve.

The ORAC assay has the advantage of measurement can be applied equally to antioxidants with long lag phases and those with very short lag phases as the area under the curve of fluorescence against time is calculated (Huang et al., 2005). Food samples may contain a variety of antioxidant compounds with varying reaction kinetics thus the ORAC assay is highly applicable to these samples (Huang et al., 2005). This means that the assay avoids assumptions of linear response of an antioxidant over time and also combines the antioxidant effect at different times into a single unit (Trolox equivalent) which is the same unit in many studies, making results easily comparable (Frankel & Meyer, 2000). The assay measures inhibitory effect against the peroxyl radical which occurs *in vivo* thus the assay measures a biologically relevant radical.

The assay has the disadvantage of being affected by the dilution factor used in the assay. Samples will often need to be diluted to have values within the range of the trolox standard curve. It is important to use the lowest dilution factor possible as the antioxidant effects of most samples unlike trolox are not linear with changes in concentration thus the final result is highly dependant on the dilution factor used in the calculation.

The total phenolic content method was originally developed for measuring protein content as the Folin-Ciocalteu (FC) reagent used reacts with the tyrosine phenol rings of proteins (Folin & Ciocalteu, 1927). Later the assay was used by Singleton and Rossi (1965) to measure the total phenol content of wine and has subsequently been utilised to measure total phenolic content for a variety of samples (Huang et al., 2005). Although the exact composition of FC reagent is not known it is hypothesised to contain heteropolytungstates-molybdates (Huang et al., 2005; Karadag, Ozcelik & Saner, 2009). As sodium carbonate is added in the assay, the pH is around 10 and the phenols can react with the FC reagent under these conditions (Huang et al., 2005). The FC reagent is reduced by the phenolate anion which is produced from the dissociation of

phenolic protons under basic conditions (Huang et al., 2005). The yellow colour of the FC reagent is changed under reducing conditions to blue measured at λ = 765 nm possibly due to the formation of the compound $(PMoW_{11}O_{40})^{4-}$ (Huang et al., 2005; Prior, Wu & Schaich., 2005). This assay has the disadvantage of the FC reagent being reduced by non phenolic compounds such as amino acids and ascorbic acid (Prior et al., 2005; Huang et al., 2005). However the assay has the advantage of being simple and reproducible as well as generally correlating to antioxidant activity (Huang et al., 2005; Magalhaes, Segundo, Reis & Lima, 2008).

Tempeh is traditionally made from soybeans, however various other pulses have been used in tempeh making (See section 2.7 for more information). The objectives of the present work are to investigate the antioxidant activities of several pulses reported to be high in antioxidant activity and examine the effects of processing and fermentation on such activities with the aim of identifying a tempeh high in antioxidant activity for use in beef patties. The antioxidant potential will be assessed by measuring the amount of phenolic compounds as well as ability to scavenge the synthetic radical DPPH and oxygen radicals which are involved in many oxidative processes in meat post-mortem (Harel & Kanner, 1985b).

3.2 Materials and Methods:

3.2.1 Materials

Butylated hydroxytoluene (BHT), fluorescein sodium, gallic acid, 6-hydroxy-2, 5, 7, 8 tetramethyl-chroman-2-carboxylic acid (Trolox) and 3, 3', 4', 5, 7-pentahydroxyflavone (quercetin) were purchased from Sigma-Aldrich (St Louis, MO, U.S.A). Disodium hydrogen phosphate, ethanol, potassium dihydrate orthophosphate, sodium carbonate and sodium chloride were purchased from BDH chemical company (Poole England). 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) was purchased from Cayman Chemical Company (Ann Arbor MI, U.S.A). Methanol was purchased from Biolab Australia (Clayton, Victoria, Australia). Diethyl ether was purchased from Riedel de Haën (Seelze, Germany). Folin Ciocalteu reagent was purchased from Merck Chemical Co (Darmstadt, Germany).

3.2.2 Sample Preparation

Dry kidney (*Phaseolus vulgaris*), azuki (also known as red, *Vigna Angularis*) and soy (*Glycine max*) beans were purchased locally from Taste nature organic store (Dunedin, New Zealand) and the antioxidant content in the dry beans was evaluated. The antioxidant of azuki and soy beans were the highest and therefore the activity was followed during processing (after soaking and boiling steps) and after tempeh making to investigate the effect of these processing steps on the antioxidant activity. Because of the potential contribution of the anthocyanins on the hull of red beans to the overall antioxidant activities, total antioxidant capacity (TAC) may be an important factor since tempeh is generally made from dehulled beans. TAC was evaluated in whole azuki beans and de-hulled azuki beans to estimate the contribution of the hull to TAC. Therefore the following treatments were used for further analyses; processed beans (azuki, dehulled azuki and soy) and tempeh (azuki, dehulled azuki and soy)(Figure 3). All samples were extracted using 70% ethanol (Chung, Ji, Canning, Shi & Zhou, 2010) and these extracts were used for the antioxidant assays. All samples were prepared in triplicate (Figure 1).

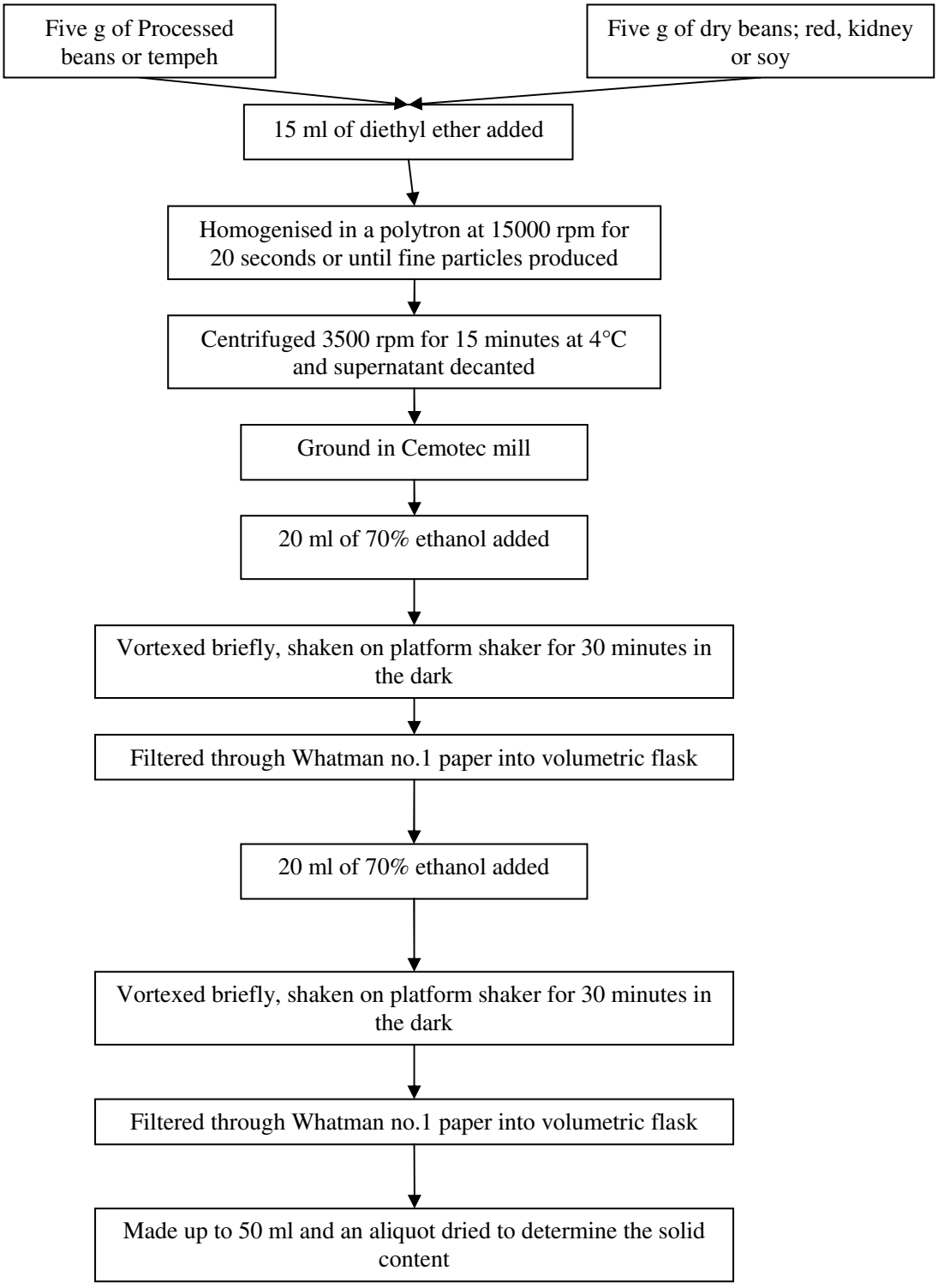

Figure 1: Extraction procedure for the samples

Processed beans

Azuki and soy beans were soaked for 2 days at 4°C. A portion of the azuki beans had the skins (hull) removed manually so that dehulled azuki beans could be obtained. Beans were cooked for 10 minutes in a pressure cooker which was heated to 100°C. The beans were drained and subsamples were cooled and stored at -80°C for subsequent analysis.

Tempeh making

Tempeh was made from processed beans prepared as described above. Processed beans were dried in towels and had white vinegar added at a concentration of 0.02 ml/g of beans. The starter culture (*Rhizopus oligosporus*) was then added at a concentration of 9.87 x 10^{-4} g culture/g beans. The mixture was then packed into perforated ziplock bags (170 x 180 mm) and incubated in a snaplock container (Klip it, 255 x 120 x 55 mm, 1.75 l, Sistema Plastics, NZ) with 1 M Potassium nitrate to create a humid atmosphere (92% relative humidity) at 31°C in an incubator (Labserve, Ontherm Scientific Ltd, Hutt City, NZ) for 24 hours (see Figure 2).

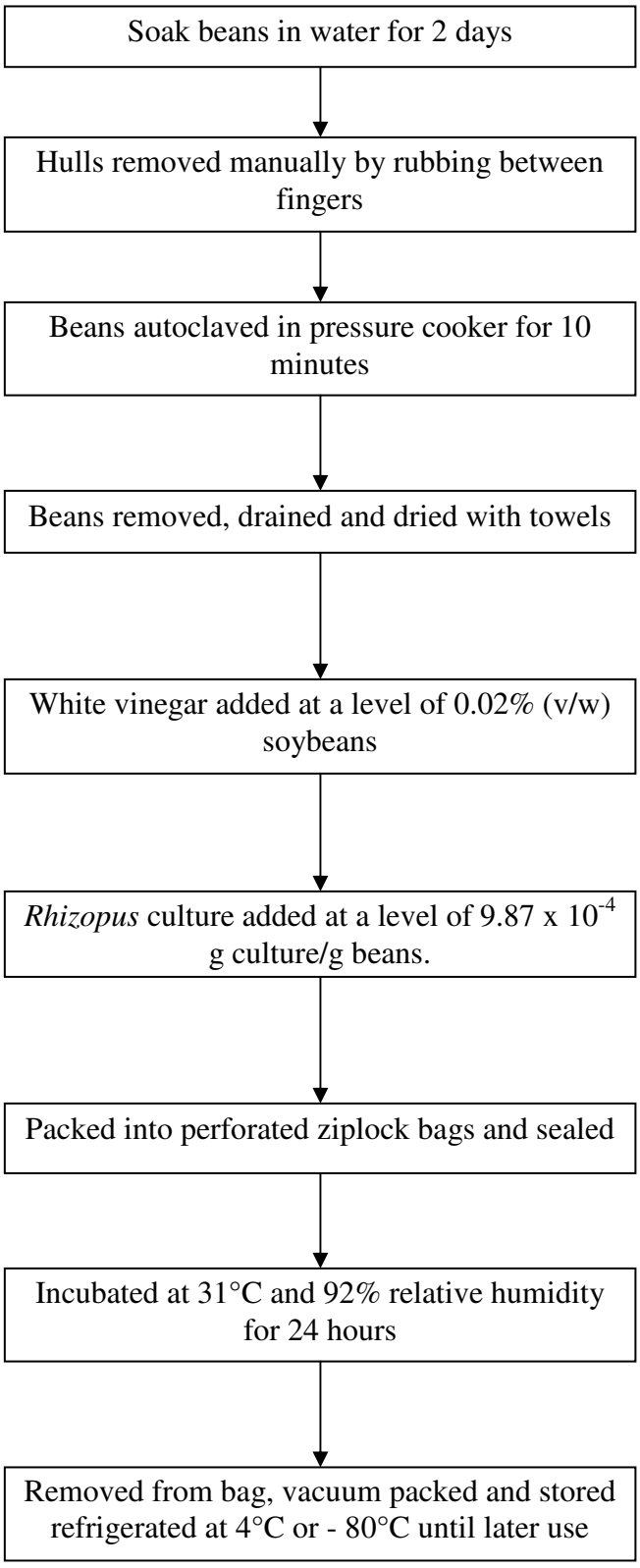

Figure 2: Tempeh making flow diagram



Figure 3: Tempeh treatments: From left to right; soybean, azuki and dehulled azuki tempeh

Sample extraction

Samples were prepared according to the method of Watanabe et al. (2007). All the samples (powder form for dry samples or minced form for wet samples) were subjected to diethyl ether wash (1:3 sample to solvent ratio) to remove the lipid fraction (Watanabe et al., 2007). Dry beans were first pulverised in a Cemotec mill (Cemotec, Tecator 1090 sample mill, 115 V, Foss, Höganäs, Sweden) on setting 1 and five gram samples were weighed into 50 ml Falcon tubes. Five grams of processed beans and tempeh were homogenised with a Polytron PT-MR 2100 (Kinematica, AG, Switzerland) at 15000 rpm for 20 second or until fine particles were produced. Samples were then centrifuged (Beckman, GPR, Palo Alto, CA, USA) at 3500 rpm for 20 minutes at 4°C. The supernatants were decanted, disposed and 20 ml of 70% (v/v) ethanol was added to the pellet and vortexed briefly (Chiltern, MT17, Sydney, NSW,

Australia). Samples were then shaken in the dark on a platform shaker for 30 minutes (Ratek Platform mixer, RM2, Boronia, Victoria, Australia). They were then centrifuged as described above and filtered through Whatman No. 1 filter paper into a 50 ml volumetric flask. Twenty ml of 70% ethanol was then added. Vortexing and shaking, centrifugation and filtering steps were repeated as described above. The funnel and filter were rinsed with 70% (v/v) ethanol solution into the volumetric flask and made up to the 50 ml mark. Samples were divided into aliquots then stored at -80°C until they were analysed. Dry matter was determined by adding aliquots of the extracts to Petri dishes. The extracts in Petri dishes were left in a fume hood to evaporate the ethanol and subsequently dried in a 70°C drying oven. Dry matter in the 50 ml extract was determined by the formula:

(Weight of Petri dish + sample after drying) - (Weight of Petri dish)/volume x 50

Samples were coded as described in (Table 4).

Table 4 : Codes of bean, processed bean and tempeh samples used for antioxidant assays.

Sample	Code
Red Kidney Bean	RKB
Dry red (azuki) bean	RB
Dry soy bean	SB
Soaked red (azuki) bean	SRB
Dehulled red (azuki) bean	DRB
Soaked soy bean	SB
Red (azuki) bean Tempeh	RT
Dehulled red (azuki) bean tempeh	DRT
Soy bean tempeh	ST

3.2.3 Total phenolic content

Total phenolic content (TPC) was determined as gallic acid equivalents as described by Singleton and Rossi (1965) modified by Makkar, Blümmel, Borowy and Becker (1993). Aliquots of 200 µl of the 70% (v/v) ethanol extracts were added to 800 µl of 70% (v/v) ethanol, then 0.5 ml of 50% Folin-Ciocalteu reagent and 2.5 ml sodium carbonate (20% w/v) were added. The sample mixtures were then vortexed and left in the dark for 40 minutes. The mixtures were then centrifuged at 2500 rpm for 10 minutes at 20°C and the supernatants were read at 725 nm in a spectrophotometer (Ultraspec 3300 Pro, Amersham Biosciences, Cambridge, England). The sample absorbance was used to calculate TPC as gallic acid equivalents by comparison with a standard curve of gallic acid prepared and measured under the same conditions used for the samples.

3.2.4 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) radical scavenging activity

The DPPH assay followed the methods of Brand Williams et al. (1995) and Sanchez-Moreno et al. (1998).Triplicate aliquots were added at various volumes to produce series of curves of DPPH inhibition (Appendix 1). Methanol was added to make the sample volume up to 1 ml. Different volumes (µl) were needed to produce optimal curves of DPPH concentration from different extracts (Appendix 1).

Two ml of $DPPH^{\cdot}$ solution (0.025 g/L) was added to cuvettes containing 1 ml of sample or a combination of sample and 70% ethanol. These were gently inverted with parafilm on the top of the cuvette and measured immediately in an Ultraspec 3300pro spectrophotometer (Amersham Biosciences, Cambridge, England) at 515 nm with a 70% ethanol blank for reference. It is necessary for the calculation to take the absorbance of the sample at time 0. Samples were then kept in the dark and subsequently analysed after 10 minutes, 1 hour and 24 hours. A standard curve of $DPPH^{\cdot}$ (0.05 mg/ml) at concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0% was prepared to compare absorbance to a known amount of DPPH for comparison. The percentage of $DPPH^{\cdot}$ remaining was determined by the equation

$$\% DPPH^{\bullet}_{R} = [(DPPH^{\bullet})_{T} / (DPPH^{\bullet})_{T=0}] \times 100$$

Where $DPPH^{\bullet}_{T}$ = $DPPH^{\bullet}$ at the time of steady state and $DPPH^{\bullet}_{T=0}$ = $DPPH^{\bullet}$ at time zero (initial concentration). The efficient concentration for 50% inhibition of DPPH (EC_{50}) was determined by plotting mg extract against % inhibition substituting and calculating the concentration required for 50% inhibtion and then converted to EC_{50} g extract/ 1 g DPPH.

Standards of gallic acid, quercetin, BHT and trolox were prepared to be tested for use as reference standards. Gallic acid and trolox were dissolved in water and BHT and quercetin dissolved in methanol at a concentration of 0.00216g/ml.

3.2.5 Oxygen radical antioxidant capacity (ORAC)

The ORAC assay was performed according to the method of Huang et al. (2005) with some modifications. Sample extracts were dried using oxygen free nitrogen (BOC gases, Auckland, New Zealand) to evaporate the solvent. Phosphate buffered saline (PBS, 1mM) was then added to make the samples to a standard concentration of 5 mg/ml.

Fluorescein stock solution was prepared (8.16 mM) in PBS, stored at 4ºC in the dark and diluted to 8.16 x 10^{-5} mM when needed for use in the assay. The stock solution at this concentration is stable over several months in the dark (Huang, Ou, Hampsch-Woodill, Flanagan & Prior, 2002). The AAPH solution was produced by dissolving 0.0414 g/ml in PBS to give a concentration of 153 mM. This solution was kept for a maximum of 8 hours before discarding the solution.

Trolox dissolved in PBS was prepared as a 2 mM working solution and diluted to make concentrations of 50, 40, 30, 20, 10, 5, 2.5 and 0 µM trolox for the standard curve.

Twenty five microlitres of sample or trolox standard were added to a 96 well black bottom plate (Greiner Bio-one, Kremsmuenster, Austria) and 150 microlitres of fluorescein were added to these. Analysis was carried out using a plate reader equipped to read fluorescence (Biotek, Synergy 2) using the Gen5 software (Biotek, Winooski, Vermont, U.S.A.). The analysis was done with an excitation wavelength of 480 nm and emission wavelength of 528 nm.

The plate was put into the platereader and incubated at 37 °C for 30 minutes with a 3 minute shaking step at the start to ensure good mixing. At the end of the incubation period, a 25 µl

aliquot of AAPH was added to each well, followed by a thirty second shaking step to mix the reagents. Readings were then taken at time 0 and at five minute intervals after that for 35 minutes to give eight readings in total.

Analysis was done in a Microsoft Excel spreadsheet. The area under the curve (AUC) was calculated using the equation (Zulueta, Esteve & Frigola, 2009):

$$AUC = (0.5 + f5 / f0 + fn / f0 \ldots\ldots\ldots\ldots f35 / f0) \times CT$$

Where

$f0$ = initial fluorescence reading at time 0,

$fn / f0$ = fluorescence at time n

CT = cycle time in minutes (5 in this case)

The net area under the curve (AUC) was calculated by subtracting the AUC for 0 mM trolox from the AUCs of the Trolox standard curve and samples. Net AUCs of samples were converted to micromoles trolox equivalents by comparison of the trolox standard curve plotted against micromoles trolox. The TAC was expressed as micromoles trolox equivalents/ g sample.

3.3 Results and Discussion:

3.3.1 Total Phenolic content:

Dry beans

On sample dry weight basis, TPC was the same for azuki, soy and kidney beans ($p < 0.05$) (Figure 4). The lack of significant difference between raw kidney and soy beans is in agreement with the results of Boateng, Verghese, Walker and Ogutu (2008).

The total phenolic contents were generally lower than those observed by others (Chung, Hogan, Zhang, Rainey & Zhou, 2008; Chung et al., 2010; Lin and Lai, 2006; Prakash, Upadhyay,

Singh & Singh, 2007; Xu & Chang, 2007a; Xu & Chang, 2008a; Devi, Gondi, Sakthivelu, Giridhar, Rajasekaran & Ravishankar, 2009). Some phenolic compounds may have been removed with the diethyl ether used to extract the sample as the solvent is able to extract phenolic compounds (Baggio, Lima, Filho, J.M & Fett, 2007; Guo, Wei, Sun, Hou & Fan, 2011). The soybeans in this study were slightly higher in TPC than seven cultivars grown in Maryland and the kidney beans in this study were higher in TPC than those extracted with 100% ethanol (Whent, Slavin, Kenworthy & Yu, 2010; Xu & Chang, 2007b). The phenolic content of azuki beans was reported to be higher compared than other beans. For instance, Amarowicz, Troszynska, Barylko-Pikielna & Shahidi (2004) reported that TPC of azuki beans to be higher than red and green lentil (*Lens culinaris*), red bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum*. L), broad bean (*Vicia faba maior Harz*) and faba bean (*Vicia faba minor*). Lin and Lai. (2006) also found that azuki beans had higher phenolic contents than soybeans. Comparison of phenolic contents reported for different biological materials, including beans, is made difficult by several factors. Differences in phenolic content may be caused by experimental procedures (different extraction temperatures, solvents and sample pre-treatments) and by variations within the species (Ninfali & Bacchiocca, 2003; Oomah, Cardador-Martinez & Loarca-Pina, 2005). Also significant differences have been observed in soybean TPCs between growing locations (Riedl, Lee, Renita, St Martin, Schwartz & Vodovotz, 2007). Solvent type was also an important factor in determining the extraction efficacy and affected TPC up to two fold under the same experimental conditions for soybean (Lee Yang & Mau, 2008b; Lee et al., 2009) and up to five fold for kidney beans (Xu & Chang, 2007a).

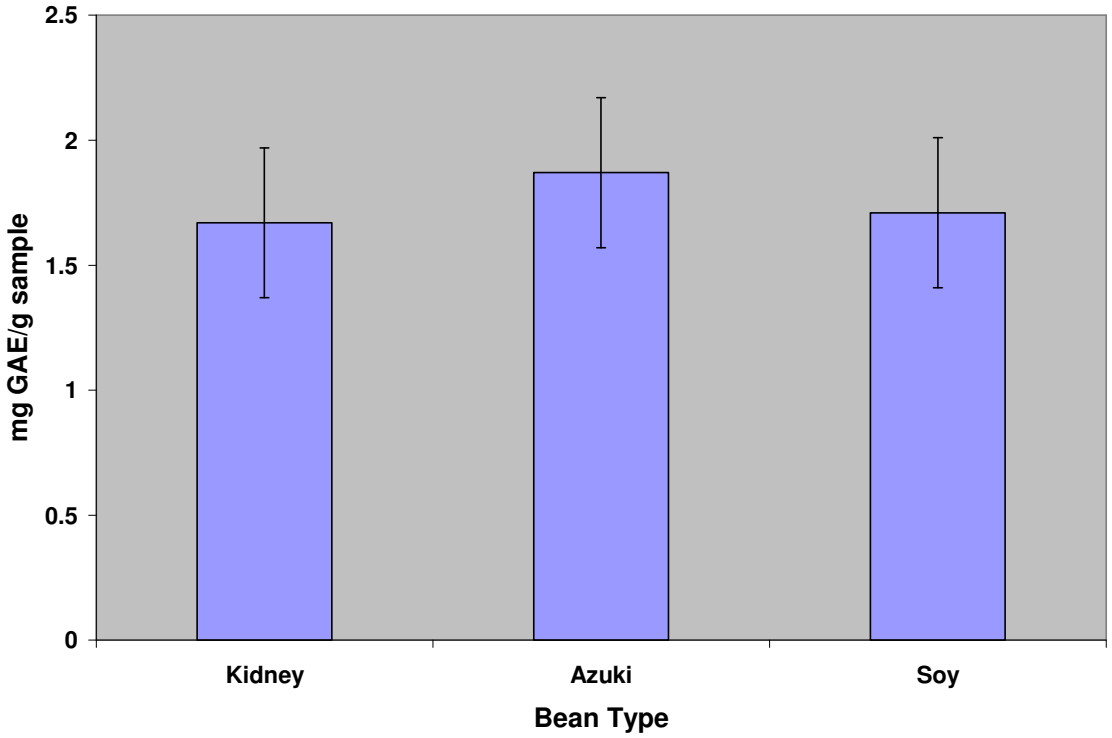

Figure 4: Total phenolic contents (expressed as mg Gallic Acid Equivalent/g sample) of dry azuki, kidney and soy beans.

Processed beans

Generally, processing (heating, soaking and de-hulling) of the azuki and soy beans decreased TPC ($p < 0.05$) due to the rehydration of the beans as well as the effect of heating and leaching into boiling water (Figure 5). Processed soy beans had a significantly higher ($p < 0.05$) TPC than the azuki beans. This is possibly due to higher rehydration level in azuki beans compared to soy beans as a result of the larger surface area per unit mass for azuki beans. A reduction in TPC of soybeans of 17% after thermal treatment was observed by Xu and Chang (2008a) and a similar reduction was also observed by Boateng et al. (2008). The soaking of soybeans weakens the hard cell wall tissues which often lead to solubilisation of bound polyphenols and causes the polyphenols to be lost into the cooking water by leaching (Boateng et al., 2008). Also the use of high temperature during cooking/processing of the beans may cause thermal degradation of polyphenols (Xu & Chang, 2008a). This suggestion is supported by the findings of Luthria

and Pastor-Corrales (2006) which could not detect more than 2% of phenolic acids in the cooking water of a total of 14% lost during boiling, suggesting that thermal degradation probably contributes more to the loss than leaching. For other samples increases in phenolic content have frequently been observed for thermal treatment (Xu & Chang, 2008a; Turkmen, Sari & Velioglu, 2005). Specifically, pressure boiling for 60 minutes was observed to increase the TPC of soybeans by 35% and steaming or microwaving of pepper, broccoli and green beans produced an increase in TPC (Xu and Chang, 2008a; Turkmen et al., 2005).

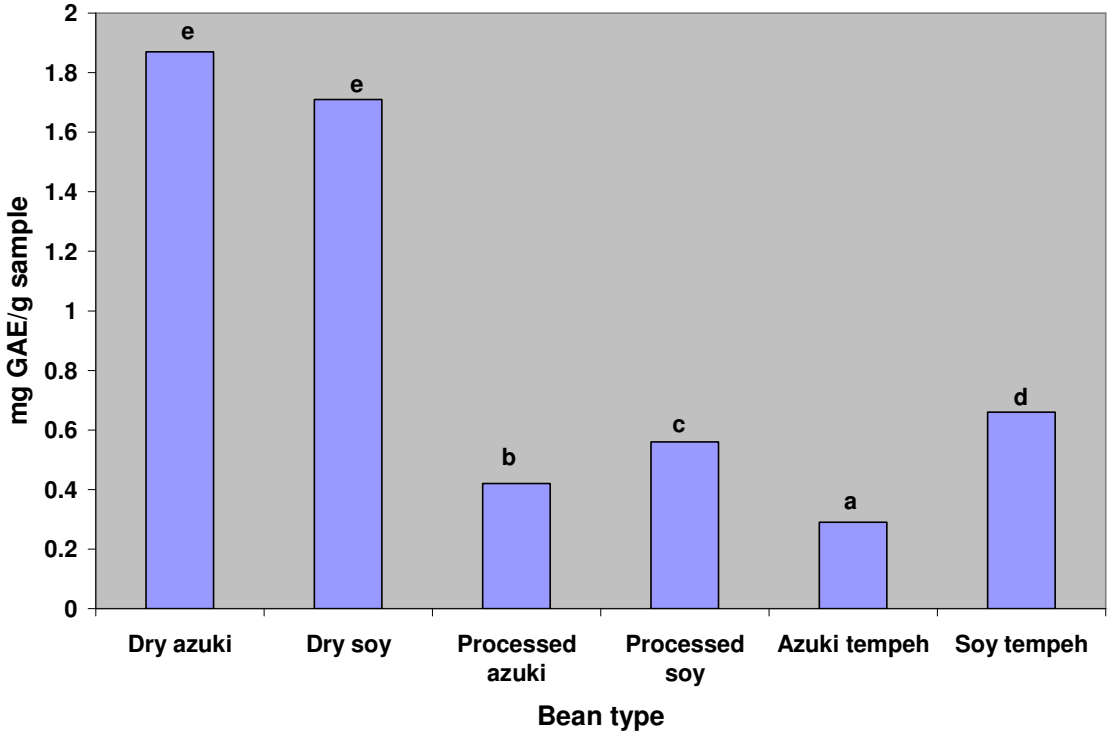

Figure 5: Total phenolic contents expressed as mg Gallic acid equivalent/g extract of dry, processed and tempeh forms of soy and red beans. a-e: different letters denote significant ($p < 0.05$) differences between treatments.

Processing and dehulling of the beans significantly ($p < 0.05$) decreased total phenolics (Figure 5). The dehulling of the beans can be expected to lower the phenolic content as it is widely recognised that the hull is more abundant in phenolic compounds than the cotyledon (Pastor-

Cavada, Juan, Pastor, Alaiz & Vioque, 2009). The hulls from the common bean (*Phaseolus vulgaris* L.) were observed to have a TPC 37 fold higher than the dehulled bean (weight basis) thus although the hull only made up 8% of the weight of the bean it had a significantly higher TPC than whole bean flour (Cardador-Martinez, Loarca-Pina & Oomah, 2002). Higher phenolic contents in bean hulls compared to the cotyledons have been demonstrated by many researchers (Cardador - Martinez et al., 2002; Oomah, Corbe & Balasubramanian, 2010; Xu & Chang 2008b). Oomah et al. (2005) found a 45% reduction after dehulling of common beans. Xu and Chang. (2008b) found that dehulling black soybeans produced a significant reduction in the total phenolic content. These authors found that the dehulled bean was lower in phenolic acids thus it is possible that higher phenolic acid concentrations in azuki bean cotyledons in relation to seed hulls compared to black soybeans account for the reduction observed by Xu and Chang (2008b) which did not occur in this experiment.

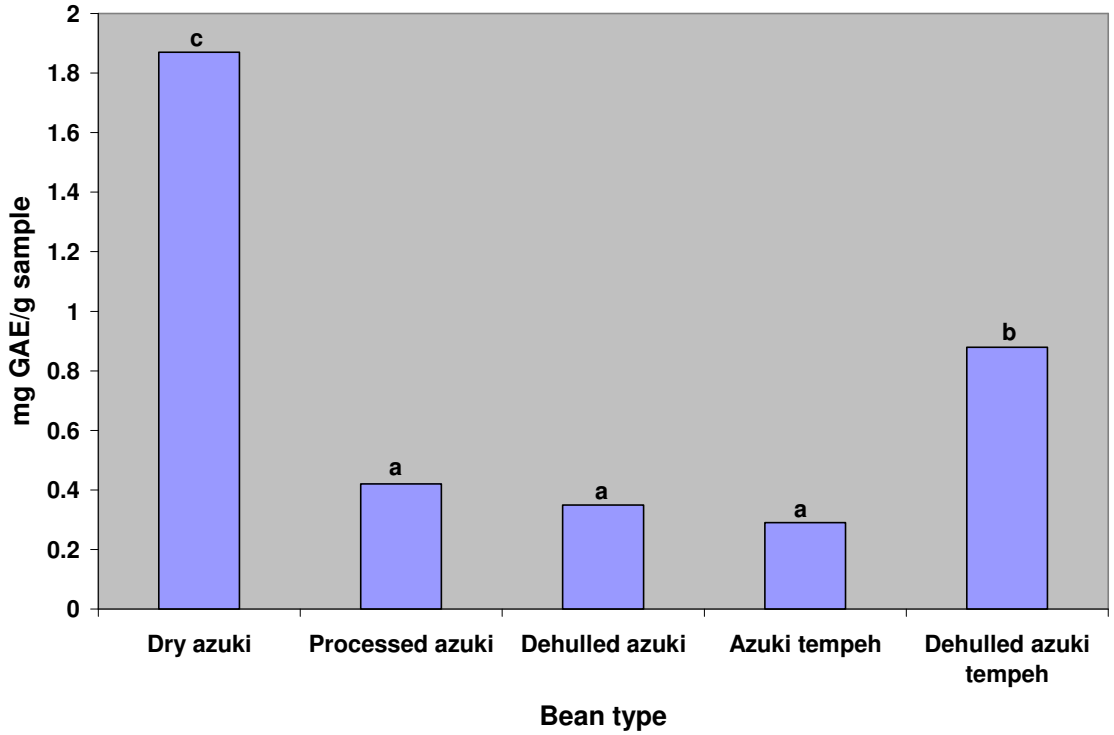

Figure 6: Total phenolic contents expressed as mg Gallic Acid equivalent/ g sample of red bean and dehulled red bean in processed form and as tempeh. Letters a-c denote significant ($p < 0.05$) differences between treatments.

Fermentation increases TPC of beans (Granito, Paolini & Perez, 2008; Oboh, Ademiluyi & Akindahunsi, 2009) and there is a plethora of evidence for this occurring in soybeans

specifically (Lee et al., 2008a & 2008b, Lee et al., 2009, McCue & Shetty, 2003a; Lin et al., 2006; McCue, Horii & Shetty, 2003b, Hu et al., 2010).

Many phenolic compounds in plants are attached to sugars, mainly as glycosides (Hu et al., 2010.). Because phenolic compounds contain glycosidic bonds they are affected by glucosidases and other carbohydrate cleaving enzymes (McCue & Shetty, 2003a; McCue et al., 2003b) resulting in the release of free phenolics in aglycone form (Hu et al, 2010). The enzyme β-glucuronidase which is used by *R.oligosporus* can change normally water insoluble polyphenolic compounds from soybean into more water soluble compounds for utilisation (McCue & Shetty, 2003a). A decrease in bound phenolic compounds accompanied by an increase in free phenolic compounds has been demonstrated for beans other than soy (Oboh et al., 2009).

3.3.2 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) radical scavenging activity

Dry beans

Due to the variety of units used for reporting DPPH radical scavenging activity (RSA) values, comparison of results from the present study and those in the literature is complex. This is compounded by environmental factors such as growing region as even common beans can vary up to 70 fold in DPPH RSA based on growing location (Heimler, Vignolini, Dini & Romani, 2005). The rate of DPPH radical scavenging activity is shown by the results at 10 minutes whilst the overall activity is shown by the results at 24 hours. Significantly lower DPPH RSA was observed for soybeans compared to kidney and red beans (Figure 7) which is comparable to the results found in the literature. Azuki beans have been reported to have significantly ($p < 0.05$) higher RSA than soy (Lin & Lai, 2006). However, Boateng et al. (2008) observed that kidney bean and soybean were not significantly different in DPPH RSA At 10 minutes dry azuki bean RSAs were not significantly different from the kidney beans but were higher than soy beans. All dry bean RSAs were significantly ($p < 0.05$) different from each other in the order azuki > kidney > soy.

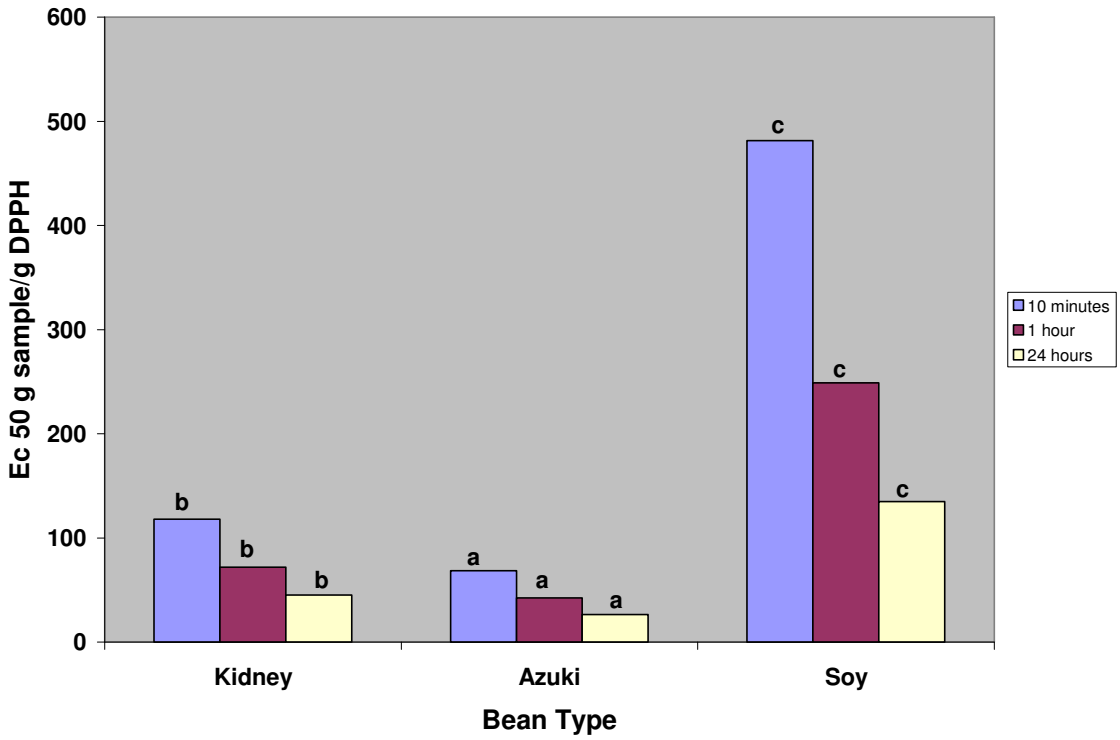

Figure 7: DPPH$^{\cdot}$ radical scavenging activities expressed as Ec50 g sample/g DPPH of three types of dry beans at 10 minutes, 1 hour and 24 hours. Letters a-c denote significant ($p<0.05$) differences between samples.

Processed beans

Processing removed the significant difference between soy and azuki bean RSAs found in the dry beans (Figure 8). Azuki beans contain tannins (Amarowicz et al., 2004) and phenolic compounds which are predominantly found in the following order of abundance; catechin glucoside, procyanidin dimers, myrecetin, quercetin glucoside and epicatechin glucoside (Amarowicz, Estrella, Hernandez & Troszynska, 2008). In addition, the seed coat contains an anthocyanin pigment (Yoshida, Sato, Okuno, Kameda, Isobe & Kondo, 1996) which also contributes to its radical scavenging activity. The antioxidant activity of soy tempeh has been attributed to isoflavones, soy proteins and peptides as well as 3HAA, as these compounds have radical scavenging activity (Esaki et al., 1996). The types of antioxidant compounds present in these beans and tempeh are important when they are considered in terms of extraction solvent used and their relative activities in different assays which will be discussed later in this chapter.

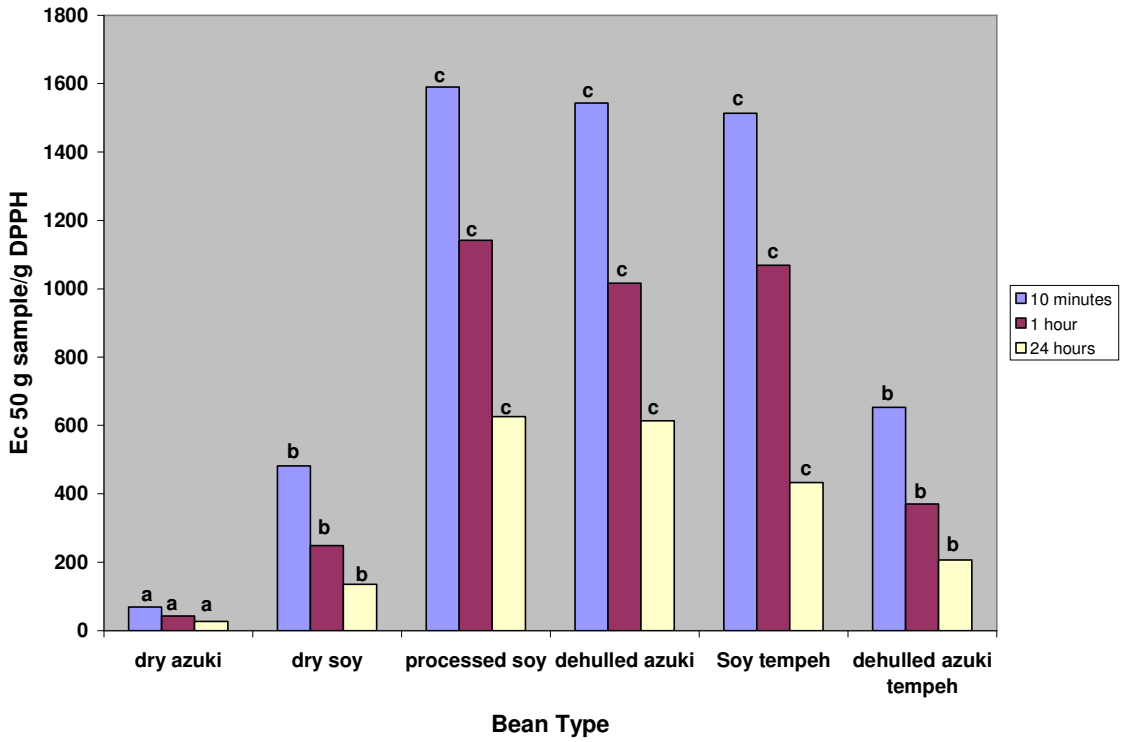

Figure 8: DPPH· radical scavenging activities of dry, processed and tempeh forms of soy and red beans at 10 minutes, 1 hour and 24 hours. Letters a-c within a given time period above treatment indicate significant ($p < 0.05$) differences between treatments

Thermal treatment of the azuki beans produced a significant ($p < 0.05$) reduction in RSA. Dehulling caused a further reduction in RSA for thermally treated beans (Figures 8 and 9). Thermal treatments can cause a decrease in anthocyanins and this may account for the loss of activity in azuki beans (Xu & Chang, 2008a). The reduction of RSA in dehulled azuki is probably due to the removal of the hulls containing anthocyanins which contribute to the DPPH RSA (Fukumoto & Mazza, 2000). Anthocyanin contents are highly correlated to DPPH RSA as Radovanovic and Radovanovic (2010) showed high correlations of different classes of anthocyanins to DPPH RSA ranging from $r = 0.8873$ to 0.9728.

Thermal treatment of soybeans significantly reduced the DPPH RSA threefold (Figure 8). Contrasting results have been reported in literature for the effect of thermal treatment on DPPH RSA of soybean. Reductions in RSA have been observed for soybean and common bean (Granito et al., 2008; Boateng et al., 2008). However increase in DPPH RSA of soybean has been observed with boiling as is normally done during tempeh making (Lee et al., 2008b, Xu &

Chang, 2008a). While boiling causes the solubilisation and leaching of phenolic compounds as mentioned earlier, it is also possible that antioxidative products are formed during heating (Acar et al., 2009). The obvious interpretation for the results in Figure 8 is that the beans in the current study suffered a loss of phenolic compounds during processing which led to lower DPPH RSA. It should be noted that the dry beans have much lower moisture contents and therefore a higher concentration per gram of compounds with DPPH RSA, thus the difference observed in the samples could be largely related to sample weight basis used. In fact, Boateng et al. (2008) observed a significant increase in DPPH RSA on a dry weight basis when soy beans were soaked.

The choice of solvent may contribute to the relatively low DPPH RSAs of soy (dry and tempeh) compared to azuki bean treatments. Hexane, petroleum ether (Chang et al., 2009) and water (McCue & Shetty, 2003a) have been shown to produce extracts with higher scavenging abilities in the DPPH assay. Therefore if these solvents were used for extraction there may not be a difference or there may be a smaller difference between the dry beans and tempeh of soy and azuki and processed soy may exhibit a higher DPPH RSA than azuki.

Fermentation did not produce a significant change in DPPH RSA for dehulled soybeans (Figure 8). Fermentation caused significant ($p < 0.05$) increases for dehulled azuki beans whilst it did not in dehulled soybeans and although dehulled azuki tempeh had a higher rate of DPPH RSA than azuki tempeh it did not have a different activity overall. Increases in DPPH RSA are normally observed after fermentation (Lee et al., 2008a and 2008b; 2009; Fan, Zhang, Chang, Saito & Li, 2009; McCue & Shetty, 2003a; Chang et al., 2009; Lin et al., 2006; Oboh et al., 2009; Hu et al., 2010), however the effect is highly dependent on the solvent used (Lee et al., 2008b; Lee et al., 2009; McCue & Shetty, 2003a; McCue & Shetty, 2003b; McCue & Shetty, 2005; Chang et al., 2009).

The dehulled azuki tempeh had an increase in DPPH RSA caused by fermentation whereas the azuki tempeh did not. Furthermore the extent of change in DPPH RSA after fermentation of dehulled azuki beans (+297%) is greater than that for whole azuki beans (-9%). As the processed azuki beans still contained the hulls the *Rhizopus* mycelium was not able to penetrate the hull and digest the cotyledon extensively. Thus, the overall effect of fermentation is greater than dehulling, which suggests that changes during fermentation contribute more to the RSA than antioxidative compounds in the hull. Proteolysis that occurs during fermentation can increase the antioxidant capacity. During fermentation with *R.oligosporus* there is an increase

in the total, non- protein (Nassar et al., 2008) and soluble (Astuti et al., 2000) nitrogen contents. An increase in free amino acids (Baumann & Bisping, 1995) and peptides is observed to occur simultaneously during fermentation (Watanabe et al., 2007). Proteolysis raises the antioxidant activity as the breakdown of protein exposes functional groups with radical scavenging properties. Increased radical scavenging of douchi (fermented soybean) has been attributed to high content of hydrophobic amino acids (Fan et al., 2009) and hydrophobic peptides (which can be formed during fermentation) have been implicated to contribute to antioxidant activity (Park, Lee, Baek & Lee, 2010). Additionally aromatic amino acids including histidine are able to donate protons to stabilise radicals by providing electrons in resonance structures (Fan et al., 2009). Unlike the work of Fan et al. (2009) soybeans did not appear to produce peptides with DPPH RSA (although it appears that this occurred in azuki bean) which maybe be due to the type and size of peptide fragments produced during *R.oligosporus* fermentation not showing high activity in this assay.

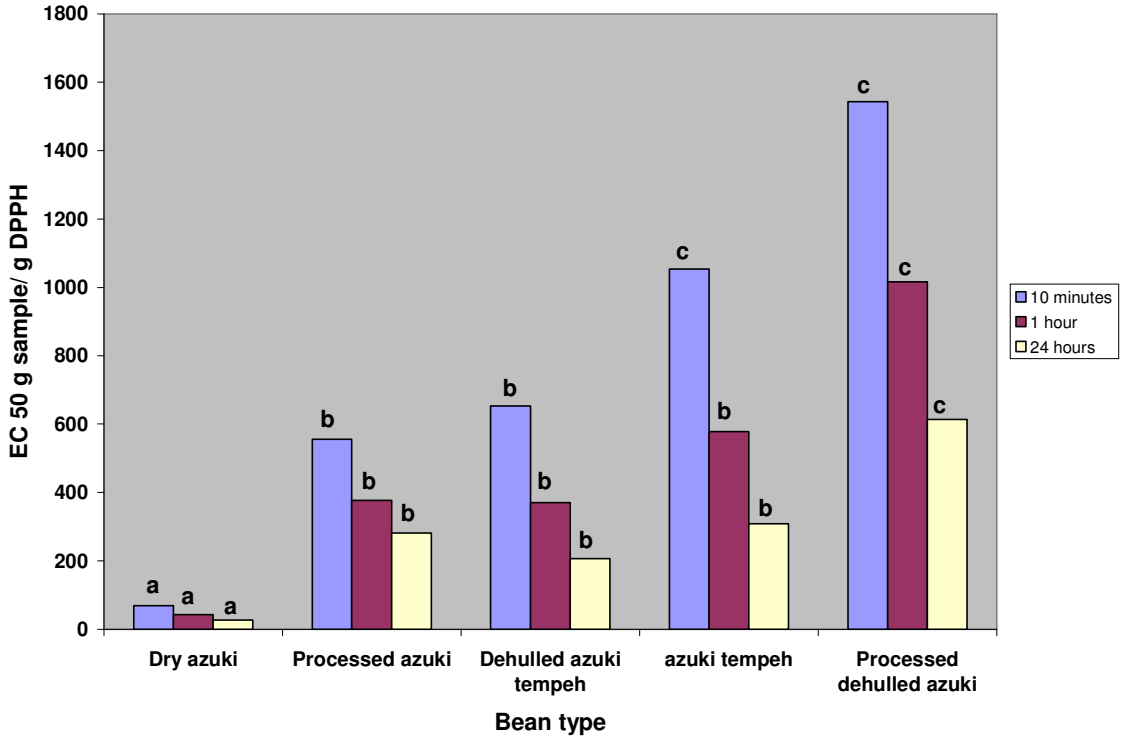

Figure 9: DPPH· radical scavenging activities of dry red beans compared to processed and tempeh forms of red and dehulled red beans at 10 minutes, 1 hour and 24 hours. Letters a-c within a given time period above treatment indicate significant ($p < 0.05$) differences between treatments

3.3.3 Oxygen radical anti-oxidant capacity (ORAC) assay

Dry beans

Only kidney beans had lower ($p < 0.05$) ORAC values than soybeans which were more than two fold higher in ORAC values (Figure 10). The ORAC of kidney beans in this study was lower than that reported by Wu et al. (2004), (although only a single sample was used in that study), but is comparable to those reported by Xu and Chang. (2007a) which used more replicates. These values were higher than those reported by Xu & Chang (2008a) but lower than those reported by Chung et al. (2010). These results indicate that environmental conditions and location can have great effect on the ORAC values of beans.

The ORAC results are different to the TPC and DPPH assays where azuki beans had higher phenolic contents and radical scavenging activities than soy. Azuki beans contain epicatechin and quercetin (Amarowicz et al., 2008) which have higher activities in the DPPH assays than isoflavones which could, along with potentially many other compounds explain the higher activities for azuki bean DPPH RSA than soy compared to ORAC (Mitchell, Gardner, McPhail, Morrice, Collins & Duthie 1998; Lee, Yang, Xu, Yeung, Huang & Chen, 2005). The variety of compounds present in the beans and their synergistic and/or antagonistic effects make it difficult to definitively explain this. No significant correlation between ORAC and DPPH (Capitani, Carvalho, Rivelli, Barros & Castro 2009; Chung et al., 2010) and between ORAC and TPC (Chung et al., 2010) was demonstrated and this was consistent with the correlations found in this work (Table 5). The ORAC assay is based on a hydrogen atom transfer whereas the DPPH assay is based on electron transfer (Huang et al., 2005). Although the DPPH assay has been classified as occurring by electron transfer it can also operate by hydrogen atom transfer depending on the solvent used (Foti, 2007). Isoflavones can contribute more to the ORAC value than the DPPH value as they are more effective at transferring hydrogen atoms but not as effective at single electron transfer (Xu & Chang, 2008b). Therefore the higher ORAC value for soy relative to the DPPH assay could be due to the isoflavone content of the beans. The isoflavone genistein was shown to have relatively high antioxidant activity by the

ORAC method but relatively low activity by the DPPH method (Capitani et al., 2009). A low DPPH RSA of isoflavones and their glycosides was observed by Lee et al. (2005) who found α – tocopherol and epicatechin to have higher RSAs.

The solvent used may also contribute to these results as 70% ethanol was found to show the highest ORAC values for soybeans (Chung et al., 2010). However extraction of the fat soluble fraction with diethyl ether may remove antioxidants such as α-tocopherol which show greater activity in the DPPH assay. For example α-tocopherol which is present in soybeans shows greater activity than the isoflavones genistein, daidzein (Mitchell et al., 1998) and their glucoside forms and glycitin and its malonylglucoside form (Lee et al., 2005) in the DPPH assay. The compound 5-(δ-tocopheroxy)-δ-tocopherol is considered to be an important antioxidant in tempeh and is ideally isolated with cyclohexane/ethyl ether (9:1) (Hoppe et al., 1997) thus this antioxidant is likely absent in the extracts tested.

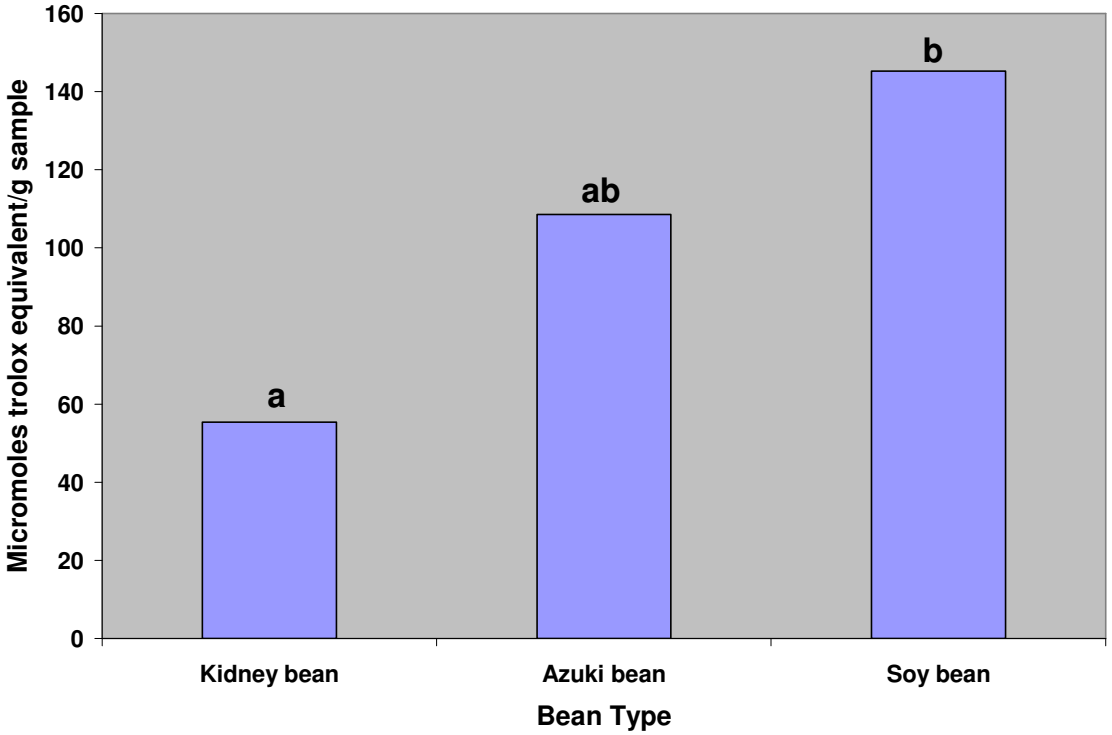

Figure 10: ORAC values (expressed as micromoles trolox equivalents/g sample) of dry red, kidney and soy beans. Different letters a-b denote significant differences at $p < 0.05$

Processed beans

Thermal processing of the dry azuki beans significantly ($p < 0.05$) reduced ORAC values (Figure 11). The reduction in ORAC values as a result of processing has been observed in pinto and black beans by Xu and Chang. (2009). This effect may be partially due to the loss of anthocyanins in the seed coat during thermal processing (Xu & Chang, 2009) and the dilution effect which occurs with rehydration of the bean. The dehulled beans however did not show a significant loss of ORAC which indicates that the effects of thermal processing are complex in terms of softening of tissue and release of phenolics, potential formation of antioxidant compounds due to heating and the degradation of antioxidants. Loss of flavonol compounds such as myrecetin present in azuki beans (Amarowicz et al., 2008) is associated with pressure boiling (Xu & Chang, 2009) which may account for the reduction in ORAC. The dehulling of the processed beans did not produce a significant ($p < 0.05$) effect on ORAC (Figure 11). However, dehulling of common bean and black soybeans has been demonstrated to produce a significant reduction in ORAC values (Oomah et al., 2010, Xu & Chang, 2008b). Black soybean ORAC values on a weight basis were reported to be in the following order; hull > whole bean > dehulled bean (Xu & Chang, 2008b) and common bean hulls had 6–8 fold higher ORAC than dehulled beans (Oohmah et al., 2010). The large standard deviations between different batches in our study probably account for the lack of difference between hulled and dehulled beans (Figure 11).

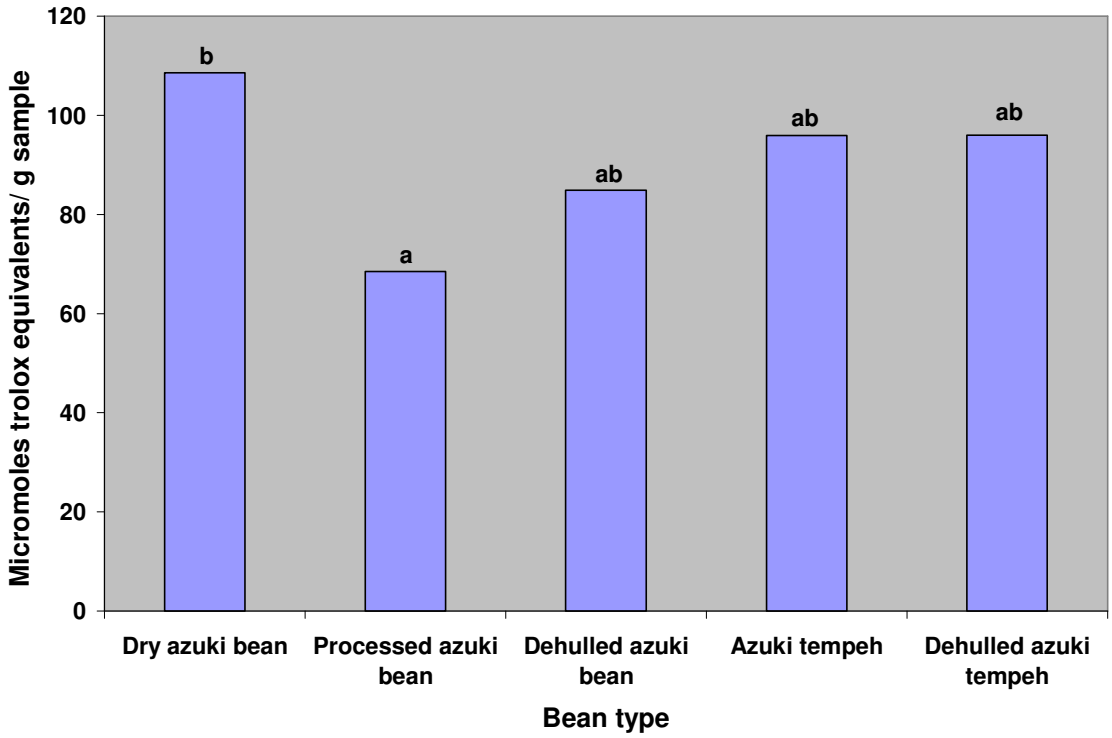

Figure 11: ORAC values (expressed as micromoles trolox equivalents/g sample) of red bean and dehulled red bean in processed form and as tempeh. Letters denote significant ($p < 0.05$) differences between treatments

Soybeans were not significantly ($p < 0.05$) affected by thermal processing and dehulling (Figure 12). A non significant effect on soy ORAC values with pressure cooking has been observed previously (Xu & Chang, 2008a). This may be due to the loss of isoflavones as a result of thermal treatment (Xu & Chang, 2008a). The processed soy beans had significantly higher ORAC values than the dehulled azuki beans also. The dry beans contain less moisture thus the change on a dry weight basis may be an increase. Fermentation of the beans did not affect ORAC values for azuki

beans but caused a significant ($p < 0.05$) decrease for soy beans. The ORAC values for azuki tempeh were not different from either dry or processed azuki beans (Figure 12).

The significant reductions in the ORAC values of soybeans may be caused by loss of components which contribute to ORAC activity in the tempeh making process. Isoflavone composition is possibly changed during the fermentation since they are easily affected by processing conditions (Wang & Murphy, 1994; Watanabe et al., 2007; Murphy, Barua & Hauck, 2002; Xu & Chang, 2008a). The amount of isoflavones in roasted soybeans has been

demonstrated to be more than twice as high as that of soy tempeh (Wang & Murphy, 1994). Furthermore changes in the form of the isoflavone also occur. After the fermentation of soy the aglycon forms of isoflavones predominate which is accompanied by the reduction of the acetyl, malonyl and β-glucoside forms (Wang & Murphy, 1994; Hubert et al., 2008). Different forms of isoflavones show variation in the ORAC assay (Rufer & Kulling, 2006), however this effect is not as pronounced in the DPPH assay (Lee et al., 2005). There is also a loss of compounds such as saponins and phytic acid which have antioxidant activity (Hubert et al., 2008).

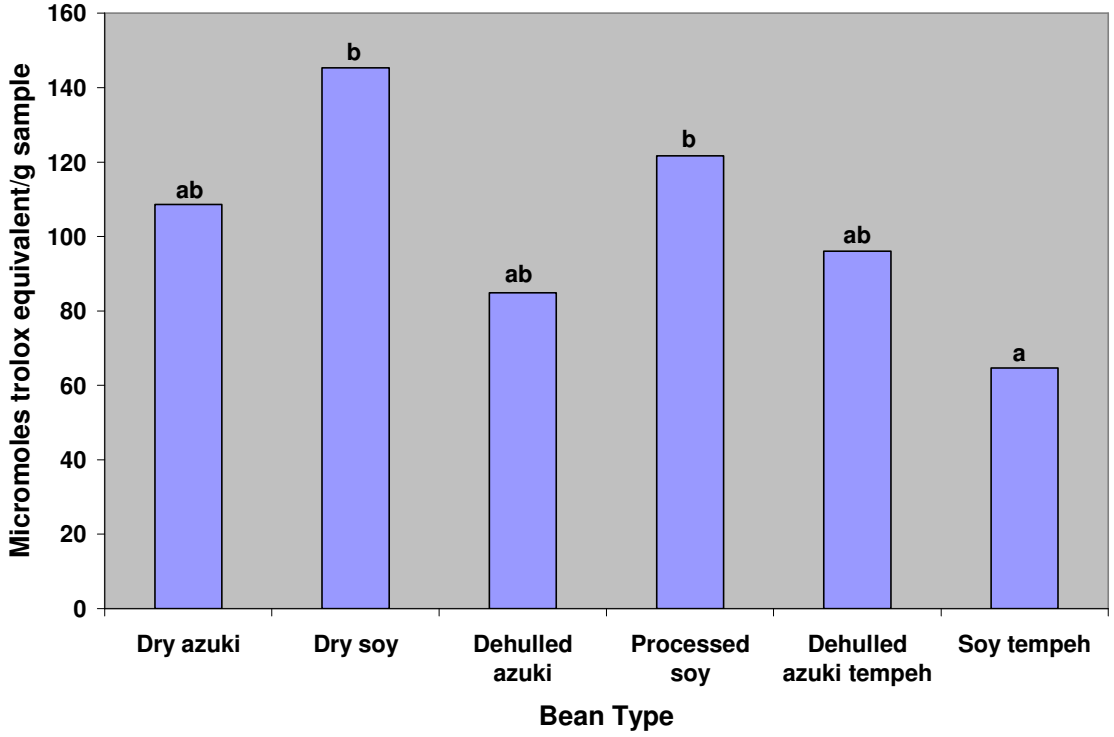

Figure 12: ORAC values (expressed as micromoles trolox equivalents/g sample) of dry, processed and tempeh forms of soy and red beans. Letters a-c denote significant ($p < 0.05$) differences between treatments.

3.3.4 Correlation of antioxidant activities and total phenolic content

There was a strong significant ($p < 0.05$) correlation between the DPPH rate and overall activity ($r = 0.94$) (Table 5). Negative correlations between DPPH and other assays are due to low values of DPPH corresponding to higher antioxidant activity. The DPPH assay was shown to have only a moderate correlation (r ranged between -0.57 and -0.56) between the total phenolic content and DPPH RSA at 10 min, 1 hour and 24 hours (Table 5). These are similar to the ($r = 0.49$) correlation between the two assays found by Heimler et al. (2005). Also, Riedl et al. (2007) found a weak ($r = 0.26$) correlation between DPPH RSA and TPC for soybeans. The relationship between DPPH RSA and TPC appears to be dependent on the extraction solvent used. However Kumar, Rani, Dixit, Pratap and Bhatnagar (2010) did not find a significant correlation between DPPH RSA and TPC in their investigation of six genotypes of yellow soybean. For instance, Devi et al. (2009) and Lee et al. (2008b) found very different correlations between water extracts of a selection of soy products and *Monascus* fermented soybeans respectively ($r = 0.81$ and $r = -0.731$ respectively). Postive and negative correlations were reported because of the different methods used to report DPPH activity between the two studies. Similarly, 70% ethanol extracts of thermally treated soybeans produced a correlation of $r = 0.76$ between the DPPH and TPC assays (Xu & Chang, 2008a). Stronger correlations have been observed for ethanol and methanol extracts of *Monascus* fermented soybeans ($r = 0.94$, $p < 0.05$), black soybeans fermented with *Bacillus natto* ($r = 0.93$, $p < 0.05$) and chungkookjang (Korean fermented soybean paste, $r = -0.90$, $p < 0.05$) (Lee et al., 2009; Hu et al, 2010; Kwak, Lee & Park, 2007). The TPC and DPPH assays both utilise an electron transfer mechanisms as they both abstract electrons from antioxidants, becoming reduced and changing colour in the process (Huang et al., 2005). For this reason some correlation is to be expected between the two assays despite a range of compounds being present in the samples as many antioxidant compounds will react similarly in the two assays. As the same extraction solvent was used for all samples the same antioxidants were likely extracted from all samples.

The ORAC assay had no correlation with rate of DPPH RSA and TPC (r ranged from 0.07 to 0.15) (Table 5). This may be due to the different reaction mechanisms utilised by the assays. Xu and Chang (2008c) reported similar lack of correlation between DPPH RSA and ORAC ($r = 0.17$) for twenty eight cultivars of soybeans. Zhang, Li and Zhou. (2010) also did not find a

correlation between the two assays when testing hydrolysates of soy protein prepared with microbial proteases and Capitani et al. (2009) tested a range of antioxidative compounds and did not find a correlation between the two assays. The tempeh in this experiment would contain hydrolysates of soy protein and there would have been a variety of antioxidative compounds in all samples tested similar to the work of Capitani et al. (2009) thus the lack of correlation between the two assays is expected. Also Xu and Chang (2007b) found correlations within the classes of soybeans and common beans tested but not within classes of green peas, yellow peas and lentils suggesting correlations between the two assays are dependant on the type of legume tested. As this experiment compares three different types of legumes a lack of correlation is not surprising.

There was no significant ($p < 0.05$) correlation between TPC and ORAC (Table 5). This is in contrast to results reported in the literature. Xu and Chang (2007b; 2008a. and 2009) found significant correlations between TPC and ORAC of ($r = 0.89$, $r = -0.79$ and $r = 0.89$) for soybeans, UHT processed soymilk and six bean types (yellow peas, green peas, lentils, common beans, soybeans and chickpeas) respectively.

As mentioned above (section 3.3.2 and 3.3.3) the samples tested contain a variety of compounds which contribute to their antioxidant activity and phenolic contents. These compounds exhibit different activities in each of the assays (see section 3.3.3) leading to a low correlation between them.

Table 5: Correlations between the total phenolic, DPPH and ORAC assays shown as Pearson correlation coefficients with P values. Negative values indicate inverse correlations between assays.* indicates a significant correlation at a level of p = 0.001**

Testing parameter	mgGAE/g	Ec50 10 min g sample/g DPPH	Ec50 1 hour g sample/g DPPH	Ec50 24 hours g sample/g DPPH
Ec50 10 min g sample/g DPPH	-0.574			
P value	0.000***			
Ec50 1 hour g sample/g DPPH	-0.563	0.972		
P value	0.000***	0.000***		
Ec50 24 hours g sample/g DPPH	-0.558	0.936	0.960	
P value	0.000***	0.000***	0.000***	
Micromoles trolox equivalent/g sample	0.073	0.133	0.130	0.148
P value	0.534	0.245	0.254	0.192

ORAC = Micromoles trolox equivalent/g sample

EC_{50} = g sample required to inhibit 50% of 1 g $DPPH^{\cdot}$

3.4: Conclusions

The samples tested responded differently in the three assays utilised due to the variety of compounds they contain and the reaction mechanisms involved in the assays.

Heating decreased TPC, DPPH RSA and ORAC for all samples with the exception of dehulled azuki beans and soybeans which were unchanged.

Fermentation increased the TPC of dehulled azuki and soy but did not affect whole azuki beans. It reduced the DPPH RSA of dehulled azuki beans and the rate of DPPH RSA of whole azuki beans but did not affect overall DPPH RSA of the beans or activity of rate of these attributes for soybeans. ORAC was not changed after fermentation for azuki beans (dehulled and whole) but was reduced for soybeans.

Soy tempeh had a higher phenolic content than azuki or dehulled azuki tempeh. Although soy tempeh had lower DPPH RSA than these treatments it was not significantly different in ORAC value. The ORAC value is the more important determinant as it measures scavenging of the peroxyl radical which occurs in biological systems. It is important to note that for the assays the lipid fraction was removed. Also the dehulling of the azuki beans was very difficult and time consuming compared to the dehulling of soybeans. Soybeans are also the traditional tempeh ingredient and several papers have been published on soybean tempeh. The azuki and dehulled azuki beans produced visually unappealing tempeh which would be unlikely to be accepted by consumers.

Therefore soy bean tempeh was chosen to be tested further by a variety and chemical, physical and consumer tests to determine an appropriate level of tempeh inclusion for a novel burger patty product and the changes which occur with substitution of beef for tempeh.

Chapter 4: Physico-chemical and sensory properties of tempeh extended beef patties

4.1 Introduction:

Much research has been conducted to investigate addition of non-meat additives or extenders to improve nutritional properties (Garcia, Calvo & Selgas, 2009; Danowska-Oziewicz, 2010; Mansour & Khalil, 1999; Bilek & Turhan, 2009; Dzudie, Kouebou, Essia-Ngang & Mbofung, 2004; Turhan, Temiz & Sagir, 2006; Turhan et al., 2009; Hashim & Khalil, 2007), shelf life (Banon, Diaz, Rodriguez, Delores Garrido & Price, 2007; Ismail, Lee, Ko, Paik & Ahn, 2009), sensory properties (Garcia et al., 2009; Das, Anjaneyulu & Kondaiah, 2006), physical parameters (Garcia et al., 2009; Das et al., 2006) and make use of by products of other food industries (Garcia et al., 2009) to improve burger patties.

Consumers have become increasingly concerned about fat consumption and have often associated red meat with high fat content. There are several classes of fat and each contributes differently to the risk of cardiovascular disease (Kris-Etherton & Yu, 1997). Cardiovascular disease is associated with atherosclerotic plaques which build up on the inside of coronary arteries which provide blood to the heart muscle (myocardium) (Mann & Chisolm, 2007). These plaques are composed mainly of cholesterol and low density lipoprotein (LDL) particles which are the main cholesterol carriers. Low density lipoproteins are oxidised and consumed by macrophages (Mann & Chisolm, 2007) which can consequently lead to increased oxidative stress and diseases.

The polyunsaturated: saturated (P: S) ratio and the Omega 6: Omega 3 ratio (n-6/n-3) are used as indices of the nutritional properties of food in terms of its fat content (Simopoulos, 2008; Fernandez-Gines et al., 2005). Omega 6 is represented by linoleic acid (18:2 n-6) and omega 3 by linolenic acid (18:3 n-3) (Simopoulos, 2008). In addition to contribution to health, fatty acid content is important to meat as there is an increased tendency for unsaturated fatty acids to oxidise (Fernandez-Gines et al., 2005) which causes several changes in flavour, nutrition and colour.

Lipid oxidation causes detrimental effects to the sensory properties of foods by discolouration and by formation of "off flavours" (Gray et al., 1996). In addition lipid oxidation negatively affects the nutritional composition by degradation of vitamins and production of toxic compounds (Gorelik et al., 2005).

Lipid oxidation is linked to the discolouration of meat as the oxidation of oxymyoglobin to metmyoglobin produces intermediate species of lipid oxidation which can promote further pigment or lipid oxidation (Section 2.3.4) (Faustman, Sun, Mancini & Suman, 2010). Shelf life as affected by oxidative processes can be measured by the 2-thiobarbituric acid reactive substances assay (TBARS) which measures the amount of malondialdehyde (MDA) formed. MDA is a useful compound to measure as it is a toxic product of lipid oxidation. During aerobic storage lipid oxidation occurs in meat products, especially comminuted products.

Colour is the most important attribute of meat for consumers for purchase intention and satisfaction (Muchenje, Dzama, Chimonyo, Strydom, Hugo & Raats, 2009). In addition to colour many other sensory attributes are important for beef patties. Texture is a sensory parameter which is important for the acceptance of foods including meat products. Producing foods with the desired textural quality is economically important (Bourne, 2002). Consumers prefer tender beef and tenderness of beef influences their purchase intention (Miller, Carr, Ramsey, Crockett & Hoover, 2001).

The experiments in this chapter examine a variety of physical, chemical and sensory properties of three levels of tempeh addition (10%, 20% and 30%) to beef patties to determine which level is optimal for a potential product and for futher testing of *in vivo* oxidation.

4.2 Materials and Methods:

Addition of a fermented vegetal product to beef patties will affect their nutrient composition, colour, shelf life due to a different profile of oxidative processes, sensory properties and consumer perception of the product. It is necessary to measure the extent of these changes.

All experiments were approved by the University of Otago Human Ethics committee.

Sample preparation

Samples of fresh beef semitendinosus (ST; eye of round, 22 kg) of normal pH (range 5.55-5.64) was obtained from a local supplier (Alliance Wholesale meats, Dunedin) (Appendix 2). The meat was separated into lean and fat and diced. Diced meat and 10% fat were added to a Kenwood blender with mincing attachment (Alp 5 blade and mincing plate, 4.5 mm diameter die) (Appendix 3). Burgers were prepared (about 4-5 kg each treatment) according to Table 6. Five experimental groups (Table 6) representing; non treated control sample (control); samples with 10% of the weight was replaced with breadcrumb (Crumb 10%); samples with the part of the weight was replaced with tempeh at level of 10% (Temp 10%), 20% (Temp 20%) or 30% (Temp 30%). Patties were made by adding 120 g of the mixture into a burger patty former and pressing into shape (Appendix 4). Fresh samples were used for the colour stability trials and for other analyses, the patties were vacuum packed and stored at -80°C.

Table 6: Composition of five burger patty treatments

Treatment Number	Treatment	Lean meat (%)	Fat (%)	Tempeh (%)	Bread crumb (%)	Salt (%)
1	Control	89	10	-	-	1
2	Control +10% breadcrumb	79	10	-	10	1
3	Control + 10% tempeh	79	10	10	-	1
4	Control + 20% tempeh	69	10	20	-	1
5	Control + 30% tempeh	59	10	30	-	1

Chemical and Biochemical analyses

Proximate analysis

Proximate analysis was carried out in duplicate for 3 samples (separate patties) per treatment. Moisture content was determined using gravimetric measurement of water content by freeze drying (AOAC, 1995). Crude lipid content was determined according to AOAC (1995) using a Tecator Soxtec System HT 1043 Extraction Unit™ (Tecator, Högänas, Sweden). Crude protein content was determined according to AOAC (1995) using a Tecator Kjeltec Auto Sampler System 1035 Analyser™ (FOSS Tecator AB, Högänas, Sweden). Ash content was determined on the freeze-dried samples mentioned above. The crucibles were placed in a muffle furnace which was set to run at an initial temperature of 200°C for one hour before being turned up to 550°C for four hours. It was found that without the hour at the lower temperature the samples had a tendency to explode out of the crucible. After the crucibles had cooled sufficiently (in a desiccator) they were weighed and returned to the oven. This process was repeated until a constant weight was achieved.

Fatty acids analysis

Sample preparation

The triglycerides were extracted as fatty acid methyl esters (FAME) as described by Bligh and Dyer (1959). Samples were freeze dried (Vertis Freezemobile 12SL, New York, U.S.A) over a 24 h period, then ground in a Breville coffee grinder. One hundred milligrams of each patty (3 patties/ treatment) was then weighed out in triplicate into test tubes. Then 3.75 ml of a chloroform/methanol solution (2:1 v/v) was added and samples were vortexed for 1 minute. A further 1.25 ml of 8% (w/v) NaCl solution and 1.25 ml of chloroform were added with 1 minute of vortexing in between. Centrifugation was then carried out at 2500 rpm for 5 minutes at 10°C. The resulting bottom layer containing triglycerides dissolved in chloroform was then removed and transferred to a new tube. The chloroform was evaporated by flushing with nitrogen gas (oxygen free, BOC gases, Auckland, New Zealand) until only the concentrated triglycerides remained. Aliquots of 3 ml of 6% sulphuric acid (v/v) in methanol were added and

tubes were vortexed for 1 minute. Then tubes were held in an 80°C oven for 12 hours to methylate the fatty acids (Welch, 1977). After methylation, 2 ml of hexane and 1 ml of distilled de-ionised water were added with 1 minute of vortexing in between. The upper layer of hexane containing the fatty acids was transferred to small vials and held at -20°C until analysis.

Sample Analysis:

The FAME analysis was carried out by Gas Chromatography with a Flame Ionisation Detector (GC-FID). The gas chromatograph was an Agilent 6890N (Agilent Technologies, Palo Alto, CA, U.S.A) equipped with a flame ionisation detector. The column was a DB-225 MS (30m x 0.25 mm, J + W Scientific, Agilent Technologies, Palo Alto, CA, U.S.A) with a 250 μm film of polyethylene glycol stationary phase. The carrier gas was hydrogen at a flow rate of 1.5 ml/minute. The injector temperature was 250°C and the oven temperature was raised from 180-210°C over 30 minutes with a detector temperature of 260°C. Lipid standards GLC 463 (Nu Check Prep Lipid Standards, Elysian, Mn, U.S.A) were also run to compare peaks to samples.

Colour:

Objective colour measurements were obtained using a Minolta colorimeter (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA) as described by (Bekhit, Farouk, Cassidy & Gilbert (2007). Duplicate readings were taken on samples (3 patties/treatment) placed in polystyrene trays and covered with oxygen permeable polyvinylchloride film (O_2 permeability >2000 mL m^{-2} atm^{-1} $24h^{-1}$ at 25°C, AEP FilmPac (Ltd), Auckland, New Zealand). The patties were exposed to fluorescent cool light (1,076 lux) and colour measurements were carried out at 0, 15, 24, 39, 48, 63, 72, 87 and 96 hours of retail display at 4°C. The colorimeter was calibrated using a black standard plate and a white standard C2-36852. Measurements were CIE L*, a* and b* values using illuminant C and a 10° observer with an aperture size of 3.5 cm. The chroma ($C = [a^{*2}+b^{*2}]^{1/2}$), and hue angle ($HA = \tan^{-1} b^*/a^*$) were calculated.

Two indices of browning, the 630/580 nm and 630-580 nm parameters were determined. The reflectance at 630 and 580 nm were recorded with the Minolta colorimeter during objective colour measurements and determined by subtracting the relfectance at 580 nm from the reflectance at 630 nm or dividing the reflectance at 630 nm by the reflectance at 580 nm.

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric Acid reactive substances (TBARS) were determined according to the method of Nam and Ahn (2003). The processed patties were sampled over a simulated display period and subsamples taken at days 0, 2, 4, 6 and 9, placed in foil vacuum packed bags (Swiss Pack NZ (19.6 x 30 cm) and stored at -80°C until analysis. Five grams of sample were put into fifty ml Falcon tubes and fifteen ml of distilled de-ionised water (DDH_20) was added. Samples were homogenised using a Polytron PT-MR 2100 (Kinematica, AG, Switzerland) at 15000 rpm for 20 seconds or until fine particles were produced. Lower weights of sample were used when the assay absorbances were too high. The meat homogenate (1 ml) was transferred to a 10 ml Falcon tube and a 50 µL aliquot of butylated hydroxytoluene (BHT) (7.2% w/v in methanol) and 2 ml thiobarbituric acid/ trichloroacetic acid (TBA/TCA, 20 mM TBA, 15% (w/v) TCA) were added and vortexed for 30 seconds. The samples were then incubated in a 90°C water bath (Grant, Cambridge, U.K.) for 15 minutes for colour development, after which they were cooled in cold water for 10 minutes. Samples were then centrifuged at 3000 rpm (Beckman, GPR, Palo Alto, CA, USA) for 15 minutes at 5°C. The resulting supernatant was read at 531 nm in an Ultraspec 3300pro spectrophotometer (Amersham Biosciences, Cambrige, England) with 3ml DDH20 as the blank. Amount of MDA (mg/ kg) meat was then determined by the equations;

$$Mg\ MDA/kg\ meat = A531 / E \times 72.063/1000 \times dilution\ factor$$

Where E is the molar extinction coefficient of MDA= 156000 $M^{-1}cm^{-1}$
72.063 is the molar mass of MDA (gmol $^{-1}$*)*
gMDA/sample volume is g of MDA in the volume of the meat slurry tested

Objective Texture measurements

Texture can be assessed both instrumentally (objectively) and by sensory assessors (subjectively). Uniaxial compression is used for measuring deformation and fracture attributes of foods and is a very commonly used and simple technique (van Vliet, 1999). The sample is compressed between two parallel plates at a constant compression rate and is uncontained in

the other two directions whilst the force of compression is measured (Bourne, 2002; van Vliet, 1999). Shearing devices which simulate the bite of the mouth are often used to measure hardness, especially in fibrous foods. Meat texture has often been measured using the MIRINZ tenderometer (Figure 13) or more sophisticated instruments such as the Instron Texture Analyser (Greaser & Pearson, 1999).

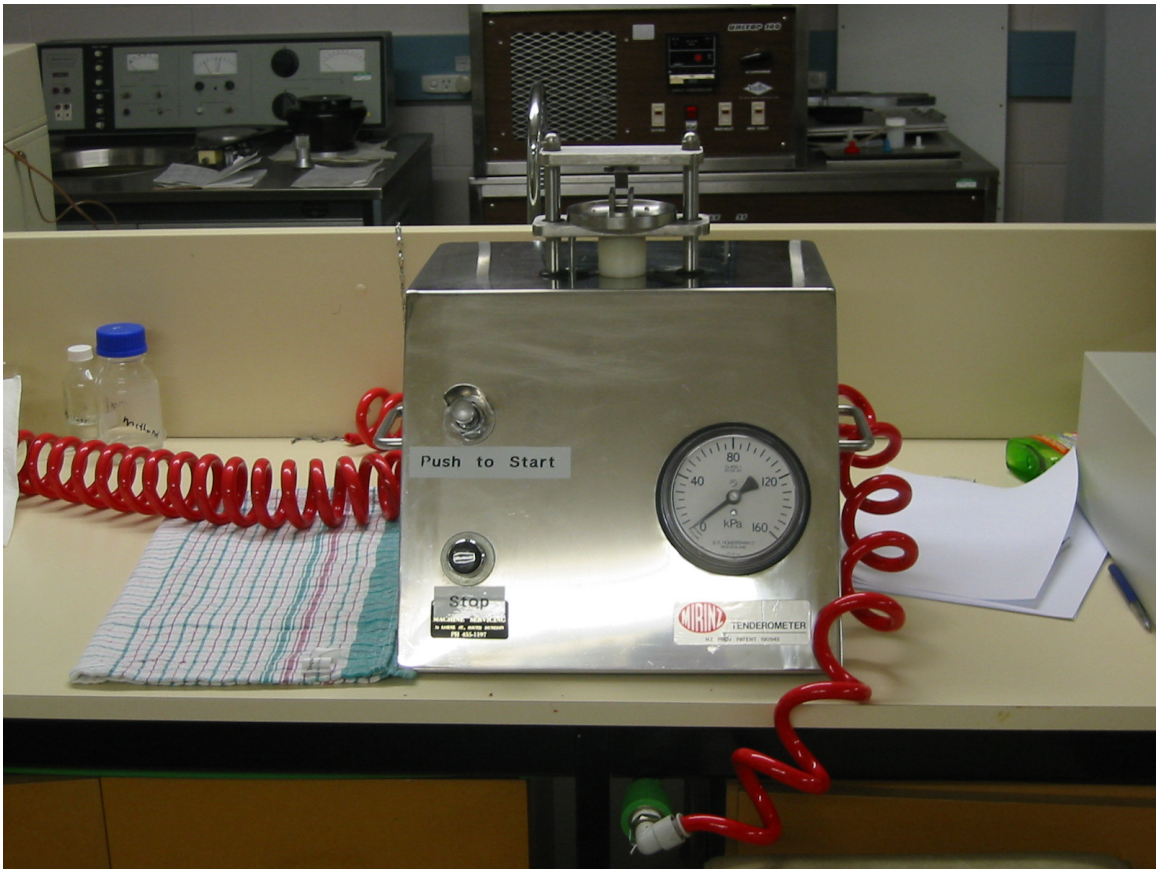

Figure 13: MIRINZ tenderometer used for shear force measurements

The texture of the patties was analysed by both shear force and compression measurements. Cooked patty samples were cut into 1cm x 1 cm x 1 cm (width x height x length) strips using a double bladed knife (see Appendix 5).

Compression measurements were performed as described by Perry, Shorthose, Ferguson & Thompson (2001) using a TA Plus texture analyser (Figure 14) (Stable MicroSystems, Surrey, UK). The sample was placed on the platform so that the probe would completely cover it. A

flat cylinder probe (TA-520 A) with a 20 mm diameter was pressed 8 mm into the sample twice at the same position at a speed of 50 mm/min (see Appendix 6).

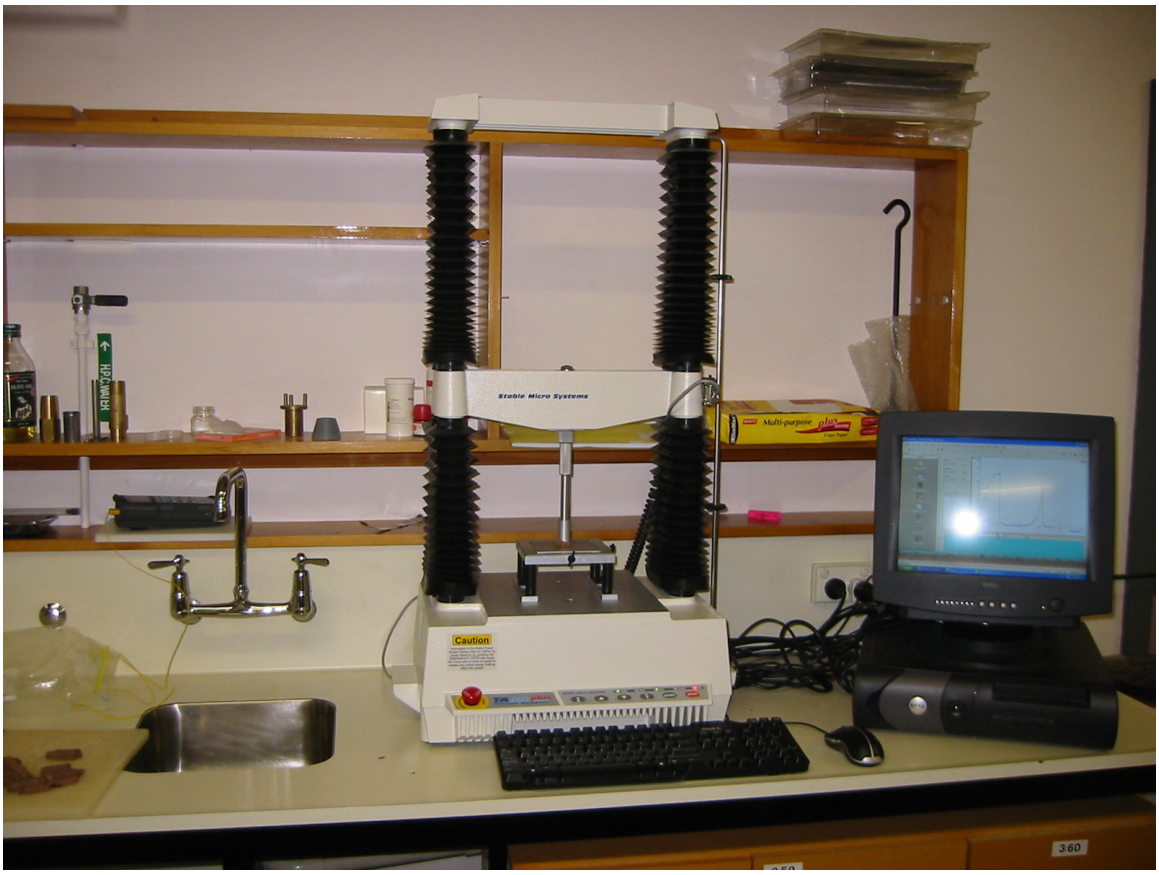

Figure 14: TA Plus texture analyser used for compression measurements

Shear force measurements hardness/tenderness was determined using a MIRINZ tenderometer (AgReasearch, Ruakura). Samples (8 bites of 1cm x 1 cm x 1 cm dimensions) were placed in the machine and the results were reported in N.

Focus group

In addition to chemical and physical characteristics consumer perceptions including attitudes and sensory perception are important characteristics of a burger patty.

Focus groups were used for exploratory research to determine level of tempeh, and serving conditions (preference to at home use vs take out). This information was used later in designing the consumer sensory analysis and to investigate consumer attitudes towards a novel product such as tempeh burgers.

Participant Recruitment

Flyers (see Appendix 7) were used to recruit participants for two focus groups. They were placed around two campuses (University of Otago, Otago Polytechnic), at supermarkets, a public library, and fish and chip shops. Restaurant franchises selling burgers were avoided as these places did not have message boards and because the product was not specifically aimed to be marketed to takeaway consumers. Flyers were placed over a period of two weeks and fifteen participants were chosen in total to take part in the two focus groups after brief screening over the phone (explained further below). The first focus groups attempted to cover the research aspects from diverse age and professional groups whereas the second focus group sought the opinions of young university students as it was clear they represent large fraction of consumers. The characteristics of the focus group participants are shown in (Tables 7 and 8).

Table 7: Summary of characteristics of focus group participants

Focus Group	Number of participants age ≤18	Number of participants age 19-25	Number of participants age 25-30	Number of participants age 30-40	Number of participants age 40-50	Number of participants age ≥50	Gender male/female
A	0	1	3	1	1	1	4/3
B	2	6	0	0	0	0	4/4
Total	2	7	3	1	1	1	8/7

Table 8: Detailed characteristics of individual focus group participants

Participant	Focus Group	Age Group	Education level
P1G1	1	19-25	Undergraduate
P2G1	1	19-25	Undergraduate
P3G1	1	25-30	Graduate /Postgraduate
P4G1	1	25-30	Graduate /Postgraduate
P5G1	1	25-30	Undergraduate
P6G1	1	40-50	Postgraduate
P7G1	1	> 50	High School
P8G2	2	18	Undergraduate
P9G2	2	19-25	Undergraduate
P10G2	2	19-25	Undergraduate
P11G2	2	19-25	Undergraduate
P12G2	2	19-25	Undergraduate
P13G2	2	19-25	Undergraduate
P14G2	2	19-25	Undergraduate
P15G2	2	19-25	Undergraduate

The majority of participants overall were within the age 19-25 group and there were almost equal numbers of males and females. Respondents were screened based on three questions to exclude those who would not be eligible for the focus group. Questions were;

1. Have you ever participated in a focus group before?
2. Are you willing to participate in a recorded discussion on this topic? The recorded data will be handled appropriately
3. Do you have any ethical or religious objections to eating beef and are you allergic to gluten and/or soy?

Participants were also recruited based on their age for the group structure in each panel. Participants who answered no to questions one and three and yes to question two were invited to participate in the focus groups.

Conducting the focus group

The focus groups were held on the 22nd and the 27th of October, 2009. The durations of the two focus groups were ninety minutes and eighty minutes for the first and second focus groups, respectively. The focus group was moderated by the author and the supervisor of the research project. Focus groups were held in a sensory lab with a table in the middle which participants sat around to encourage interaction and to have the best recording environment. A tape recorder

with external microphone was used to record the answers of participants. Participants read an information sheet and signed a consent form (Appendices 8 and 9 respectively). The participants engaged in a discussion with each other and with the moderator for five minutes before the focus group officially started. The focus group was divided into five parts and guided by the focus group protocol (see Appendix 10). At the conclusion of the focus group participants put their name in a basket to be drawn at random for a prize of a $50 grocery voucher.

Sample Preparation

The five patty treatments were prepared as described in section 4.2. Patties were then placed in a refrigerator at 4°C on a tray lined with wax paper and had wax paper put on the surface to prevent drying until they were later cooked on the same day. One patty of each formulation was also placed on polystyrene trays wrapped with gladwrap and stored at 4°C in a refrigerator until they were later shown to participants for evaluation. Burger patties were cooked in canola oil on a Kambrook Banquet electric frypan for two minutes on each side. They were then put into a fan forced oven for ten minutes at 180°C which was sufficient to have internal temperature > 75°C. Burgers were removed, cut into quarters and wrapped in aluminium foil. These were put into labelled trays and held in an oven until later served. The internal temperature of the patties was tested with a thermal probe before serving.

Focus group Protocol

The focus group consisted of five parts that dealt with consumption of hamburgers, attitudes towards processed meat, attitudes towards negative aspects of red meat consumption and positive attitudes towards tempeh consumption as well as sensory perceptions of the cooked patties and visual acceptance of the raw patties. The focus group protocol used by the moderators (the author and the research supervisor) to guide the discussion is available in Appendix 10.

Part 1: Attitudes towards consumption of hamburgers

The first set of questions was about the participants' normal consumption habits with regards to takeaways, especially burgers. Participants were asked about when and where they normally consume burgers and which factors influenced their choice of takeaways and/or burgers

Part 2: Consumer perception and knowledge of processed meats

At the start of this section the participants were shown an information sheet (see Appendix 11) on processed meat and the addition of non-meat ingredients to meat products. This section was important to evaluate the consumers' knowledge of extenders and their attitude towards products containing non-meat components as burger patties may contain up to 30% tempeh. Questions aimed at exploring whether participants realised how many non-meat ingredients are included in processed meat, understanding of why producers do it, and if it seems deceptive or not.

Part 3: Preliminary sensory analysis

During this section the participants analysed the patties for sensory attributes. Quarter sections of patties, temperature tested with a thermometer were brought from the warming oven in a separate kitchen and were served as 3 digit coded samples simultaneously to participants. Attributes assessed were intensity of beef odour, intensity of other (non beef) odours, tenderness, juiciness, chewiness, beef flavour intensity, intensity of other (non beef) flavour, acceptance of flavour and overall acceptance. Attributes were rated on paper ballots (see Appendices 12 and 13) with five point word anchored scales with the exception of acceptance of flavour and overall acceptance which were assessed on seven point word anchored scales. This section served as exploratory research for sensory analysis to choose an acceptable level for tempeh inclusion in a burger.

Part 4: Effect of information of health benefits on consumer perception of novel beef patty

This section began by providing participants with an information sheet (Appendix 14) on published research suggesting negative aspects of red meat consumption and potential health benefits of tempeh (see sections 2.3. and 2.7.3). The objective was to see how health information impacts attitudes towards adding a vegetal antioxidant source to the burgers. Questions during this section were based around previous knowledge of a link between red meat and cancer and if this link led to a change of diet. Participants were also asked if this would increase likelihood of eating meat with an antioxidant source and if they had tried tempeh. Participants were asked whether they would make an attempt to consume antioxidants with red meat either as a separate part of a meal or the inclusion of the antioxidant source (such as in the tempeh) to a patty would be a convenient option. They were also asked if they would be willing to purchase a burger containing tempeh.

Part 5: Evaluation of raw burger patties

One patty of each formulation (freshly prepared) was displayed to participants in raw form on a polystyrene tray, as it would normally be presented for retail sale. The objective was to determine the attitudes towards the product in the form it is sold at retail after the health information is given. This was decided as a better method to assess purchase intention than consumption of the cooked patties alone as consumers base meat purchase on visual cues (Troy & Kerry, 2010; Sanders, Morgan, Wulf, Tatum, Williams & Smith, 1997).

Focus group Analysis:

The focus group discussions were recorded with Audacity software (v 1.2.6) and were later transcribed (Appendices 15 and 16). Participants and responses were coded according to Tables 7 and 8. The transcripts were analysed by sorting participant quotes thematically according to the insights they provided into consumer attitudes and were analysed to write the discussion.

Sensory analysis:

Pilot sensory analysis study

A smaller pilot sensory study (n = 14) was carried out before a full scale sensory study in order to aid in the design of the larger experiment. Participants were students and staff members from the University of Otago Food Science department.

Consumer sensory analysis study

Recruitment:

The panellists for sensory analysis (n = 118) were recruited by different contact and advertisement methods. The sample size is considered adequate to avoid type I and type II errors in a consumer sensory test (Hough, Wakeling, Mucci, Chambers, Mendez Gallardo & Rangel Alves, 2006). Panellists were recruited from a database kept by the Food Science department, University of Otago and from fliers placed around the University campus, Otago Polytechnic campus, Halls of residence (Appendix 17), an advertisement at lectures and by emails circulated by the administrators of University departments. Respondents arranged a time to come in and taste the burgers and were asked a set of questions (see section 4.2 participant recruitment for focus group) to screen out participants unable to participate based on personal beliefs, allergies or lack of familiarity with the product. The gender and age categories of panellists are shown in the table below (Table 9)

Table 9: Gender and age group composition of consumer sensory experiment

Gender	Age category					
	18 - 24	25 - 30	31 - 40	41 - 50	> 50	Total
Male	17	12	8	6	5	48
Female	26	8	16	10	10	70
Total	43	20	24	16	15	118

Study Design:

The questionnaire (see Appendix 18) for participants was created in Compusense Five (version 4.8.8, Guelph, Ontario, Canada). The panellists were asked to declare their age and gender categories and then for each sample questions they were asked for the attributes of overall acceptability, intensity of beef odour, tenderness, chewiness, juiciness, intensity of flavour, level of non meat flavour and acceptance of flavour. Following these questions for all samples, the consumers were asked about the frequency of hamburger consumption and of soy product consumption. The questionnaire design was for a maximum of 120 participants served according to a Williams design for 3 treatments, with coded samples. The data of two participants were omitted due to missing samples in order to balance the model for PCA.

Sample Preparation:

Samples were prepared as described in section 4.2 above for focus groups. The results obtained from the focus groups and pilot sensory studies guided the treatments chosen for consumer sensory analysis to be; a control, 10% breadcrumb and 10% tempeh containing patties. The samples were defrosted in a refrigerator overnight prior to the sensory analysis. The following day the patties were cooked according to the method described for focus groups above. After cooking they were cut into quarters, wrapped in tinfoil and placed in casserole dishes inside an oven set to 100°C.

Sensory Analysis:

The analysis was performed in sensory booths in the Sensory Science Research centre at the Food Science Department of the University of Otago (see Appendices 20-21). Participants first read an information sheet and signed a consent form and were then served the samples. The samples were coded with randomised numbers according to the serving order devised by Compusense. The samples were served under ambient light in a booth under positive pressure. Participants took one minute breaks and drank water between assessing samples to cleanse palettes.

Statistical Analysis

Statistical analysis of sensory attributes was analysed as one way analysis of variance (ANOVA) with treatments were the independant variable and significant differences at a level of $P < 0.05$ identified by post hoc Tukey's tests. Minitab software version 16 (Minitab, State College, PA, U.S.A).

Twelve physical and chemical properties were correlated in Minitab using Pearson correlations. properties which changed over time were plotted in Sigma Plot (Stystat Software Inc, San Jose, California, U.S.A) and the equation of the slope obtained used for correlation analysis.

4.3 Results and Discussion:

4.3.1 Proximate Analysis:

The proximate analysis results are difficult to compare with those in literature as other researchers have used more complex formulations, different fat contents and different levels of inclusion of extenders. The 10% tempeh patties were comparable in moisture, protein and ash contents to patties extended with 10% wheat fibre (Mansour & Khalil, 1999). Compared to patties extended with 9% flaxseed flour, 10% tempeh patties were higher in moisture and lower in fat, protein and carbohydrate contents (Bilek & Turhan, 2009).

The control patties had significantly ($p < 0.05$) lower moisture, and carbohydrate content and significantly ($p < 0.05$) higher protein and ash contents compared to patties containing tempeh (Table 10). Substitution of 10 % of the meat by breadcrumbs significantly ($p < 0.05$) reduced the water and protein contents and significantly ($p < 0.05$) increased fat, carbohydrate and ash contents (Table 10). Substituting the same amount of tempeh significantly ($p < 0.05$) increased water and carbohydrate contents and significantly ($p < 0.05$) decreased protein content but had no significant ($p > 0.05$) effect on fat or ash contents (Table 10).

An increase in moisture content occurred with addition of 10% date fibre in beef patties (Hashim & Khalil, 2007); however addition of 10% common bean flour or addition of 9% flaxseed flour decreased moisture content in beef sausages (Bilek & Turhan, 2009; Dzudie et al., 2002). A decrease in protein content and no change in ash content were also observed with 10% substitution of common bean flour (Dzudie et al., 2002), however 10% bean flour lowered

fat content which did not occur with substitution of tempeh. Similar to tempeh, addition of 9% flaxseed flour increased carbohydrate content but in contrast to tempeh had no effect on protein content and significantly ($p < 0.05$) increased fat and ash contents (Bilek & Turhan, 2009). Increases in fat content of meat are viewed negatively by consumers however this is dependant on the type of fatty acid predominant in the fat (see Sections 2.2.2 and 4.3.2) (Biesalski, 2005).

Due to the extensive use of soy extenders in processed meat (Singh et al., 2008) there are several studies on the use of soy extenders in beef patties (Angor & Al-Abdullah, 2010; Kassama et al., 2003; Turhan et al., 2006; Turhan et al., 2009). Adding dry okara (by-product of soy milk manufacture) significantly ($p < 0.05$) increased the protein, carbohydrate, fat and ash contents whilst reducing the moisture content (Turhan et al., 2009). Adding wet okara had the exact same effect on these chemical properties as adding tempeh (Turhan et al., 2006).

Substitution with tempeh or breadcrumbs significantly ($p < 0.05$) lowered the protein content of the patties (Table 10). Protein has a satiating effect thus it can help with weight loss as eating protein decreases the sensation of hunger whilst providing fewer calories than fat (Higgs, 2000). Although tempeh has lower protein content than meat it is considered to have high protein content for a non meat product. There were no significant ($p > 0.05$) differences between 10% and 20% tempeh, however addition of 30% tempeh caused a further reduction in protein content (Table 10). The substitution of breadcrumbs increased ($p < 0.05$) the fat content of the patties. This is not the expected outcome as the breadcrumbs have a low fat content. Similar results were reported for chicken balls (Huda et al., 2009) and breakfast sausage (Aleson-Carbonell, Fernandez-Lopez, Perez-Alvarez & Kuri, 2005) which was attributed to the ability of the extenders to bind fat. Incorporation of breadcrumb caused the largest increase in carbohydrate content due to level of carbohydrates in breadcrumb (45%). The carbohydrate content in the patties was increased by the increase in the level of added tempeh (Table 10). The ash content was highest in the breadcrumb treatment and the addition of tempeh increased the ash content only at 30% level (Table 10).

Few studies include levels of extender larger than 10% because of the changes which occur to processed meat products with substitution of meat for non meat ingredients. In Australia and New Zealand regulations state that processed meat must contain at least 30% meat or 66% meat for manufactured meat products (FSANZ). Processed meat is defined by FSANZ as “a meat product containing no less than 300 g/kg meat, where meat either singly or in combination with other ingredients or additives, has undergone a method of processing other than boning, slicing,

dicing, mincing or freezing, and includes manufactured meat and cured and/or dried meat flesh in whole cuts or pieces". A manufactured meat is defined as "processed meat containing no less than 660 g/kg of meat."

The tempeh prepared in the present study had similar fat content but higher moisture and ash contents and lower protein and carbohydrate contents than a recently reported study (Haron, Ismail, Azlan, Shahar & Peng, 2009). On a dry weight basis this tempeh (Appendix 22) was similar in protein and ash contents, higher in fat and lower in carbohydrate than other papers in the literature (Van der Riet et al., 1987; Zamora & Veum, 1978). This soy tempeh is higher on a dry weight basis in protein and fat, comparable in ash content and lower in carbohydrate content than tempeh made from combinations of faba bean, lupine, chickpeas and peas (Nassar et al., 2008).

Table 10: Proximate composition of beef burger patties, tempeh and patties with partial substitution of tempeh and breadcrumbs. Different letters a-f denote significant ($p < 0.05$) differences between treatments. Values are the mean ± standard deviation

Treatment	Water	Protein	Fat	Carbohydrate	Ash
Control	68.18±0.01d	23.38±0.51e	6.48±0.44a	0.00±0.00a	1.97±0.02b
10% breadcrumb	61.23±0.12a	22.22±0.16d	7.11±0.05b	7.19±0.01b	2.25±0.03d
10% tempeh	68.80±0.27c	21.71±0.20cd	6.37±0.30a	1.14±0.23c	1.98±0.16b
20% tempeh	68.30±0.27b	21.40±0.22c	6.51±0.14a	1.81±0.17d	1.99±0.02b
30% tempeh	68.80±0.20c	20.10±0.21b	6.59±0.09a	2.51±0.07e	2.03±0.02c
Tempeh	71.57±0.35e	13.14±0.31a	9.30±0.05c	4.93±0.08f	1.06±0.01a

4.3.2 Fatty acids analysis:

The control patties were composed mainly of 18:1 n-9, 16:0 and 18:0 (Table 11). The profile and concentration of fatty acids were not affected generally with the substitution of 10% breadcrumbs (Table 11) confirming that breadcrumbs were not contributing to the fatty acids profile. The predominance of 16:0, 18:0 and 18:1 fatty acids in control burger patties is consistent with beef patties containing 10% fat (Bilek & Turhan, 2009; Hur, Lim, Park & Joo, 2009).

Tempeh contained 18:2 n-6 as more than half its fatty acid composition, followed by 18:1 n-9, 16:0 and 19:0 in smaller amounts (Table 11). The control patty was significantly ($p < 0.05$) higher in saturated and monounsaturated fatty acids than tempeh, whilst tempeh was higher in polyunsaturated fatty acids and had a significantly ($p < 0.05$) higher P/S ratio. The substitution of tempeh in the formulation caused significant ($p < 0.05$) dose dependent reductions in the major fatty acids 16:0 18:0 and 18:1 n-9 and significant ($p < 0.05$) increases in 18:2 n-6 and 19:0 fatty acids. The saturated fatty acid content of 10% tempeh was the same as the substitution of 9% flaxseed flour (Bilek & Turhan, 2009).

Increased consumption of the fatty acids 12:0, 14:0, 16:0 and 18:0 are associated with increased risk of coronary heart disease (Hu et al., 1999; Mann & Chisolm, 2007) and tempeh addition reduced the amounts of these in a dose dependent manner. Therefore tempeh addition reduced amounts of atherogenic fatty acids producing a healthier patty.

Linoleic acid (18:2 n-6) is associated with a reduced risk of cardiovascular disease compared to other fatty acids (Mann & Chisolm, 2007; Kris-Etherton & Yu, 1997). Linolenic acid raises HDL cholesterol concentrations and lowers total and LDL cholesterol concentrations in comparison to 18:0 (Kris-Etherton & Yu, 1997). The high amount of 18:2 n-6 in tempeh gives the burgers a high n-6/n-3 ratio which is sometimes used in literature as an indicator of health properties of food (Simopoulos, 2008). The U.K Food Standards Agency has recommended against using this ratio as an indicator of the nutritional value of a food (Stanley et al., 2007). This is because the ratio fails to differentiate between different n-6 and n-3 fatty acids which have different effects and cardiovascular risk can be decreased with high amounts of both classes of fatty acids (Stanley et al., 2007).

Polyunsaturated fatty acids are better for health than other classes as they exert an hypo-cholesterolaemic effect (Kris-Etherton & Yu, 1997) and higher intake of polyunsaturated fat relative to saturated fat is beneficial to health (Kris-Etherton, Fleming & Harris, 2010). This ratio is strongly inversely associated to risk of CVD (Hu et al., 1999) and Wood et al. (2003) recommend a P: S ratio higher than 0.4 which is achieved with addition of 20% tempeh or more.

There is little information in the literature on the fatty acid composition of tempeh. Sudarmadji and Markakis (1978) also showed that tempeh contained predominantly 18:2, 18:1, 16:0, 18:0 and 18:3 although these authors did not provide a complete fatty acids profile

20:2 n-6	0.18 ± 0.03a	0.02 ± 0.03a	0.05 ± 0.00ab	0.05 ± 0.00ab	0.07 ± 0.03ab	0.21 ± 0.14b
20:3 n-3	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.03 ± 0.04a
20:3 n-6	0.12 ± 0.01d	0.13 ± 0.00d	0.12 ± 0.00d	0.11 ± 0.00c	0.09 ± 0.01b	0.00 ± 0.00a
20:4 n-6	0.34 ± 0.03c	0.40 ± 0.01d	0.39 ± 0.00d	0.32 ± 0.01bc	0.28 ± 0.01b	0.00 ± 0.00a
20:5 n-3	0.19 ± 0.10b	0.30 ± 0.03bc	0.32 ± 0.00c	0.26 ± 0.00bc	0.23 ± 0.01b	0.00 ± 0.00a
22:0	0.00 ± 0.00a	0.00 ± 0.00a	0.05 ± 0.05b	0.12 ± 0.01c	0.16 ± 0.00c	0.38 ± 0.01d
22:5 n-3	0.32 ± 0.02c	0.35 ± 0.02d	0.36 ± 0.01d	0.30 ± 0.01c	0.27 ± 0.01b	0.00 ± 0.00a
24:0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.16 ± 0.02b
Unknown Peak	6.28 ± 0.99b	5.87 ± 1.44b	4.57 ± 0.02b	3.93 ± 0.13a	3.25 ± 0.11a	1.87 ± 1.40a
Saturated	42.95 ± 0.66e	42.88 ± 0.06e	41.65 ± 0.11d	38.86 ± 0.10c	36.64 ± 0.09b	25.28 ± 0.30a
Monounsaturated	47.78 ± 1.44e	46.97 ± 1.16e	43.57 ± 0.24d	41.04 ± 0.43c	37.81 ± 0.38b	18.82 ± 0.49a
Polyunsaturated	2.99 ± 0.06a	4.28 ± 0.21b	10.21 ± 0.18c	16.17 ± 0.56d	22.31 ± 0.43e	54.04 ± 0.64f
Unknown class	6.28 ± 0.99b	5.87 ± 1.44b	4.57 ± 0.02b	3.93 ± 0.13ab	3.27 ± 0.11a	1.87 ± 1.40a
Omega 3	0.67 ± 0.09bc	0.81 ± 0.06cd	0.85 ± 0.06d	0.69 ± 0.01c	0.60 ± 0.01bc	0.05 ± 0.04a
Omega 6	2.32 ± 0.04a	3.47 ± 0.16b	9.32 ± 0.12c	15.48 ± 0.57d	21.68 ± 0.38e	53.99 ± 0.68f
P/S	0.07 ± 0.00a	0.10 ± 0.00b	0.25 ± 0.00c	0.42 ± 0.02d	0.61 ± 0.01e	2.14 ± 0.01f
S/P	14.37 ± 0.39f	10.03 ± 0.50e	4.08 ± 0.07d	2.41 ± 0.09c	1.64 ± 0.03b	0.47 ± 0.00a
Omega 3/Omega 6	0.29 ± 0.04d	0.23 ± 0.01c	0.09 ± 0.01b	0.04 ±0.00ab	0.03 ± 0.00a	0.00 ± 0.00a
Omega 6/Omega 3	3.51 ± 0.57a	4.29 ± 0.12a	10.94 ± 0.72a	22.50 ± 1.27a	36.23 ± 1.38a	2203 ± 2077a

Fatty acid (%)	Control	Control + 10% breadcrumb	Control + 10% tempeh	Control + 20% tempeh	Control + 30% tempeh	Tempeh
12:0	0.06 ± 0.00de	0.06 ± 0.00e	0.06 ± 0.00d	0.05 ± 0.00c	0.04 ± 0.00b	0.00 ± 0.00a
12:1	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.01 ± 0.01b
13:0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.01 ± 0.00a
13:1	0.05 ± 0.00a	0.05 ± 0.00b	0.05 ± 0.00c	0.04 ± 0.00d	0.03 ± 0.00e	0.00 ± 0.00e
14:0	2.59 ± 0.05a	2.58 ± 0.05b	2.33 ± 0.00c	2.01 ± 0.02d	1.66 ± 0.05e	0.08 ± 0.01e
14:1	0.77 ± 0.08de	0.77 ± 0.07e	0.65 ± 0.01cd	0.60 ± 0.01bc	0.50 ± 0.05b	0.00 ± 0.00a
15:0	0.41 ± 0.01f	0.41 ± 0.01e	0.37 ± 0.01d	0.31 ± 0.00c	0.26 ± 0.00b	0.00 ± 0.00a
16:0	23.17 ± 0.29e	23.12 ± 0.07e	21.67 ± 0.04d	20.12 ± 0.11c	18.70 ± 0.00b	10.98 ± 0.00a
16:1 n-7	3.56 ± 0.28d	3.48 ± 0.25d	2.95 ± 0.03c	2.76 ± 0.05bc	2.36 ± 0.07b	0.08 ± 0.01a
16:1 T	0.23 ± 0.06c	0.23 ± 0.04c	0.19 ± 0.01bc	0.17 ± 0.00bc	0.14 ± 0.00c	0.00 ± 0.00c
17:0	0.93 ± 0.03e	0.91 ± 0.00e	0.85 ± 0.00d	0.75 ± 0.01c	0.64 ± 0.00b	0.13 ± 0.00a
17:1	1.01 ± 0.02e	1.01 ± 0.03e	0.90 ± 0.01d	0.82 ± 0.01c	0.70 ± 0.00b	0.07 ± 0.00a
18:0	14.70 ± 0.42e	14.57 ± 0.16e	13.96 ± 0.07d	12.19 ± 0.07c	10.81 ± 0.07b	3.73 ± 0.06a
18:1 n-7	1.99 ± 0.22c	1.92 ± 0.06bc	1.81 ± 0.06bc	1.69 ± 0.02b	1.68 ± 0.02b	1.22 ± 0.02a
18:1 n-9	39.94 ± 0.95e	39.26 ± 0.81e	36.75 ± 0.21d	34.75 ± 0.37c	32.19 ± 0.28b	17.29 ± 0.45a
18:2 n-6	1.31 ± 0.08a	2.45 ± 0.19a	8.33 ± 0.11b	14.65 ± 0.58c	20.93 ± 0.41d	53.56 ± 1.04e
18:2 n-6 TT	0.42 ± 0.02b	0.38 ± 0.00ab	0.35 ± 0.00ab	0.28 ± 0.01ab	0.24 ± 0.00ab	0.17 ± 0.18b
18:3 n-3	0.16 ± 0.00c	0.16 ± 0.00bc	0.18 ± 0.06bc	0.13 ± 0.00bc	0.11 ± 0.01b	0.02 ± 0.01a
18:3 n-6	0.11 ± 0.03c	0.09 ± 0.00bc	0.08 ± 0.01bc	0.07 ± 0.00b	0.07 ± 0.03b	0.02 ± 0.01a
19:0	0.99 ± 0.06a	1.12 ± 0.09a	2.21 ± 0.03b	3.16 ± 0.07b	4.18 ± 0.06c	9.48 ± 0.22d
20:0	0.10 ± 0.01a	0.11 ± 0.01a	0.13 ± 0.01ab	0.15 ± 0.00b	0.19 ± 0.00c	0.32 ± 0.00d
20:1	0.15 ± 0.01a	0.17 ± 0.01a	0.15 ± 0.00ab	0.15 ± 0.00ab	0.15 ± 0.00a	0.15 ± 0.01b
20:1 n-9	0.09 ± 0.01d	0.09 ± 0.00d	0.07 ± 0.00c	0.07 ± 0.00c	0.06 ± 0.01b	0.00 ± 0.00a

Table 11 Individual fatty acid and fatty acid class percentages of beef patties, beef patties with addition of either breadcrumbs or tempeh, or tempeh alone. Different letters a-e denote significant ($p < 0.05$) differences between treatments for a given fatty acid

4.3.3 Colour

Lightness (L* values)

The incorporation of both 20% and 30% tempeh significantly ($p < 0.05$) increased L* values of the patties compared with the control (Figure 15). The 10% breadcrumb and control patties were not different ($p > 0.05$) with the exception of the 24 hour time point. The control and 10% tempeh patties were not significantly ($p > 0.05$) different at 15, 48, 87 and 96 hours, but were different at all other times.

The L* values of burgers extended with tempeh were higher than most other L* values reported in the literature for other meat extenders. The 10% tempeh patties were higher than other patties extended with similar amounts of dry tomato peel, dry okara powder, wet okara, flaxseed flour and sorghum flour (Garcia et al., 2009; Turhan et al., 2009; Bilek & Turhan, 2009; Huang et al., 1999; Turhan et al., 2006). Patties made with 20% and 30% tempeh also had higher L* values than those made with similar amounts of sorghum flour and wet okara (Huang et al., 1999; Turhan et al., 2006). Tempeh has a white colour, is lighter than all of these ingredients and contains higher moisture compared to dry flours and powders which accounts for the higher L* values with tempeh. Hazelnut pellicle added at 5%, however was found to have higher L* values than tempeh (Turhan, Sagir & Ustun, 2005).

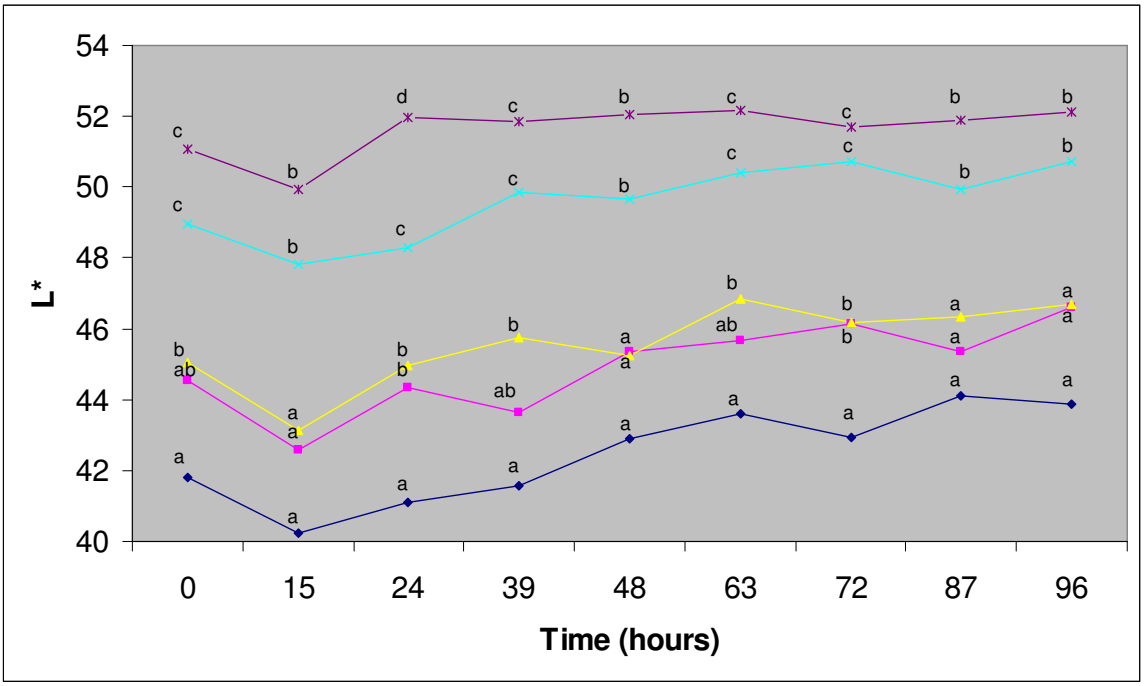

Figure 15: L* values of Control (♦), Control+10% breadcrumb (■), Control + 10% tempeh (▲), Control + 20% tempeh (x) and Control + 30% tempeh (*) over a 96 hour storage period. Different letters a-d denote significant ($p < 0.05$) differences between treatments for each time

Redness (a* values)

All a* values decreased over the 96 hours storage period which means a loss of the fresh red colour (Figure 16). The fresh patties showed no significant ($p > 0.05$) differences and at the end of testing only 30% tempeh was significantly ($p < 0.05$) increased compared to other treatments. Generally, all treatments had higher ($p < 0.05$) a* values than the control at 24, 39, 48 and 63 hours of display at 4°C. There were no differences ($P > 0.05$) among 10% breadcrumbs, 10% tempeh and 20% tempeh treated patties throughout the display period (Figure 16).

The a* values were higher in the 10% tempeh burgers than in burgers extended with okara powder, flaxseed flour, sorghum flour, hazelnut pellicle or wet okara (Turhan et al., 2009; Bilek & Turhan, 2009; Huang et al., 1999; Turhan et al., 2005; Turhan et al., 2006).

They were however lower than the values of burgers incorporating dry tomato peel, paprika, tomato oleoresin and lycopene rich tomato pulp (Garcia et al., 2009; Sanchez-Escalante, Torrescano, Djenane, Beltran & Roncales, 2003). These ingredients have a red pigment and

impart this colour on the patties leading to higher a* values. The important pigment for fresh beef colour is oxymyoglobin which gives the characteristic colour. Oxygen attached to the ferrous iron atom in the porphyrin ring gives this characteristic colour (high a* values). However the meat will oxidise over time and the iron atom will convert to the ferric state which causes the brown metmyoglobin colour (low a* value). This is important for the sale of meat products as colour is the first attribute noticed by the consumer and indicates the freshness (Troy & Kerry, 2010). Meat colour was the most important attribute for selecting beef products in a sample of Japanese consumers (n = 10,941) (Sanders et al., 1997). In a sample of New Zealand consumers up to 85% were willing to buy beef steaks on the basis of colour (Farouk, Bekhit, Dobbie & Waller, 2007).

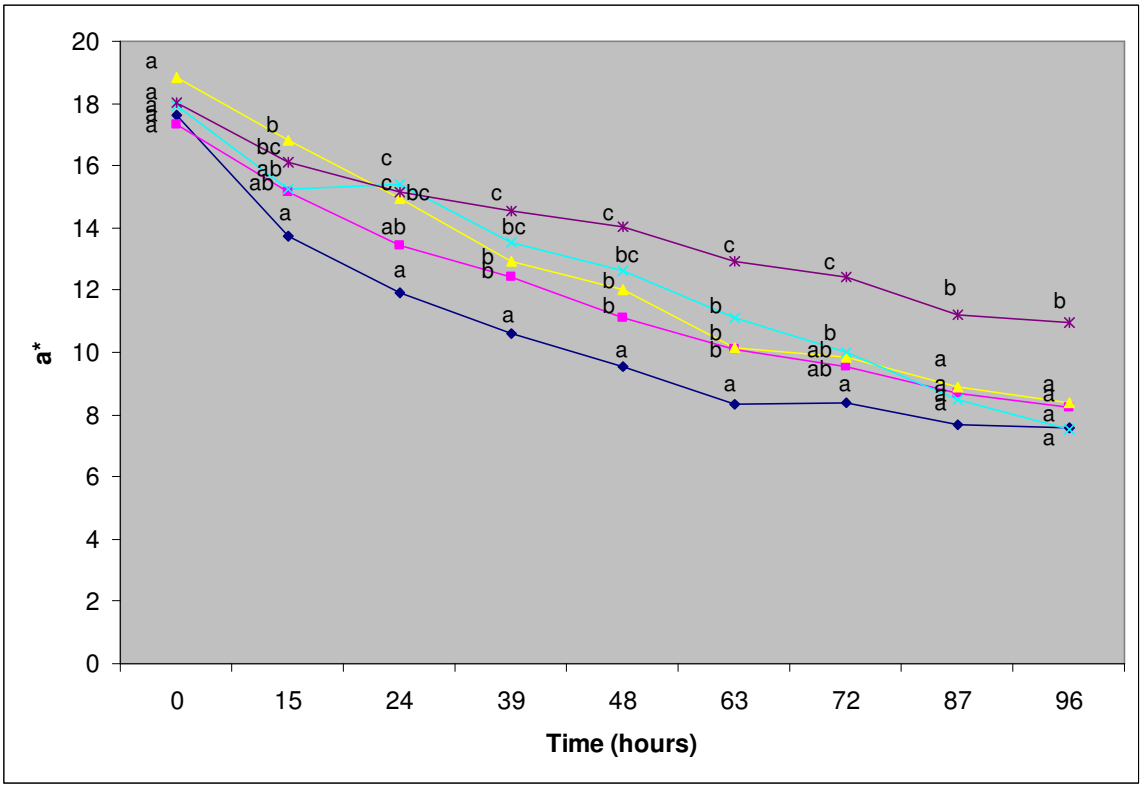

Figure 16: a* values of Control (♦), Control+10% breadcrumb (■), Control + 10% tempeh (▲), Control + 20% tempeh (x) and Control + 30% tempeh (*) over a 96 hour storage period. Different letters a-c denote significant ($p < 0.05$) differences between treatments for each time.

Yellowness (b* values)

Yellowness values for 10% breadcrumb, 10% tempeh and 20% tempeh patties were not different ($p < 0.05$) from the control throughout storage with the exception of 10% tempeh treatment at 15 hours and 20% tempeh treatment at 24 hours (Figure 17). The 30% tempeh treatment had the highest b* values throughout storage period and was the only treatment significantly higher than the control patties.

The b* values for tempeh patties were also higher than most values reported in the literature for other meat extenders added at similar levels. The tempeh patties were higher in b* values than those of burgers extended with dry tomato peel, okara powder, flaxseed flour, sorghum flour, hazelnut pellicle and wet okara at similar levels of inclusion (Garcia et al., 2009; Turhan et al., 2009; Bilek & Turhan, 2009; Huang et al., 1999; Turhan et al., 2005; Turhan et al., 2006). As the soybeans are white yellowish in colour and part of this colour is retained in the tempeh they contributed to the elevated b* values. Increases in b* relative to control patty (which was observed with addition of 30% tempeh) with addition of other soy products and legume and seed flours to beef patties have been observed previously (Turhan et al., 2009; Bilek & Turhan, 2009, Huang et al., 1999).

When comparing CIE parameters for meat colour it is important to recognise the natural variation in values which is caused by meat type, processed form and ingredients and the colorimeter used for measurement. Even within beef cattle, colour can vary according to the animal age, diet, muscle type and metabolism, enzymes, endogenous antioxidants and activity prior to slaughter (Bekhit & Faustman, 2005; Muchenje et al., 2009). The colorimeter used influences the values obtained as well. For example, Minolta colorimeters have been demonstrated to produce higher chroma and L* values and lower hue angles and a* and b* values relative to a Hunter colorimeter when measuring CIE values for beef steaks (Farouk et al., 2007). This may create some problems in comparing the results from different studies.

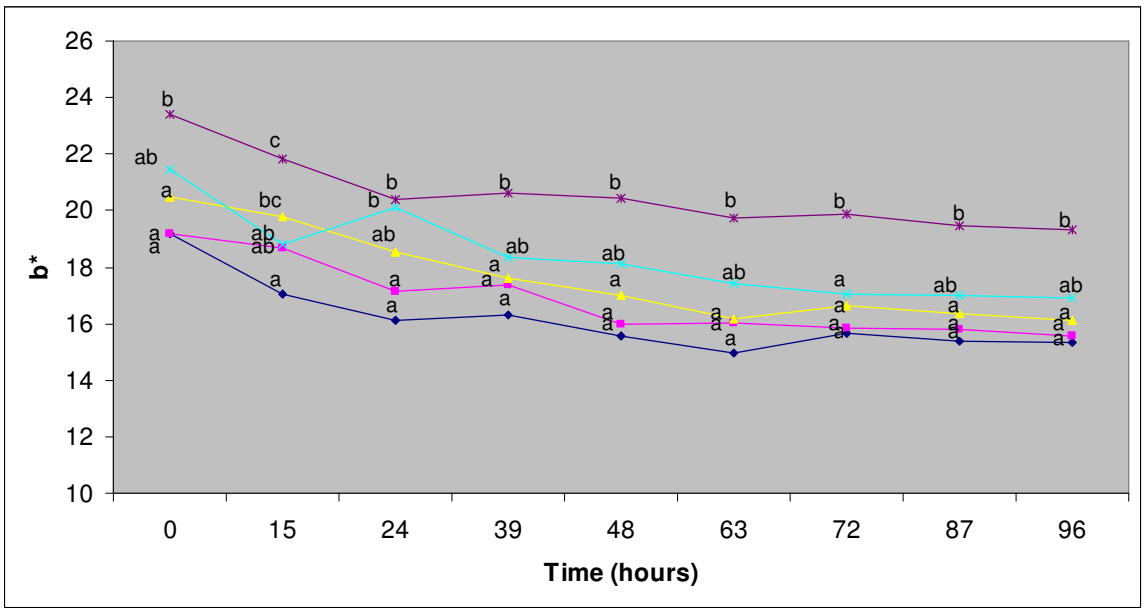

Figure 17: b* values of Control (♦), Control+10% breadcrumb (■), Control + 10% tempeh (▲), Control + 20% tempeh (x) and Control + 30% tempeh (*) over a 96 hour storage period. Letters a-c denote significant ($p < 0.05$) differences between treatments

Chroma (C* values)

C* values for all samples were decreased over the storage period, following the trend described for a* values (Figure 18). The patties from 10% breadcrumb treatment only were higher ($p < 0.05$) than the control at 72 hours of testing and the C* values of 10% tempeh were only higher from the control at 15, 24 and at 72 hours. The 20% tempeh treatment was observed to be significantly ($p < 0.05$) higher than the control between 24 and 72 hours. The C* of 30% tempeh was generally the highest of all treatments throughout storage and significantly ($p < 0.05$) higher than the control for the whole period.

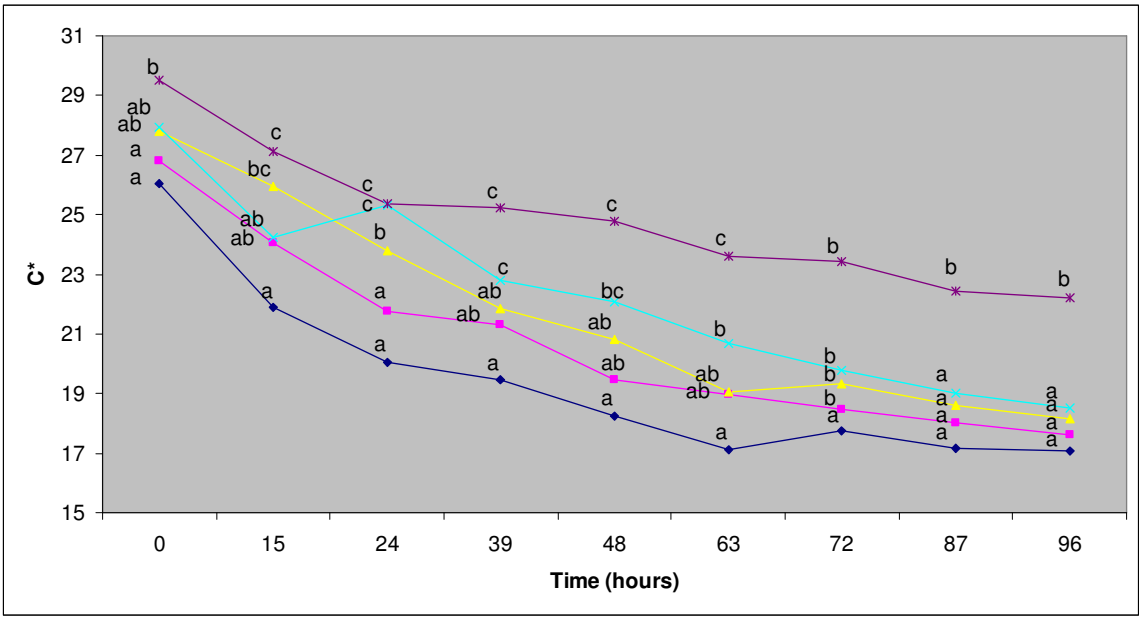

Figure 18: C* values of Control (♦), Control+10% breadcrumb (■), Control + 10% tempeh (▲), Control + 20% tempeh (x) and Control + 30% tempeh (*) over a 96 hour storage period. Letters a-c denote significant ($p < 0.05$) differences between treatments

Hue angle (H* values)

Initially, the addition of 20% and 30% tempeh produced patties with significantly ($p < 0.05$) higher H* values (Figure 19). After 24 hours the control was significantly higher ($p < 0.05$) than patties containing 10% tempeh and retained the highest value for most of the storage period until the 96th hour when 20% tempeh patties had higher H* values (Figure 19). The addition of tempeh prevented increases in H* which were significant compared to the control. Throughout most of the storage period 10% breadcrumb patties had higher hue angles than patties produced with the same amount of tempeh due to the brown colour of breadcrumbs.

Storage time and time post-mortem increases H* values of beef patties (Bekhit, Geesink, Ilian, Morton & Bickerstaffe, 2003a) during display and as the hue angle increases the willingness to purchase of the consumer decreases (Farouk et al., 2007).

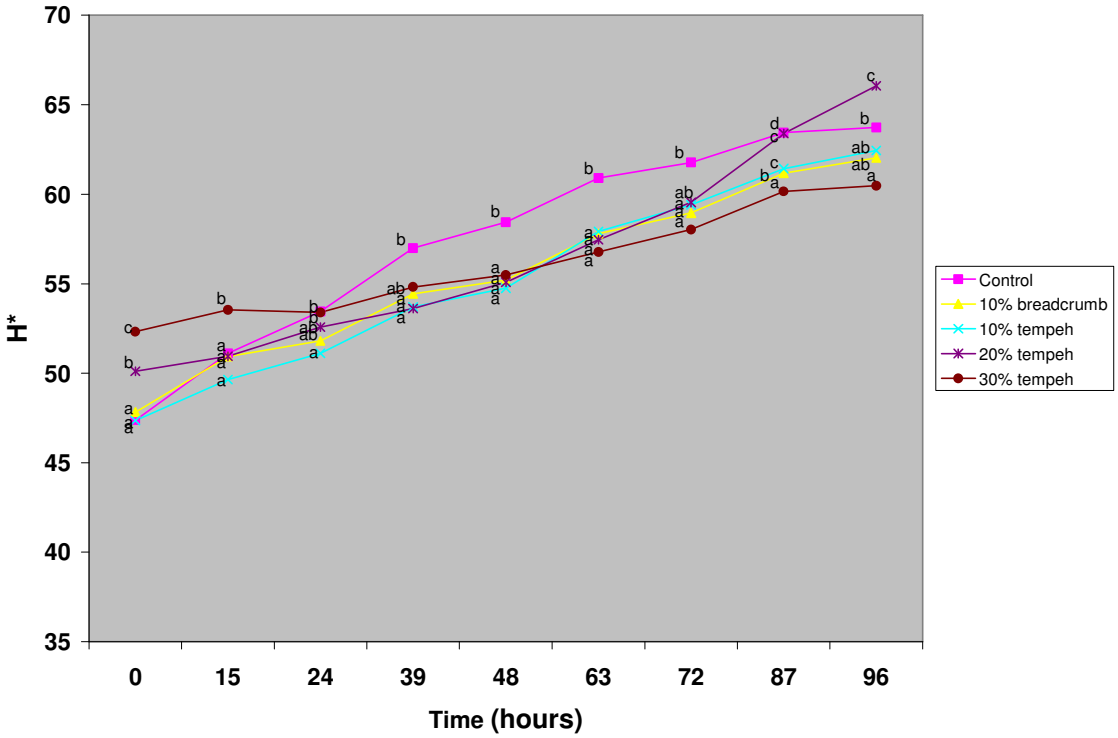

Figure 19: H* values of five burger patty treatments over a 96 hour storage period. Letters a-c denote significant ($p < 0.05$) differences between treatments

Browning index

Reflectance indices 630-580 nm and 630/580 nm can be used to demonstrate loss of fresh red colour in meat products (Fernandez-Lopez, Perez-Alvarez, Sayas-Barbera & Aranda-Catala, 2000). Addition of tempeh produced patties with 630-580 nm indices higher than those of control and 10% breadcrumb patties. Values for 630-580 nm indices increased with increasing amounts of tempeh (Figure 20).

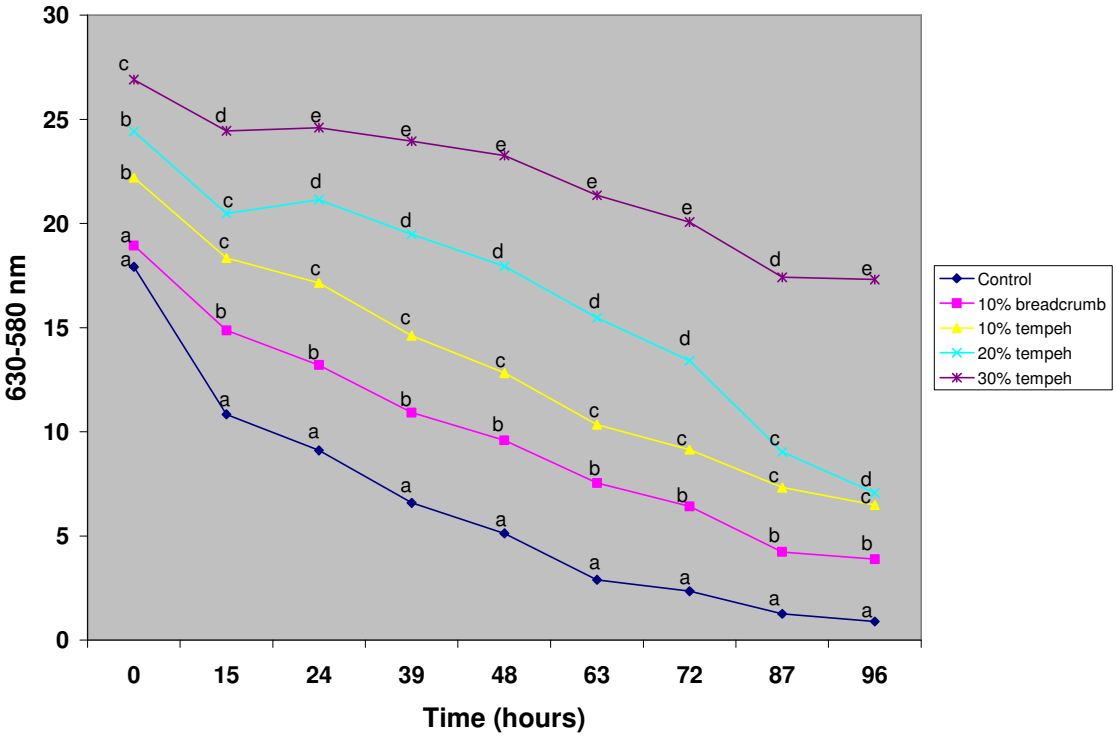

Figure 20: The reflectance ratio 630-580 nm of five hamburger patty treatments over a 96 hour storage period. Different letters a-e denote significant ($p < 0.05$) differences.

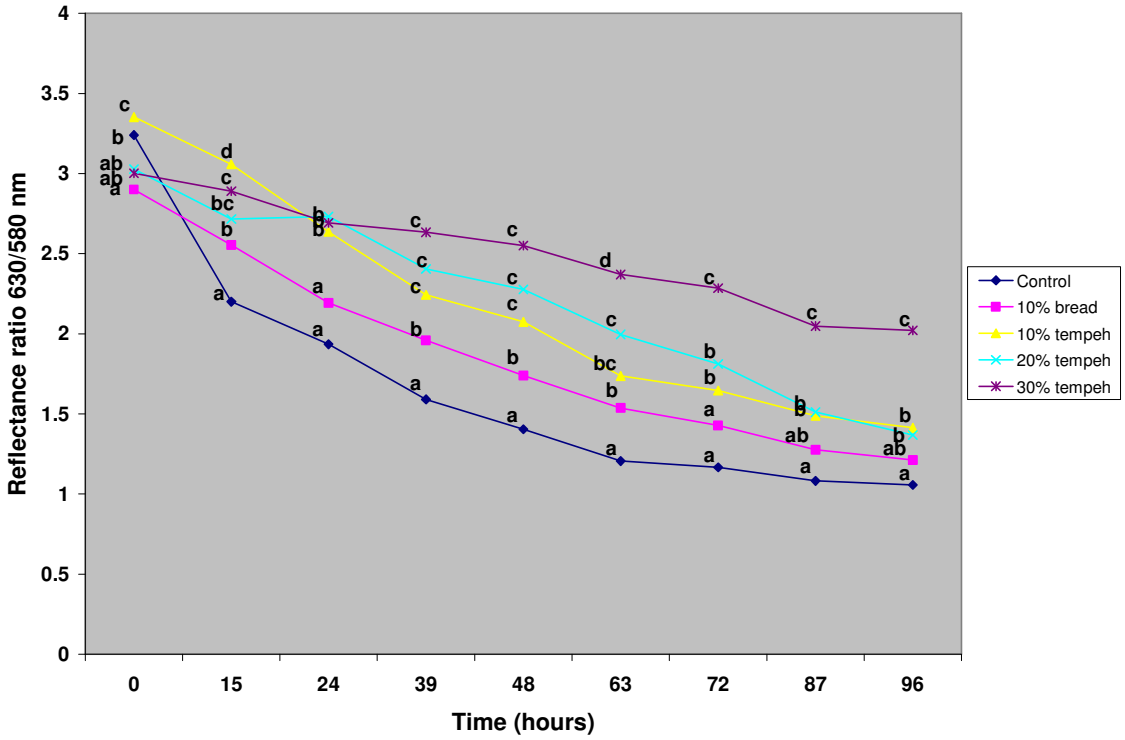

Figure 21: The reflectance ratio 630/580 nm of five hamburger patty treatments over a 96 hour storage period. Letters a-d denote significant ($p < 0.05$) differences.

The 630/580 nm index produced values with less differences between each other. Initially the 20% and 30% tempeh patties were not significantly ($p > 0.05$) different from the control or breadcrumbs and the 10% tempeh patties were significantly ($p < 0.05$) higher than all other treatments (Figure 21). All patties treated with tempeh were significantly ($p < 0.05$) higher than the control throughout the storage period.

For both ratios, 10% breadcrumb addition produced lower values indicating faster browning of the patties over storage compared to 10% tempeh.

Patties in this trial decreased in 630/580 nm index faster than patties containing 1% chitosan stored at 1°C in the dark (Suman et al., 2010). This may be due to the lower temperature used in that experiment and probably more effective antioxidant activity of chitosan. The chitosan patties were significantly higher in 630/580 nm index than the control after 3 days of storage whereas addition of 10% or more tempeh produced values significantly higher than the control from the beginning of storage (Suman et al., 2010).

630/580 nm and 630-580 nm indices have been used to demonstrate the increase in metmyoglobin and browning of fresh red meat (Fernandez-Lopez et al., 2000). A decrease in

the 630/580 nm ratio over storage was parallel to increased browning in retail displayed lamb (Ponampalam et al, 2010).

With the exception of 30% tempeh patties the browning indices were highly correlated (R^2 = 0.9599–0.9797) (Figures 22-26). Fitness coefficients decreased with increasing substitution of meat for tempeh (Figures 22-25) or breadcrumbs and were reduced to R^2 = 0.86 (Figure 26). Thus the correlations between these browning indices could be used interchangeably for beef patties up to a meat substitution level of 20%.

Overall the two browning indices were strongly correlated to each other (R^2 = 0.91). The 630-580 nm and 630/580 nm ratios were strongly correlated to a* values (R^2 = 0.85 and R^2 = 0.98 respectively) and the H* value (r = -0.67 and r = -0.86 respectively).

Due to the very strong correlation of 630/580 nm ratio to a* value and the stronger correlation to the H* value which are indices of browning (Larrain et al., 2008; Bekhit et al, 2003a; Bekhit, Geesink, Ilian, Morton, Sedcole & Bickerstaffe, 2003b) this ratio can be considered more reliable as a browning index for burger patties.

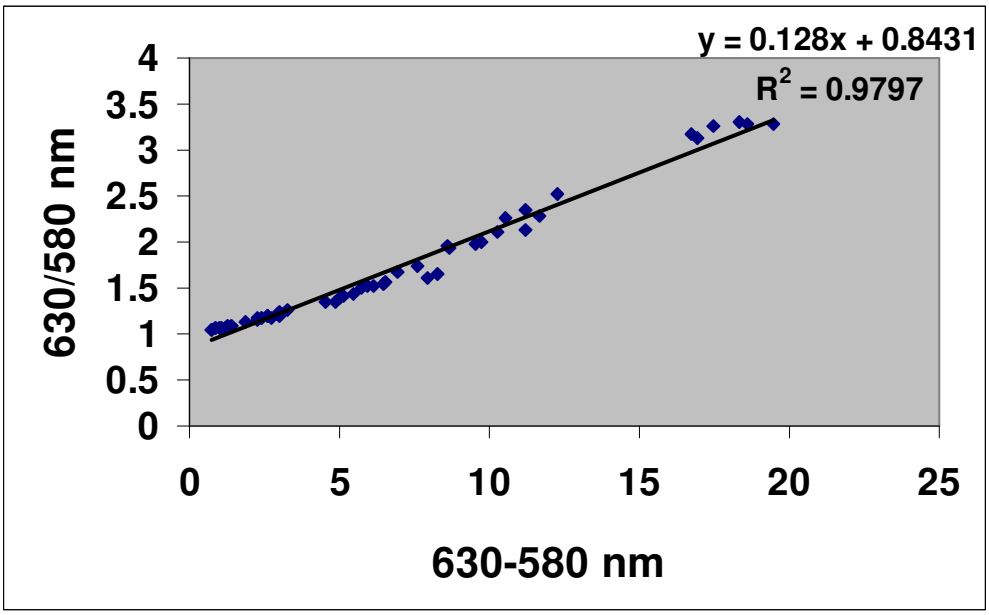

Figure 22: Fitness coefficients (R^2) values of the relationship between the browning indices 630/580 nm and 630-580 nm for control patties

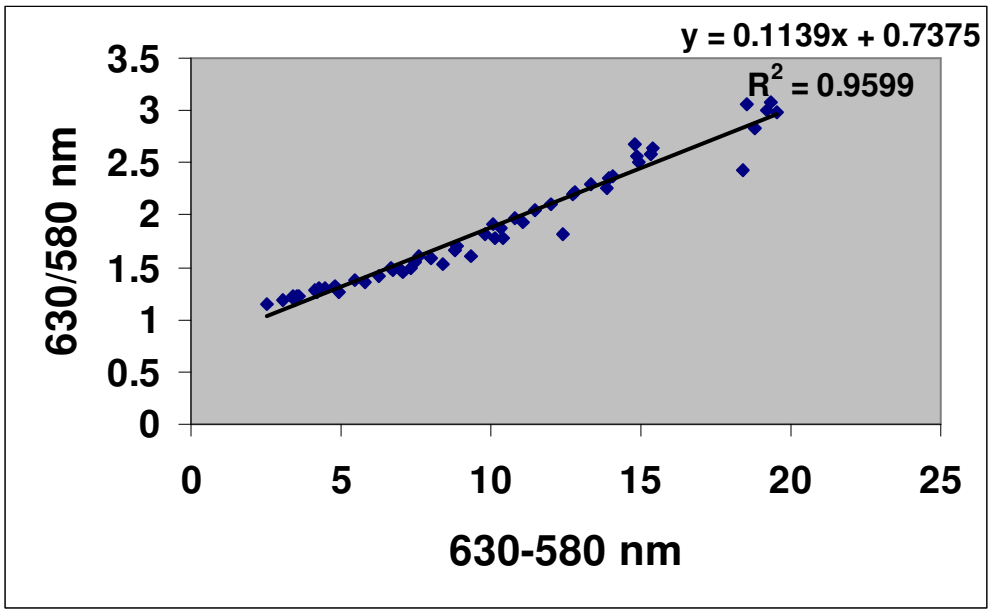

Figure 23: Fitness coefficients (R^2) values of the relationship between the browning indices 630/580 nm and 630-580 nm for 10% breadcrumb patties

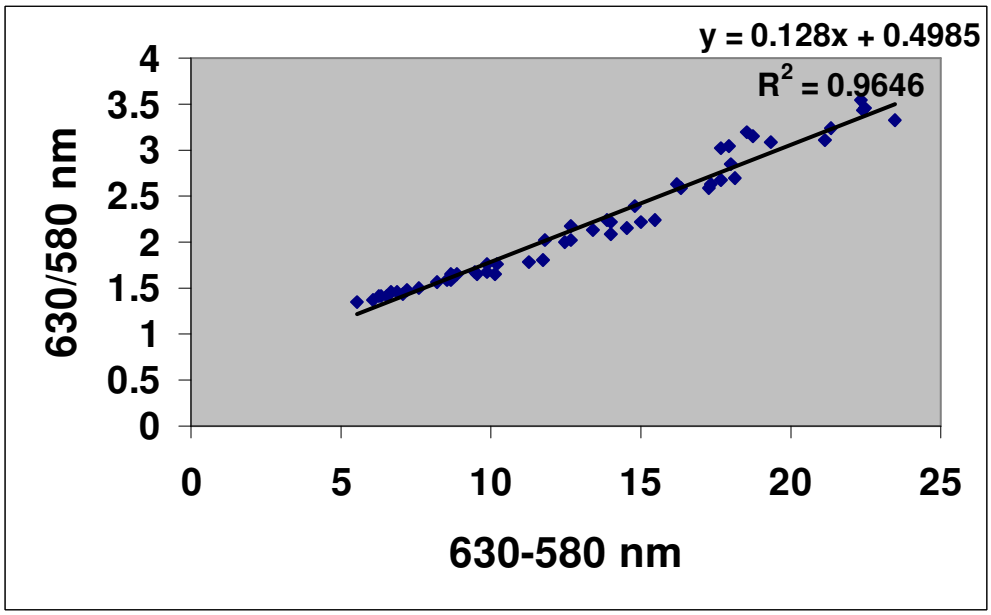

Figure 24: Fitness coefficients (R^2) values of the relationship between the browning indices 630/580 nm and 630-580 nm for 10% tempeh patties

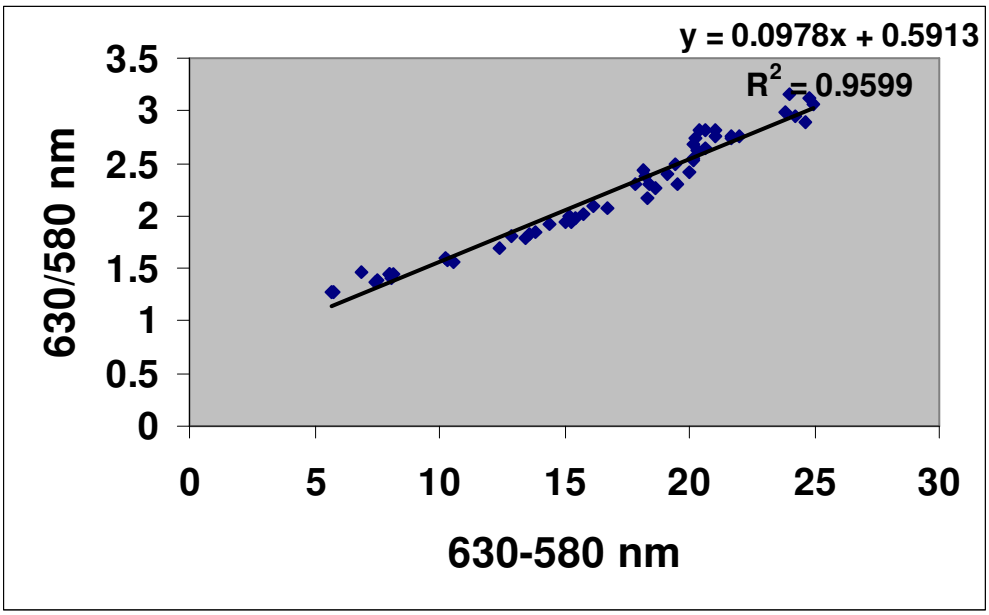

Figure 25: Fitness coefficients (R^2) values of the relationship between the browning indices 630/580 nm and 630-580 nm for 20% tempeh patties

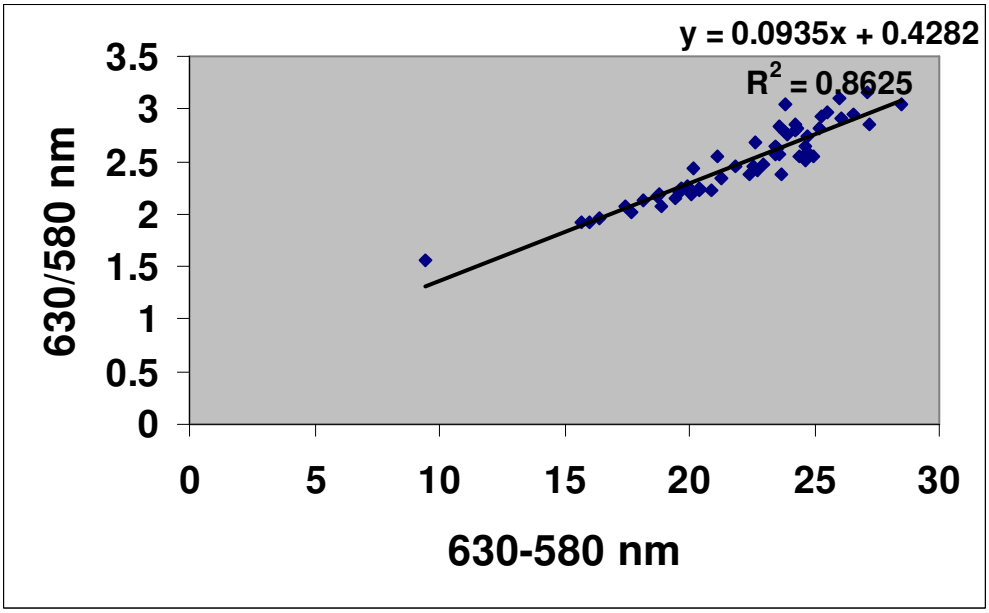

Figure 26: Fitness coefficients (R^2) values of the relationship between the browning indices 630/580 nm and 630-580 nm for 30% tempeh patties

4.3.4 Lipid Oxidation: thiobarbituric acid reactive substances (TBARS)

The lipid oxidation (determined as TBARS) of all treatments were similar until after day 2 when 20% and 30% tempeh increased significantly ($p < 0.05$) compared to the other treatments (Figure 27). These treatments accumulated MDA at an even greater rate after days 4 and 6 to levels around four fold higher than the control. The 10% tempeh patties were not significantly different from 10% breadcrumb or control patties until after day 4 when they increased significantly compared to control and breadcrumb treatment (Figure 27).

Comparisons to other TBARS values in patties formulated with extenders during refrigerated storage in the literature is complicated due to the fact that often there are smaller amounts of other additives incorporated, different fat contents and the different treatments applied to patties such as cooking, irradiation and modified atmosphere storage, all of which affect the TBARS values (Aleson-Carbonell et al., 2005; Alp & Aksu, 2010; Angor & Al-Abdullah, 2010; Banon et al., 2007; Dzudie et al., 2004; Ismail et al., 2009; Lund, Hviid & Skibsted, 2007; Martinez et al., 2009; Sanchez-Escalante et al., 2003; Suman et al., 2010; Tang, Ou, Huang, Li, Kerry & Buckley, 2006).

At day 0 all the patties had TBARS values similar to those of other studies (Dzudie et al., 2004; Lowder & Osburn, 2010; Bond, Marcello & Slanger, 2001; Sanchez-Escalante et al., 2003; Tang et al., 2006; Hur, Ye, Lee, Ha, Park & Joo, 2004; Candogan, 2002; Britt, Gomaa, Gray & Booren, 1998). In many of these studies the TBARS values were lower for treated patties at the time points tested than the tempeh patties from this experiment (Lowder & Osburn, 2010; Bond et al., 2001, Tang et al., 2006; Hur et al., 2004: Candogan, 2002; Britt et al., 1998).

The tempeh extended patties were lower in TBARS than a lean meat control prepared by Tang et al. (2006) stored under the same conditions until the sixth day of storage when 10% tempeh was comparable and 20% and 30% tempeh were higher. At four days of storage 10% tempeh patties were comparable to patties containing 2% cayenne pepper or 0.55% tomato oleoresin (Sanchez-Escalante et al., 2003).

The TBARS value of around 2.0 has been determined previously to be a limit of acceptability for oxidised beef flavour (Campo, Nute, Hughes, Enser, Wood & Richardson, 2006). Accordingly patties containing 20 and 30% tempeh were unacceptable at the fourth day of storage and 10% tempeh patties were unacceptable at the sixth day.

The TBARS were higher during the latter part of storage in tempeh patties because they contain significantly larger amounts of PUFAs which are more susceptible to lipid oxidation. The TBARS were significantly ($p < 0.05$) correlated to PUFA ($r = 0.86$) and significantly negatively correlated to SFA ($r = - 0.82$) (Table 12). This is because carbon-hydrogen double bonds are weaker when the carbon atom is next to a double bond because it delocalises electrons, and in polyunsaturated fatty acids there are even weaker methylene-interrupted carbon atoms with double bonds on either side (McClements & Decker, 2008).

Water and ethanol extracts of tempeh (Chang et al., 2009) and isoflavone extracts of soy bean press cake (by-product of oil extraction) (Kao & Chen, 2006) have been demonstrated to have an inhibitory effect on TBARS however this is obviously not enough to completely inhibit formation of lipid oxidation in burger patties. Other studies which involve adding PUFA sources to meat patties protected these with added antioxidants (Lowder & Osburn, 2010; Martinez et al., 2009).

TBARS values showed an inverse correlation with H* values ($r = - 0.52$) and a weak correlation with the 630/580 nm ratio ($r = 0.61$) (Table 12) indicating that about 52.0% of the browning measured by the H* parameter and 61.0% of browning measured by the 630/580 nm ratio can be explained by lipid oxidation as measured by TBARS.

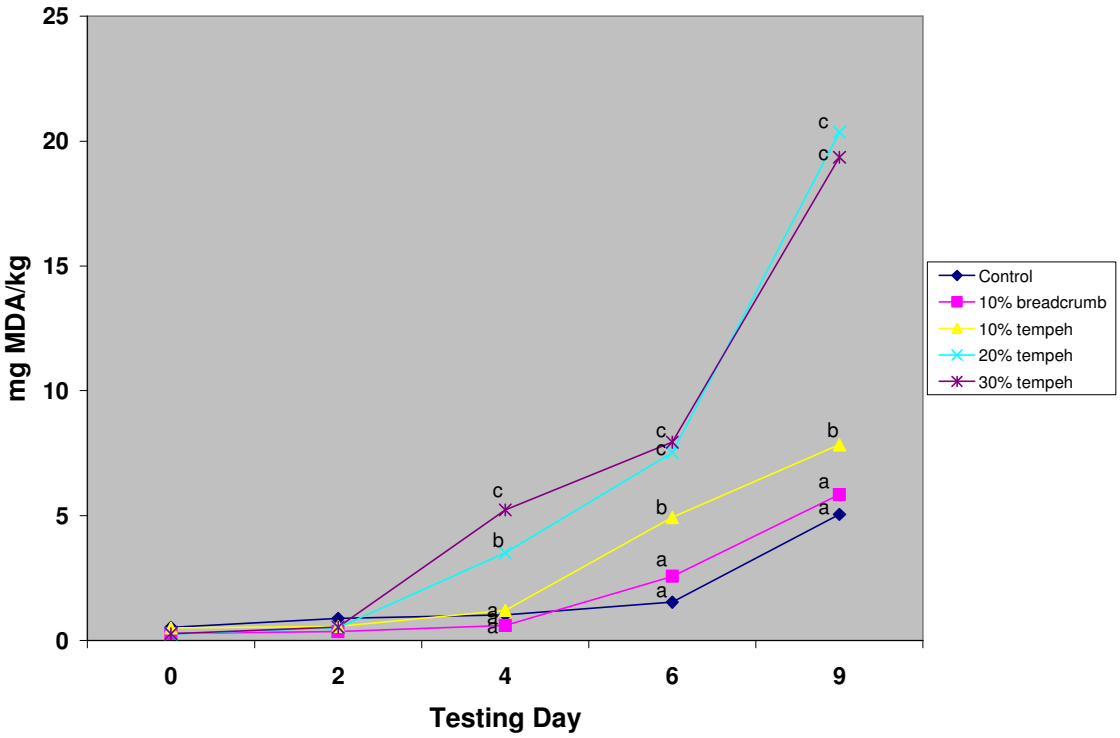

Figure 27: Least square means of thiobarbituric acid reactive substances (TBARS) expressed as mg MDA/kg meat values of five burger patty treatments over a 9 day storage period. Different letters a-c denote significant ($p < 0.05$) differences between treatments

Table 12: Pearson correlations of 12 physical and chemical attributes.

	Monounsaturated	Saturated	Polyunsaturated	Omega 3	Omega 6	S/P	P/S	TBARS	a*	H*	630-580 nm
Saturated	0.95***										
Polyunsaturated	- 0.98***	-0.98***									
Omega 3	0.43	0.60*	-0.45								
Omega 6	-0.98***	-0.98***	0.10*	-0.50							
S/P	0.90***	0.83***	-0.91*	0.11	-0.90***						
P/S	-0.98***	-0.99***	0.10*	-0.52*	0.10***	-0.88***					
TBARS	-0.86***	-0.82***	0.86*	-0.23	0.85***	- 0.87***	0.85***				
a*	-0.11	-0.04	0.15	0.36	0.08	-0.17	0.08	0.08			
H*	0.69	0.71*	-0.68	0.46	-0.69***	0.48	-0.72***	-0.52*	-0.90***		
630-580 nm	0.36	0.37	-0.37	0.28	-0.38	0.33	-0.37	-0.39	0.85***	-0.67***	
630/580 nm	-0.70*	-0.80***	0.72*	-0.57*	0.74***	-0.48	0.77***	0.61*	0.98***	-0.86***	0.91***

Significant correlations are indicated with an asterisk (*) at p = 0.05, (**) at p = 0.01 and (***) at p = 0.001

4.3.5 Focus Group:

A focus group is an interview based on a set of predetermined open ended questions which aims to generate discussion amongst participants to gain insights into consumer behaviour (Tremblay, Hevner & Berndt, 2010). It is based around a small number of issues with the aim to understand how the behaviour of individuals is influenced by their beliefs, attitudes and feelings (Tremblay et al., 2010; Rabiee, 2004).

Section 1: Consumption of takeaways

Most focus group participants consumed takeaways at least once a week and the younger university students generally consumed takeaways more often. The proximity of the takeaway outlet and the price seemed to be the most important factors for increased consumption of takeaways.

Younger adult participants have been reported to be more frequent consumers of takeaways than older adults, possibly due to a more positive perception of convenience foods (Hunter & Worsley, 2009). Full time workers consumed takeaways twice as often as non full time workers (Hunter & Worsley, 2009) although this was not the case in this focus group study.

Two of the second focus group consumers said that they normally buy burgers at fast food outlets. Although in the groups there was a majority of fast food hamburger consumers amongst some of the participants in both groups there was a definite preference for the homemade burgers " *they just taste nicer normally, home made patties and stuff* '- P2G1; "*But, I love homemade hamburgers the best*" – P9G2. One of the reasons mentioned for this is that it was a "*pretty easy meal to prepare*" - P2G1 and "*quite filling as well*" - P1G1. In the second focus group the reasons for making homemade were that "*it tastes way better*" - P10G2. There was a noticeable lack of trust in fast food outlets for some of the young female consumers. Reasons given for lack of trust were "*because I know whats inside*" - P15G2 as the participant studied Human Nutrition papers and had a knowledge of some ingredients used in beef patties and their nutrient contents. Another did not trust that burgers were made from the ingredients that they were claimed to be made from*" with mince you know it is mince…rather than I don't know like in chicken burgers and you are like is it actually chicken"* – P10G2. For one

participant it was previous work in the fast food industry which influenced their beliefs "*You know, how long the meat sits there*" - P9G2.

Female consumers preferred to eat burgers from the higher quality takeaway outlets and one stated *"... willing to pay a bit more for a really good burger"*- P4G1.

For the desirable attributes of the hamburgers younger females placed more emphasis on health whilst the male consumers did not. One male participant said "*I don't think that healthy comes into it when I eat hamburgers personally*" - P1G1 and conversely two female consumers cited health as one of the desirable attributes for a burger. The meat content was also stated as being important " *I definitely think that all meat kind of burgers not like probably 30% meat and the rest is other things*" - P15G2.

2. Adding non meat ingredients and processed meat

The participants were overall quite sceptical about processed meat and related this to the profiteering of the producers. "*They are not adding ingredients because they want to make a consumer happy (but) because they see that they can add value to the product*" - P6G1. European consumers have also expressed views of meat processors working only for their benefit rather than that of the consumer (de Barcellos et al., 2010). Some of the consumers accept this as a way of getting lower priced meat products and are not so concerned; the younger male students in the second focus group are in this category. These products were recognised as a way of selling second grade meat which is in agreement with the perception by European consumers (de Barcellos et al., 2010) although some of the consumers accept this as part of buying cheaper meat products. Overall the preference was for non-processed meat forms and was similar to the consumers interviewed by de Barcellos et al. (2010). For the second focus group especially the word processed had negative connotations. "*I don't think I have ever bought the frozen patties because I just think they look so yuck……. like it just looks so processed* " – P9G2 and two of the consumers are influenced by growing up with home killed meat on a farm.

3. Sensory testing of the burgers

One of the panellists was very familiar with tempeh whilst most were not and used a variety of words to describe the flavour that was foreign to them. It was described as "*vegetabley*" - P2G1, and smelling like "*warm pecans*"- P4G1 or having a "*beaniness*" – P4G1.

4. The link between red meat and colon cancer.

A couple of the panellists had previously heard about a link between red meat and cancer. One was a Human Nutrition student but was more familiar with the link of heterocyclic amines and processed meats to colorectal cancer. The panellists were overall quite sceptical about this link as there were many factors reported as being linked to cancer in the media. In the two separate groups a panellist said "*everything causes cancer these days*" – P1G1 and "*but they link everything to cancer*" – P10G2. Participants generally did not care or were not willing to change consumption habits over this fact and one panellist said "*it's more dangerous to dye your hair*" – P8G2. In contrast a separate study found that beef consumers were quite health conscious although this study had a more varied age structure (van Wezemael, Verbeke, de Barcellos, Scholderer & Perez-Cueto, 2010). A study of European consumers found that a number of the consumers had concerns about beef carcinogenicity and its long term health effects (van Wezemael et al., 2010).

5. Consuming patties with antioxidant source or balancing yourself.

Participants seemed more willing to choose how to balance their diet with an antioxidant source than buy a burger patty with added antioxidant. Some participants perceived this as unnatural and said "*Normally I would rather I think something more natural*" - P14G2 and "*I don't want people chopping and changing my food*" – P11G2. Consumers react negatively towards a perceived 'interference' to food products including manipulation of beef which is perceived as 'unnatural' which may explain these answers (de Barcellos et al., 2010; van Wezemael et al., 2010). The ability to sell tempeh patties may be enhanced by the inclusion of a health claim on the packaging as these claims are able to positively influence the consumer perception of a health benefit (Bech-Larsen & Grunert, 2003).

However frequent takeaway consumers are significantly less likely to try to achieve dietary requirements of fruits and vegetables (Smith, McNaughton, Gall, Blizzard, Dwyer & Venn, 2009) which may make it more difficult to sell tempeh patties through a takeaway outlet. This was stated by the participants; "*to go to eat to McDonalds to have a healthy hamburger, it seems a bit paradoxal* " – P6G1.

Participants expressed that a potential consumer would need to be informed of the health benefits in order to be willing to purchase the product. "*If you outlined the ingredients its all like, you need to have that in it maybe, otherwise it would be like why change?* " - P8G2.

6. Consuming tempeh

There were contrasting attitudes towards consuming tempeh. Three of the participants had tried it but those who had not generally were not accepting of the description of tempeh. The description was unappealing for younger consumers not familiar with the product and elicited responses such as "*That doesn't sound good*" – P14G2 and "*that doesn't sound appealing*" – P9G2. For an idea of what tempeh was two participants asked if it was similar to tofu. Food neophobia has a negative effect on the acceptance of functional food products such as tempeh burgers (Labrecque, Doyon, Bellavance & Kolodinsky, 2006). Food neophobia was not explored in this focus group however these consumers may be more neophobic than the public in general. Food neophobia in this group could be due to a lack of exposure to novel and foreign foods.

Evaluation of the beef patties

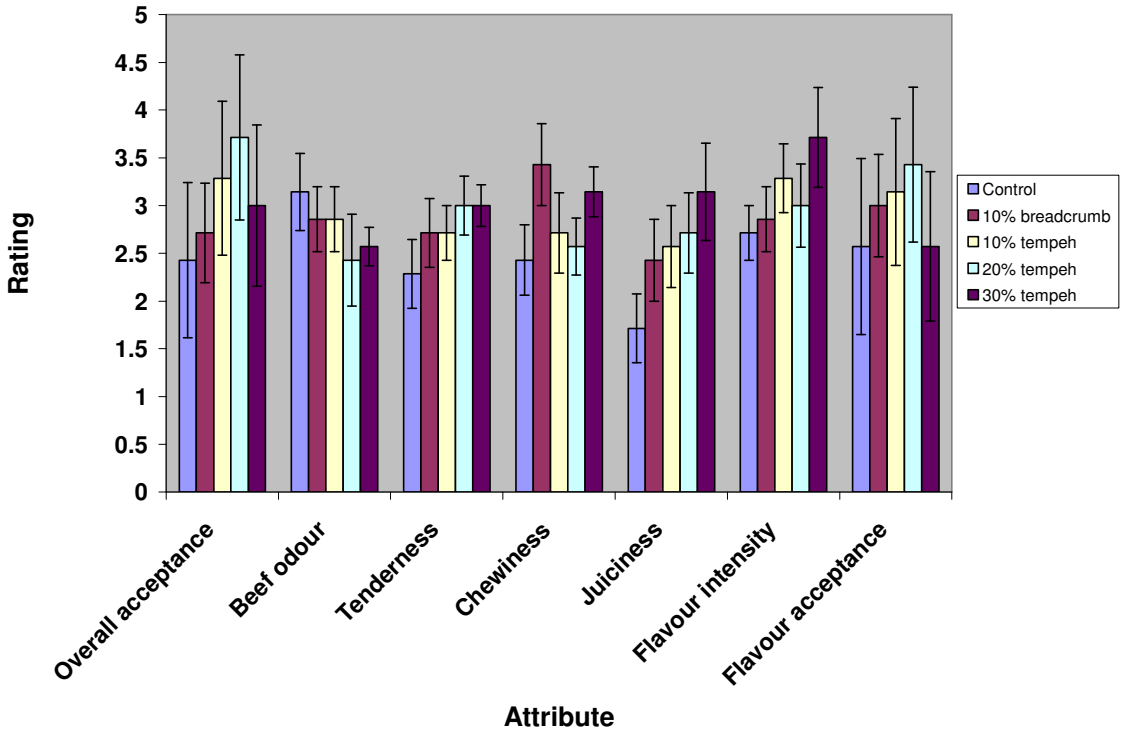

Figure 28: Participant ratings of sensory attributes of five tempeh patties during the first focus group

Overall not many differences among the evaluated attributes could be found within the data set (Figures 28 and 29). For the second focus group there were significant differences within the tenderness with 10% breadcrumb the least tender and chewy and 30% tempeh the most tender and chewy (Figure 29). The intensity of beef flavour was significantly ($p < 0.05$) higher in 10% breadcrumb and 30% tempeh patties than in 10% tempeh ones. However there was no difference ($p > 0.05$) in the intensity of beef flavour between 10% tempeh and control patties (Figures 28 and 29).

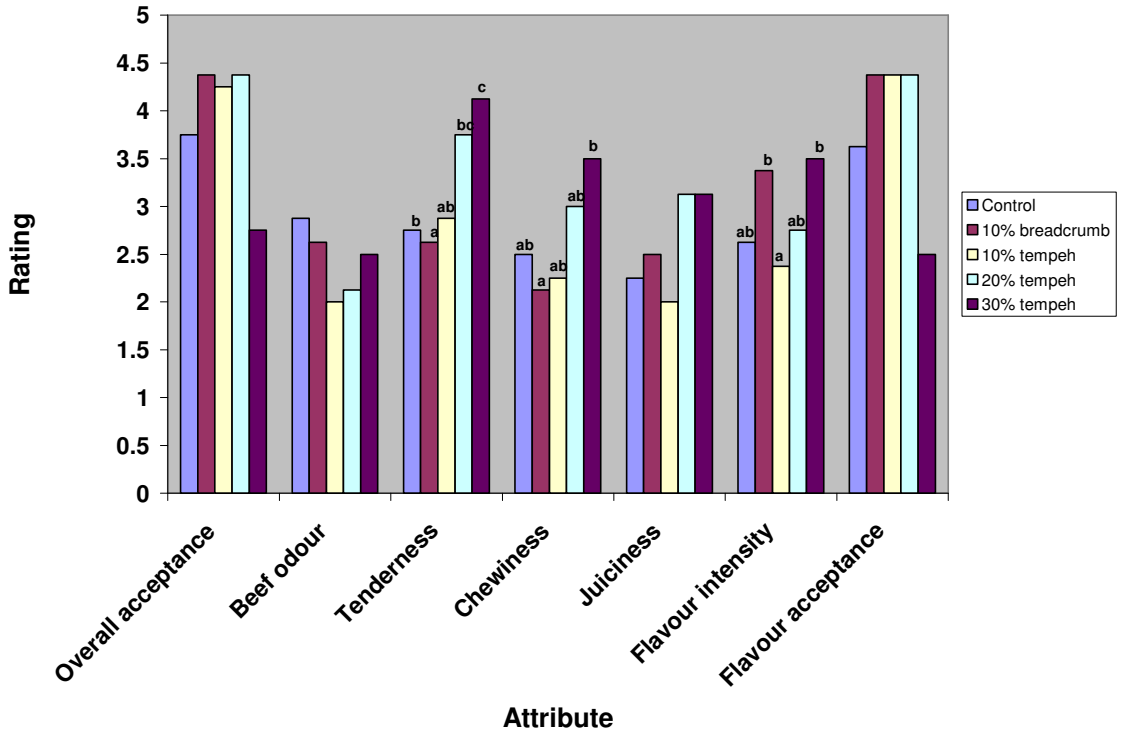

Figure 29: Participant ratings of sensory attributes of five tempeh patties during the second focus group. Letters a-c denote significant differences between treatments.

In the first focus group participants were asked to choose and rate visual attributes for one patty that they considered best among all treatments. In the second focus group the assessment of raw patties included all treatments. The 20% tempeh and 30% tempeh patties were consistently rated lower ($p < 0.05$) than all others (Figures 28 and 29). The 10% tempeh patties were not different ($p > 0.05$) from the 10% breadcrumb patties and were not significantly different from the control in colour or appearance. There is a relatively large decrease in ratings of visual attributes when increasing tempeh incorporation from 10 to 20% (Figure 30). Ratings of appearance are important as this attribute influences consumer purchase decision (Sanders et al., 1997; Troy & Kerry, 2010). Data from the second focus group suggests that temp 10% is the tempeh containing patty most likely to be purchased.

A pilot sensory study was necessary following the focus groups to provide a larger sample size to select an appropriate patty.

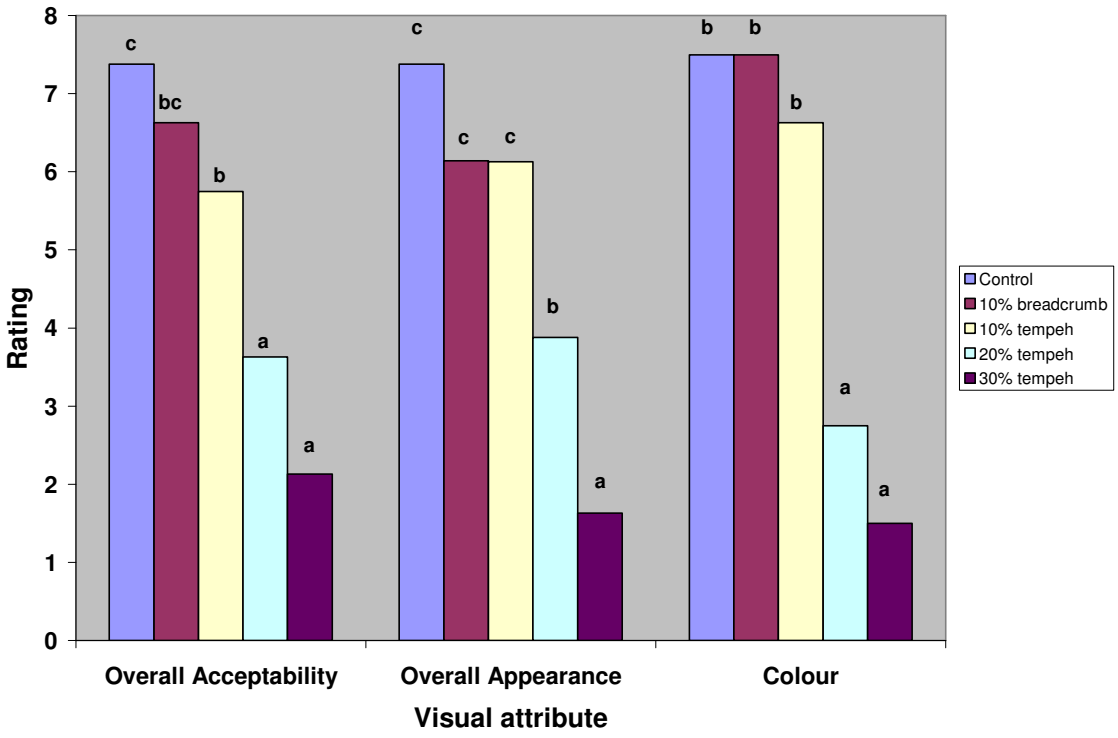

Figure 30: Participant ratings of visual attributes of five tempeh patties during the second focus group. Letters a-c denote significant differences between treatments.

4.3.6 Pilot Sensory Study

From the pilot sensory study it was observed that the 10% tempeh patties had rating scores closer to the control than 20% and 30% tempeh regardless of significance (Table 13). Most importantly it was closest in overall acceptability and there were decreased ratings with additional tempeh incorporation (Table 13). This evidence combined with the focus group quantitative data which suggested that in the sample size tested the 10% tempeh patties were most similar to the control led to the decision to take the 10% tempeh patty to the full size sensory trial. The 10% tempeh patty was more likely to be accepted and less likely to have significant differences in sensory attributes in the larger sample size of the full size sensory trial than patties containing higher levels of tempeh incorporation.

Table 13: Mean Scores and analysis of variance (ANOVA) of sensory attributes for five burger patties in the pilot sensory study. Different letters a-c indicate significant (p<0.05) differences between treatments

Attribute	Sample Type					F ratio	P value
	Control	Control + 10% breadcrumb	Control + 10% tempeh	Control + 20% tempeh	Control + 30% tempeh		
Overall Acceptability	4.21	4.21	3.93	3.5	2.93	1.73	0.155
Intensity of non meat odours	1.71a	2.57ab	2.43ab	2.57ab	3.00b	2.45	0.045
Tenderness	2.79a	4.29c	2.86ab	4.00c	3.79bc	8.6	0.000
Chewiness	2.57a	3.86b	2.36a	3.57b	3.57b	9.12	0.000
Juiciness	2.57	3.21	2.36	3.14	2.50	2.12	0.088
Flavour intensity	3.57	3.29	3.43	3.5	3.36	0.41	0.802
Non-meat flavour	1.86a	2.5ab	2.64ab	2.64ab	3.43b	3.89	0.007
Acceptance of Flavour	4.29	4.36	3.29	3.71	3.00	1.97	0.110

4.3.7 Full consumer Sensory

Differences were perceived by participants in flavour and texture sensory attributes but not for overall hedonic attributes. Mean sensory scores of participants of overall acceptability and acceptance of flavour did not significantly ($p > 0.05$) differ between the three burger treatments (Table 14).

The overall acceptability values were lower than some previous studies with other extenders for the tempeh containing patties (Angor & Al-Abdullah, 2010; Turhan et al., 2006; Yildiz-Turp & Serdaroglu, 2010). The substitution of 10% tempeh was more acceptable than substitution of

3% lemon albedo, 2% hazelnut pellicle or 9% flaxseed flour (Garcia et al., 2009; Turhan et al., 2006; Bilek & Turhan, 2009), however it was similar to the substitution of 10% okara powder, an olive oil/corn oil/fish oil blend, 0.5% carrageenan or 7.5% okara substitution did not affect the overall acceptability of beef patties (Bilek & Turhan, 2009; Martinez et al., 2009; Angor & Al-Abdullah, 2010; Turhan et al., 2006).

The acceptance of tempeh patty flavour was higher than patties extended with 4% hazelnut pellicle, or 37.5% wet okara, but were lower than patties extended with carrageen, textured soy protein, tri sodium phosphate or 10% plum puree (Turhan et al., 2005; Angor & Al Abdullah, 2010; Turhan et al., 2006; Yildiz-Turp & Serdaroglu, 2010). Partial substitution of the fat with a olive oil/corn oil/fish oil blend or 3% flaxseed flour similarly did not significantly affect the acceptability of flavour (Martinez et al., 2009; Bilek & Turhan, 2009). Decreases in flavour acceptability have been observed with substitution of 3% hazelnut pellicle, 1.5% texturised soy protein, 30% wet okara, 6% flaxseed flour and increased with addition of 10% plum puree (Turhan et al., 2005; Angor & Al-Abdullah, 2010; Turhan et al., 2006; Bilek & Turhan, 2009; Yildiz-Turp & Serdaroglu, 2010).

Similar to tempeh containing patties there were no significant differences in perception of intensity of beef odour with 15% date fibre (Hashim & Khalil, 2007).

Table 14: Mean Scores and analysis of variance (ANOVA) of sensory attributes for three burger patties. Different letters a-c indicate significant ($p < 0.05$) differences between means

Attribute	Sample Type			F ratio	P value
	Control	Control + 10% breadcrumb	Control + 10% tempeh		
Overall Acceptability	5.42	5.44	5.38	0.03	0.97
Intensity of beef odour	4.19b	3.53a	3.78a	8.66	0.00
Tenderness	3.23a	4.64c	4.14b	43.86	0.00
Chewiness	2.65a	4.05c	3.43b	38.85	0.00
Juiciness	3.66a	3.95ab	4.10b	3.22	0.04
Flavour Intensity	4.31	4.32	4.25	0.22	0.81
Non-meat flavour	2.43a	3.41b	3.11b	12.81	0.00
Acceptance of Flavour	5.62	5.60	5.42	0.44	0.64

The control was higher ($p < 0.05$) in beef odour than both 10% breadcrumb and 10% tempeh (Table 14). The inverse trend was observed for non-meat flavour as odour is a major component of overall flavour perception (Lawless & Heymann, 1999). Despite lower beef odour, 10% breadcrumb and 10% tempeh did not differ from the control in overall flavour intensity (Table 14).

The 10% breadcrumb treatment was rated most tender followed by 10% tempeh and then by control and all were significantly ($p < 0.05$) different (Table 14). Increases in tenderness have occurred with addition of 15% date fibre and 10% carbohydrate-lipid composites (Hashim & Khalil, 2007; Garzon, McKeith, Gooding, Felker, Palmquist & Brewer, 2003). Increased tenderness was also observed in instrumental texture measurements (see section 4.3.8) and was

attributed to weakening of the cohesive meat protein network with addition of tempeh. This same trend was observed for chewiness.

Juiciness was rated highest for 10% tempeh while the control was rated significantly ($p < 0.05$) lower and 10% breadcrumb did not differ significantly ($p > 0.05$) from either treatment (Table 14). Increases in juiciness with 10% substitution of carbohydrate-lipid composites or 10% tomato paste have also been reported (Garzon et al., 2003; Candogan, 2002). However, substitution of up to 30% sorghum flour did not produce any significant difference in juiciness (Huang et al., 1999; Serdaroglu et al, 2006).

Principal component analysis showed that PC1 explained 30% of the variance among the treatments and PC2 explained 23% of the variance (Figures 31 and 32). PC1 was explained by the texture attributes of chewiness, tenderness and juiciness on its positive axis. The flavour attributes of flavour intensity, acceptance of flavour and intensity of beef odour were also located on the positive axis of PC1, however unlike the texture attributes they were on the negative axis of PC2.

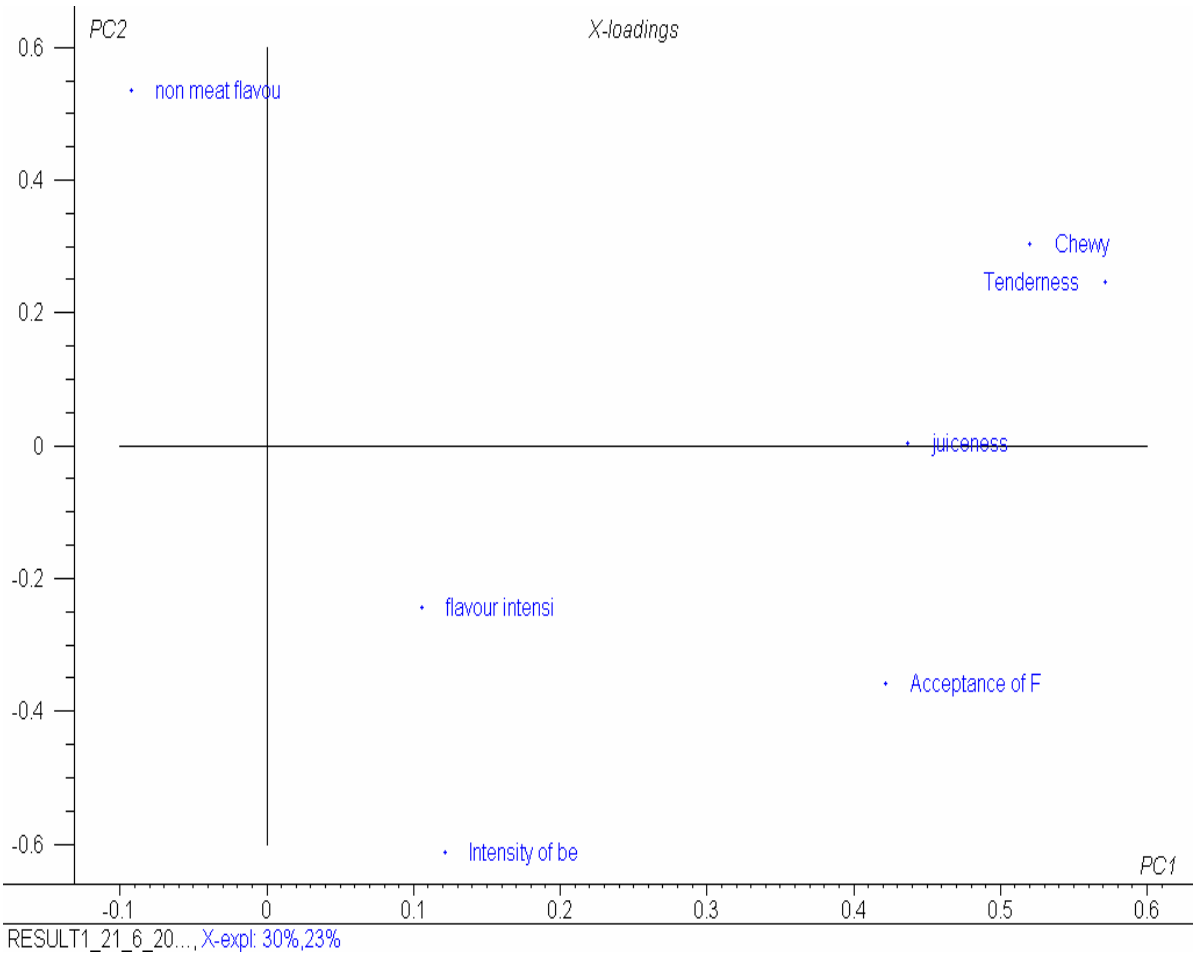

Figure 31: Principle Component Analysis of sensory attributes for three burger patties

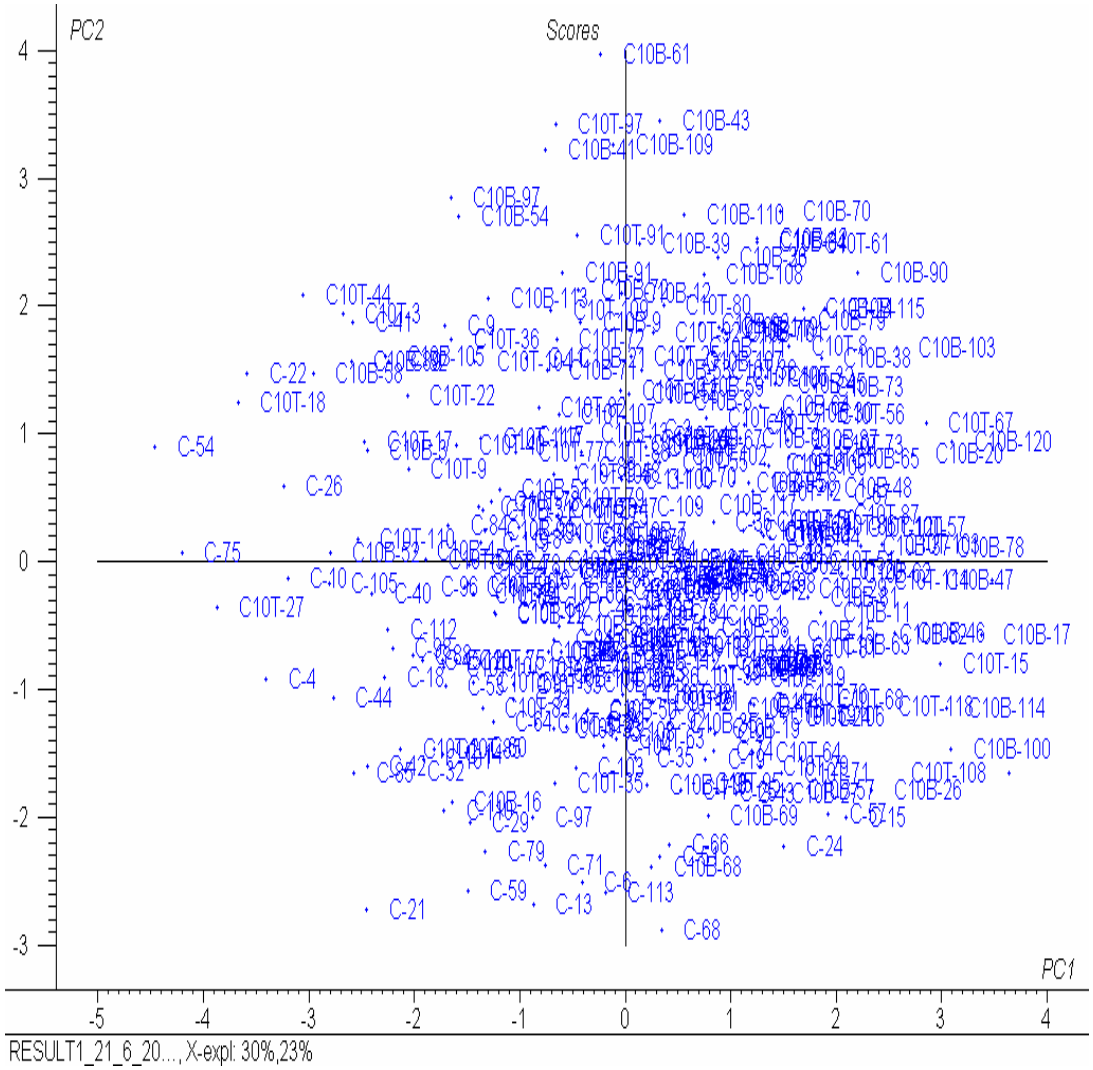

Figure 32: Principal Component Analysis of consumer sensory scores for three burger patties

Unlike many of the studies mentioned above which use trained sensory assessors (Angor & Al-Abdullah, 2010; Turhan et al., 2006; Yildiz-Turp & Serdaroglu, 2010; Bilek & Turhan, 2009; Hashim & Khalil, 2007; Garzon et al., 2003; Candogan, 2002; Huang et al., 1999) this study

used a larger untrained consumer sensory trial. To test the market potential this study used untrained consumer assessors and had a sample size was around three times the size of other studies using consumer assessors (Martinez et al., 2009; Garcia et al., 2009).

4.3.8 Textural analysis:

All patties tested varied significantly in hardness with the control patties were the hardest and 10% tempeh patties were the softest as measured by the compression test. The shear force as determined by the MIRINZ tenderometer was lower ($p < 0.05$) for 10% tempeh patties compared to the controls and 10% breadcrumb patties were not different from either (Table 15).

Addition of 7.5% lemon albedo, 6% tomato peel or 5% soy protein flour also reduced hardness of beef patties (Aleson-Carbonell et al., 2005; Garcia et al., 2009; Kassama et al., 2003). Inclusion of 10% of composites of high amylose starch, canola oil and tapioca, potato or corn maltodextrin or 5% textured soy protein did not affect hardness, (Garzon et al., 2003; Kassama et al., 2003) whilst addition of 10% waxy hulless barley increased hardness (Bond et al., 2001). Similar to the results in this study shear force was decreased by addition of 10% sorghum flour or 13.45% oat soluble fibre (Huang et al., 1999; Pinero et al., 2008).The different fatty acid profile may also contribute to the lower hardness of tempeh patties. Different fatty acids have different melting points thus incorporation of tempeh which has increased unsaturated fat content may introduce more fats which melt at lower temperatures and thus give a softer cooked texture (Wood et al., 2008).

Addition of 10% tempeh had no significant effect on the springiness of patties (Table 15). which is the same effect observed with addition of up to 7.5% raw albedo or dehydrated raw albedo and 10% addition of composites of high amylose starch, canola oil and tapioca, potato or corn maltodextrin (Aleson-Carbonell et al., 2005; Garzon et al., 2003). In other studies significant increases in springiness have been observed with addition of up to 6% dried tomato peel, 7.5% cooked and 7.5% dried cooked lemon albedo and 10% waxy hulless barley (Aleson-Carbonell et al., 2005; Garcia et al., 2009; Bond et al., 2001).

Addition of 10% tempeh significantly ($p < 0.05$) decreased the cohesiveness. Decreased cohesiveness was also observed with addition of tempeh to ham and was attributed to tempeh proteins not binding as strongly as meat proteins (Kuo et al., 1989).

Cohesiveness was increased by addition of 6% dried tomato peel, 10% waxy hulless barley or 10% addition of composites of high amylose starch, canola oil and tapioca, potato or corn maltodextrin (Garcia et al., 2009; Bond et al., 2001; Garzon et al., 2003). Addition of 5% soy protein did not significantly ($p < 0.05$) affect the cohesiveness of burger patties (Kassama et al., 2003).

Chewiness was significantly ($p < 0.05$) decreased by addition of 10% tempeh (Table 15). This is similar to the effect observed by addition of 7.5% dehydrated raw or dehydrated cooked lemon albedo (Aleson-Carbonell et al., 2005) but different to results of other studies. The addition of 7.5% raw or cooked lemon albedo had no effect on chewiness and addition of 6% dried tomato peel or 10% waxy hulless barley increased chewiness (Aleson-Carbonell et al., 2005; Garcia et al., 2009; Bond et al., 2001).

The decreased chewiness may be due to the increased water content of the patties (see proximate results section: 4.3.1) and the water binding properties of the soy proteins in tempeh (Singh et al., 2008).

Table 15: Textural parameters of Control, 10% breadcrumb and 10% tempeh cooked burger patties. Letters a-c denote significant ($p < 0.05$) differences between treatments

Treatment	Hardness (N) MIRINZ tenderometer	Hardness (N) compression	Cohesiveness	Springiness	Chewiness
Control	17.46b	85.33c	0.36c	0.75b	23.53c
10% breadcrumb	16.09ab	34.43a	0.14a	0.49a	2.55a
10% tempeh	13.64a	73.65b	0.31b	0.71b	16.23b

4.4 Conclusions:

Incorporation of tempeh was not detrimental to the nutritional profile and although protein content was decreased tempeh is still a relatively rich source of protein for a non meat

ingredient. Unsaturated fatty acids were greatly increased in the fatty acid profile and the tempeh increased amounts of fatty acids which decrease the risk of heart disease as well as decreasing the amounts of fatty acids which increase risk of heart disease.

Tempeh patties had better colour stability as it maintained a lighter colour throughout storage as measured by L* values and had lower reduction in redness as measured by a* values than the control. They were also slower to turn brown as measured by 630-580 nm and 630/580 nm indices. Therefore tempeh containing patties kept a desirable colour for longer than control patties. These indices correlated well with each other up to the addition of 20% tempeh and could therefore be used interchangeably at lower levels of tempeh addition.

The TBARS measurements were significantly ($p < 0.001$) correlated to the PUFA content ($r = 0.86$) and as the tempeh addition increased PUFA content tempeh containing patties were significantly higher in PUFA content from 2 days onwards. According to a previously determined limit of 2.0 mgMDA/kg 20% and 30% tempeh patties were unacceptable after 2 days storage, 10% tempeh patties were unacceptable after 4 days storage and control patties unacceptable after 6 days storage. Therefore tempeh addition decreased shelf life as measured by TBARS.

Information from the focus group suggested that consumers are not very concerned with a link between red meat and colon cancer, although several participants had heard of this link. They were sceptical about media reporting of cancer risk. This gives the impression that there is little potential market for a novel product such as the tempeh patty, however the participants were mainly young people who did not have a healthy diet. Quantitative data did not show great differences between the patties tested. For appearance, however, 10% tempeh patties were rated closer to control and breadcrumb patties which suggests that they are more likely to be purchased than other tempeh containing patties.

The 10% tempeh patties had better eating properties. For example, these patties were more tender, juicier and had more flavour, but they were however lower in intensity of beef odour.

Overall the 10% tempeh patty was the tempeh containing patty with the most positive attributes. Adding 10% tempeh did not diminish the nutritive value of the patty and produced a healthier fatty acid profile. This patty did not deteriorate in colour parameters as fast as the control and had values of L*, a*, b*, C* and H* which are considered more appealing

throughout storage. It was lighter, redder and slower to darken by oxidative processes as shown by the L*, H*, 630-580 and 630/580 nm parameters.

The 10% tempeh patty oxidised faster than the control due to its higher proportion of polyunsaturated fatty acids, however this could be overcome with addition of an antioxidant, use of vacuum packaging/modified atmosphere or storage in frozen form. It was not significantly different than a control patty for overall acceptance and acceptability of flavour and is comparable to a control for visual attributes and more acceptable visually than patties containing more tempeh. For these reasons the 10% tempeh patty can be considered to have market potential.

In addition to the information already gathered, an important objective of this study was to investigate whether there are any health benefits of adding tempeh to the beef patty during a simulated digestion.

Chapter 5: Effect of extender addition on *in vitro* oxidative processes of beef patties

5.1 Introduction:

In the stomach densely packed gastric glands containing several types of epithelial cells secrete the gastric juice (Okamoto, Karvar & Forte, 2006). Chief cells and mucous neck cells secrete pepsinogen which is the zymogen (pre-active form) of the digestive enzyme pepsin. The parietal cells make up 50-60% of the mass of the secretary gland and are large acid producing cells (Okamoto et al., 2006). These cells are pyramid shaped and contain many tubular and vesicular membrane invaginations. The most abundant protein in this group of membranes is the H^+/K^+-ATPase which is the main gastric proton pump. This pump uses the hydrolysis of ATP to exchange K^+ for H^+ (Okamoto et al., 2006). It releases H_3O^+ to a concentration of 160 mM and absorbs K^+ to the cytosol at a concentration of 140 mM (Sachs, Shin, Briving, Wallmark & Hersey, 1995). When the stomach is maximally stimulated it secretes hydrochloric acid at a concentration of 150 mM, which is nearly isotonic (Okamoto et al., 2006).

In some pathologies there is an over secretion of acid and these can be treated with a class of drugs called proton pump inhibitors (PPIs) which have been very successful in clinical application (Okamoto et al., 2006). The commonly prescribed compounds are omeprazole, lansoprazole and pantoprazole which have the common structure of 2-pyridyl-methylsulfinyl benzimidazole (Sachs et al., 1995; Wolfe & Sachs, 2000; Biswas, Bandyopadhyay, Chattopadhyay, Varadaraj, Esahak & Bannerjee, 2003) which is highly membrane permeable (Okamoto et al., 2006). The weakly basic properties of these compounds are exploited to target the canicular spaces of the stomach (Sachs et al., 1995; Okamoto et al., 2006). The N of the pyridine has a pKa of around 4.0 and the N of the benzimidazole has a pKa of around 2.0 and are both protonatable (Sachs et al., 1995). In the acidic secretary spaces which have a pH around 1 the compounds are protonated and accumulate to a theoretical level of more than a thousand fold (Sachs et al., 1995; Okamoto et al., 2006). They are then converted to thiophilic sulfenamide or sulfinic acid in an acid catalysed reaction in the canilicular space or on the

surface of the H^+/K^+-ATPase (Wolfe & Sachs, 2000). These reactive compounds form disulfide bonds with the cysteine groups on the external surface of the H^+/K^+-ATPase which faces the lumen of the parietal cell secretory space covalently inhibiting it (Wolfe & Sachs, 2000).

The medicinal properties of these compounds are at least partially due to their free radical scavenging abilities (Becker et al., 2006; Biswas et al., 2003; Kedika, Souza & Spechler, 2009; Natale et al., 2004; Pozzoli et al., 2007; Simon, Sturm, Hartmann & Weser, 2006). The PPIs omeprazole and lansoprazole increase expression of heme-oxygenase-1 which catalyses the breakdown of haem to bilirubin, carbon dioxide and iron (Becker et al., 2006). At physiological plasma concentrations bilirubin exerts a strong antioxidant effect (Becker et al., 2006). Also the PPIs omeprazole and pantoprazole have been demonstrated to scavenge hydroxyl radicals (Lapenna, de Gioia, Ciofani, Festi & Cuccurullo, 1996; Biswas et al., 2003; Simon et al., 2006). Reactive oxygen species are involved in the pathogenesis of some gastric disorders (Suzuki et al., 1996, Das, Bandyopadhyay, Bhattacharjee & Banerjee, 1997; Naya, Pereboom, Ortego, Alda & Lanas, 1997, Dvorak, Fass, Dekel, Payne, Chavarria, Dvorakova et al., 2006) and increased lipid peroxidation has been observed experimentally in gastric ulcers (Das & Bannerjee, 1993).

Reactive oxygen species are able to cause cell death by either necrosis or apoptosis (Bandyopadhyay, Das & Banerjee, 1999). Necrosis is cell death occurring in an uncontrolled manner when cell constituents can be released through the ruptured cell membrane (Alberts, Bray, Lewis, Raff, Roberts & Watson, 1983). Apoptosis is a controlled form of cell death which occurs by the cell and its nucleus shrinking and then being phagocytosed by macrophages or other cells (Alberts et al., 1983). Necrosis and lipid peroxidation of membranes can release transition metal ion catalysts (such as iron and copper), lysosomal enzymes and other cellular constituents leading to increased oxidation and cell damage (Naya et al., 1997; Bandyopadhyay et al., 1999). The hydroxyl radicals, generated through Haber–Weiss and Fenton reactions, can also diminish cellular antioxidants such as glutathione and oxidise important cellular constituents such as structural and functional proteins (Das et al., 1997). Thus the gastric fluid of a patient normally taking PPI medication would be expected to be a more pro-oxidative medium than gastric fluid of a healthy individual. The current experiment was designed to investigate the oxidative processes (as indicated by TBARS) during the digestion of beef patties by gastric fluid from healthy individuals and patients on PPI

medication. Furthermore the effect of inclusion of tempeh on the oxidative processes was investigated.

5.2 Methods:

Gastric fluid collection:

The collection of gastric fluid was approved by the combined Otago District Health Board and Dunedin School of Medicine, Research Advisory Group (LRS/09/08/033). Gastric fluid was collected from twenty eight patients during their scheduled endoscopy appointments at the Dunedin Hospital gastroenterology ward. Of these participants eight were taking proton pump inhibitor (PPI) medication prior to the endoscopy. All participants read an information sheet (Appendix 23) and signed a consent form (Appendix 24) before the fluid was collected. The gastric fluids were filled into sterile biological sample containers, stored on ice and later frozen at -80°C until analysis (Figures 33 and 34).

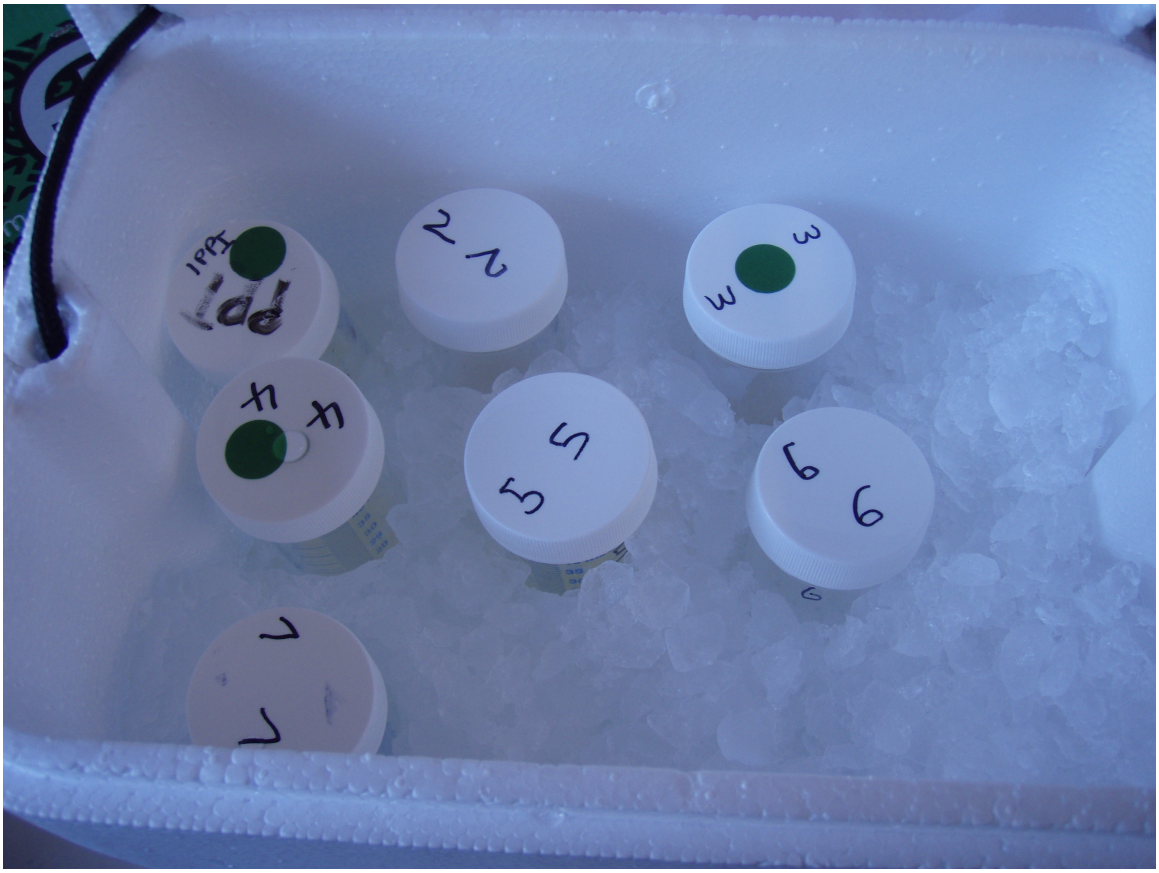

Figure 33: Gastric fluid samples in ice chilled while transported from Dunedin hospital to Department of Food Science

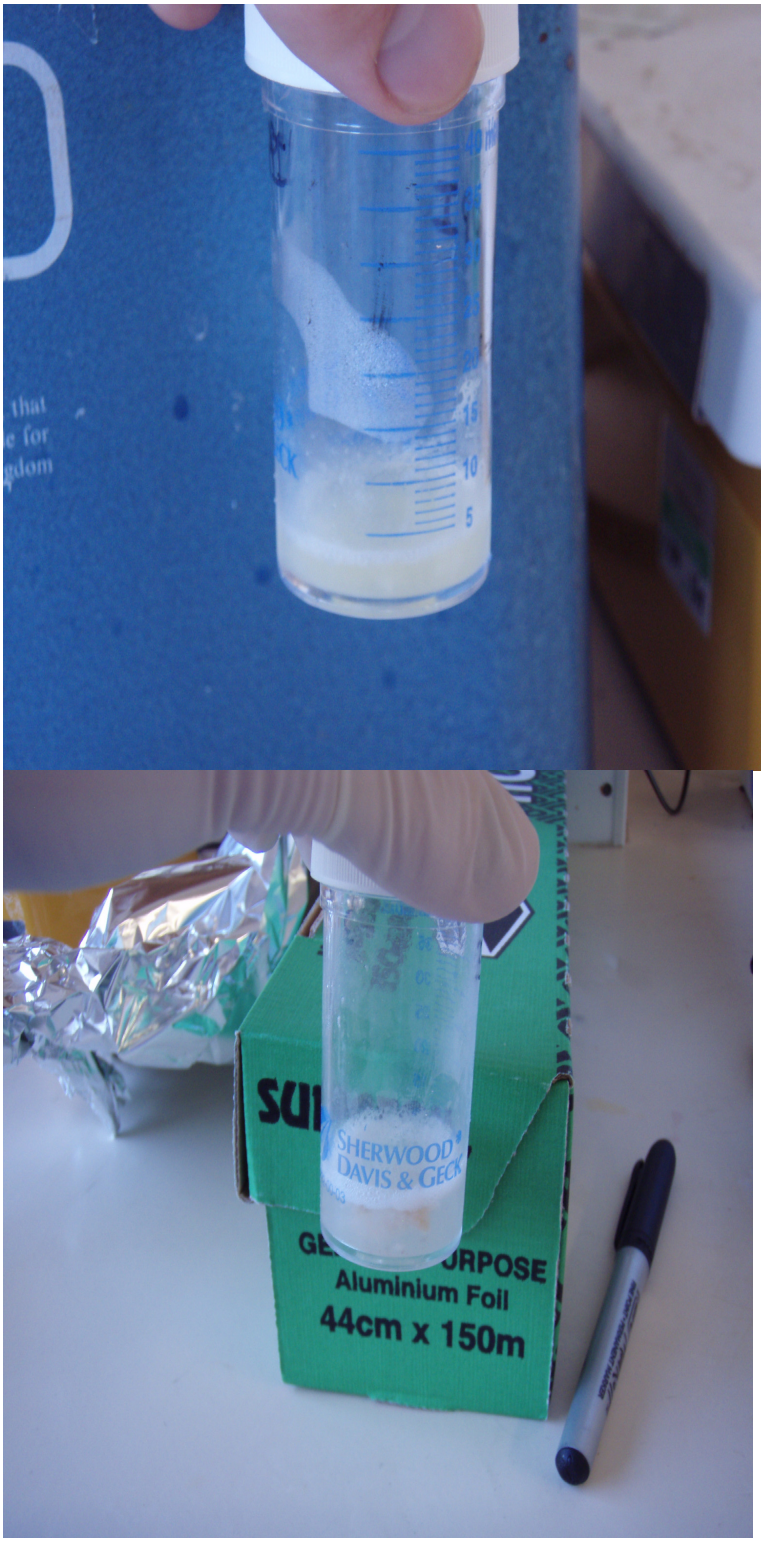

Figure 34: Two individual gastric fluid samples shown above

Sample Preparation:

Burger patties of the treatments Control, 10% breadcrumb and 10% tempeh were prepared, subjected to simulated retail display as described in Section 4.2 for 6 days at 4°C and cooked as described earlier (Section 4.2). Samples (One each of control, 10% breadcrumb and 10% tempeh were obtained from the displayed patties at 1 and 6 days of display time. Burger patties were cut into thin pieces and were snap frozen in liquid nitrogen. The frozen pieces were pulverized with a mortar and pestle with the aid of liquid nitrogen, filled into 50 ml Falcon tubes, flushed with nitrogen gas and frozen at -80°C until analysis.

Gastric digestion:

Burger patty powders (0.33g) from different samples (Control, 10% breadcrumb and 10% tempeh) were individually weighed into glass kimax tubes. One ml of gastric fluid was added into tubes containing each of the three treatments to obtain 1:3 w/v sample to gastric fluid ratio. Burger patty samples from day 1 and day 6 had gastric fluid samples from individual patients added to them as described in Table 16. The tubes were wrapped in tin foil and shaken at 37°C for 180 min in an orbital shaker incubator (Ratek Platform mixer, RM2, Boronia, Victoria, Australia) set at 180 rpm. After shaking the digested samples were immediately placed on ice. Distilled water (up to nine mls) was added to dilute the samples to a level suitable for TBARS analysis.

Table 16: Day of testing and gastric fluid type of the samples tested for TBARS after in vitro gastric digestion

	Number of samples	
Testing Day	**Healthy**	**PPI**
Day 0	10	3
Day 6	11	4

2-Thiobarbituric acid reactive substances (TBARS):

One ml of the gastric fluid homogenate was added in duplicate into fifteen ml falcon tubes. The TBARS assay was then performed as described previously (section 4.2). Results were expressed as milligrams of malondialdehyde/100g of meat (mg MDA/100g meat).

5.3 Results and Discussion:

5.3.1 Effect of display time on TBARS

The overall mean TBARS values from the simulated gastric digestion of patties stored for six days was significantly higher ($p < 0.05$) than that of fresh (day 1) patties (Figure 35). The day 6 patties would contain more MDA initially as this is produced in stored meat over time as well as a higher content of lipid hydroperoxides, hydrogen peroxide and other species which could be oxidised during digestion (Kanner et al., 1988; Harel & Kanner, 1985 a and b; Rhee, Ziprin & Ordonez, 1987; Gatellier et al., 1995; Lorrain, Dangles, Genot & Dufour, 2010; Kanner & Lapidot, 2001). The mincing of the meat disrupts cell membranes which releases cytosolic contents and exposes new surfaces to oxygen contact increasing the lipid oxidation (Harel & Kanner, 1985a).

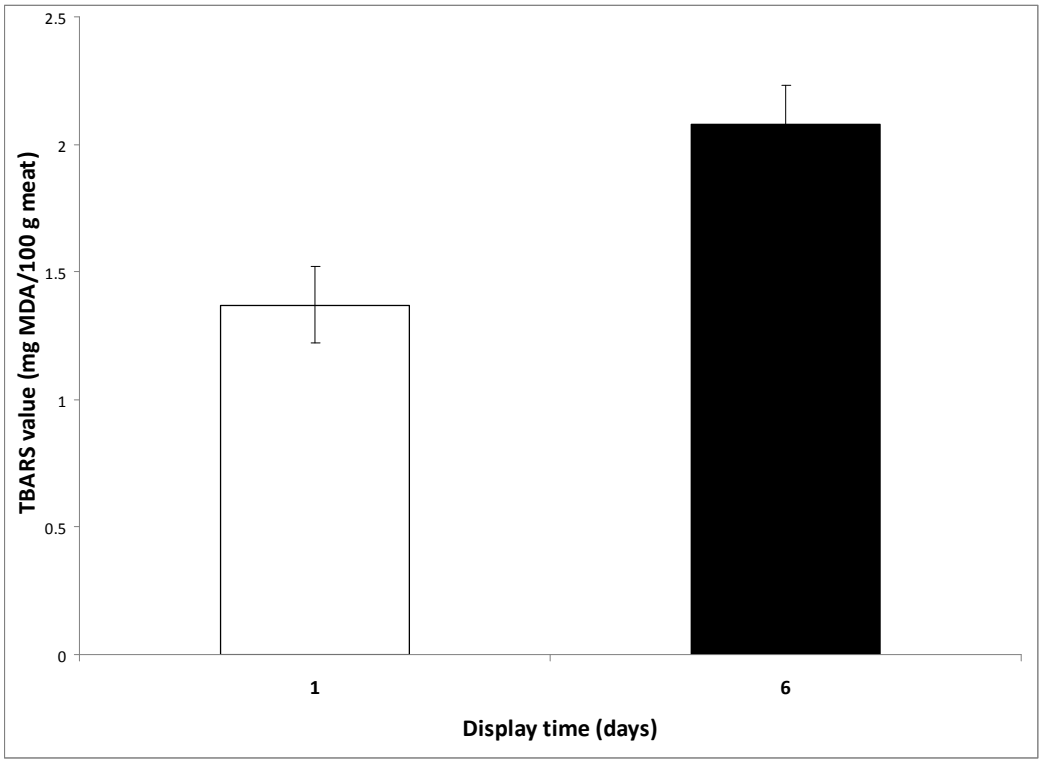

Figure 35: Least square means of thiobarbituric acid reactive substances (TBARS) expressed as mg malondialdehyde (MDA)/ 100g meat for all treatments on testing in beef patties samples across the treatments outlined in section 5.2 at days 1 and 6 of display at 4°C. Error bars are the standard error of the difference (SED).

The lipid oxidation can be initiated and catalysed by free iron ions which are released during storage by post-mortem biochemical changes (Kanner et al., 1988) and also during cooking and digestion of myoglobin (Gorelik & Kanner, 2001). Iron is not soluble above pH 3 and unable to take part in reactions unless it is chelated in the form of a soluble ligand (Kanner et al., 1988). For this reason the stomach may be the site of Fenton reactions between iron and lipid hydroperoxides (Das et al., 1997). However, myoglobin rather than free iron was observed to be responsible for lipid oxidation in cooked meat (Kristensen & Andersen, 1997) and in a model system of pepsin proteolysed myoglobin (Carlsen & Skibsted, 2004). As this experiment contains heated meat, low pH and possibly pepsin it is likely that myoglobin induced lipid oxidation is the mechanism responsible for lipid oxidation.

Myoglobin induced lipid oxidation has mainly been studied in model systems such as linoleic acid (Baron et al., 1997) and phospholipids (Maiorino et al., 1994). More recently, studies have focused on the stomach as a bioreactor for the interaction of many food constituents including myoglobin and lipids in model systems of simulated and human gastric fluid mixtures (Kanner and Lapidot, 2001, Gorelik et al., 2005, Gorelik et al., 2007, Gorelik et al., 2008a; Gorelik et al., 2008b; Lapidot et al., 2005a; Lapidot et al., 2005b). The present study may be the first to measure lipid oxidation in gastric fluid containing a novel processed food product with functional components.

There is more than one mechanism by which myoglobin can promote oxidation and the generation of hydroperoxides. Separate mechanisms (mentioned below) have been determined for myoglobin induced peroxidation of linoleic acid (Kanner and Lapidot, 2001; Lapidot et al., 2005b; Reeder & Wilson, 1998) and of phospholipids (Kanner and Lapidot, 2001; Lapidot et al., 2005b; Maiorino et al., 1994; Kanner & Harel, 1985). The burger patties contain both these types of lipids as phospholipids are present in the cell membranes of the meat (Maiorino et al., 1994) and the tempeh patties contain linoleic acid as a significant amount of their PUFA profile (see section 4.3.2).

The mechanism for metmyoglobin catalysed peroxidation of linoleic acid relevant to gastric conditions (equations 1 and 2) was suggested by Kanner and Lapidot (2001) and Lapidot et al. (2005b) is based on the work of Reeder and Wilson (1998) and Walters, Kennedy & Jones (1983).

$$MbFe^{3+} + LOOH \rightarrow MbFe^{4+}\text{-}OH + LO^{\cdot} \quad (1)$$

$$MbFe^{4+} + LOOH \rightarrow MbFe^{3+} + LOO^{\cdot} + H_2O \quad (2)$$

Kanner and Lapidot (2001) and Lapidot et al. (2005b) suggested similar mechanisms (equations 3 and 4) as the pathway for myoglobin induced peroxidation of phospholipids in the gastric environment. The suggested mechanism was based on the work of Maiorino et al. (1994). In this reaction ferryl myoglobin is not generated.

$$MbFe^{3+} + LOOH \rightarrow MbFe^{2+} + LOO^{\cdot} + H+ \quad (3)$$

$$MbFe^{2+} + LOOH \rightarrow MbFe^{3+} + LO^{\cdot} + OH^{-} \quad (4)$$

5.3.2 Effect of addition of extenders on TBARS

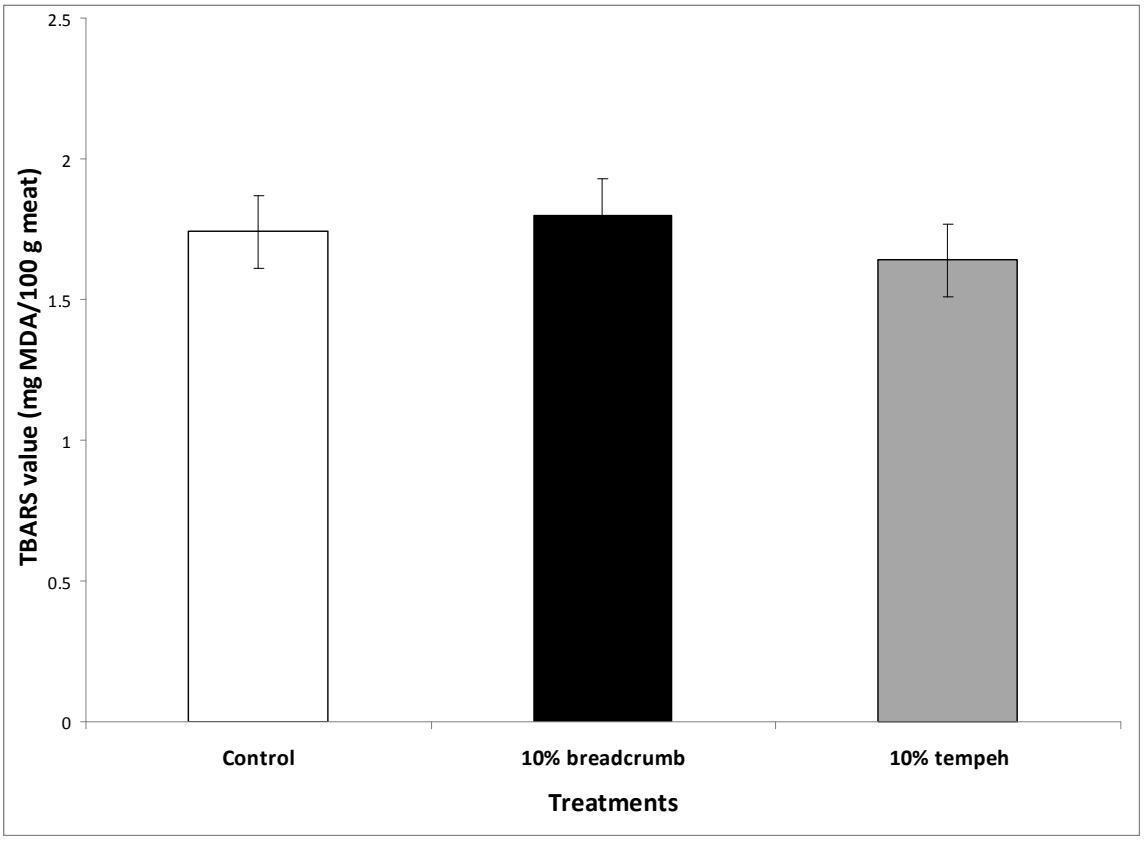

Figure 36: Overall main effects of addition of breadcrumb (10% w/w) and tempeh (10% w/w) on mean values of thiobarbituric acid reactive substances (TBARS) expressed as mg malondialdehyde MDA/kg in beef patties. Error bars are the standard error of the difference (SED).

There were no significant ($p < 0.05$) differences in TBARS between the three treatments tested after digestion (Figure 36). The mean values for TBARS in Figure 36 include samples from day 1 and day 6 display time.

On day 1 and day 6 none of the treatments are significantly ($p > 0.05$) different in TBARS. The 10% tempeh patties digested at day 6 are not significantly different from patties digested at display day 1 whilst the other treatments at day 6 are significantly higher (Figure 37).

For patties digested on day 1 this was expected as the treatments have similar TBARS (Section 4.3.2) on day 1, however the 10% tempeh patties had significantly higher TBARS at day 6 (Section 4.3.2). The 10% tempeh patties would be expected to have a significantly ($p < 0.05$)

higher TBARS than the other treatments. They have a higher PUFA content (section 4.3.2) and had TBARS values two and three fold higher than 10% breadcrumb and control patties at 6 days of storage (section 4.3.4). As the 10% tempeh patties are not significantly higher in TBARS than control and 10% breadcrumb treatments during *in vitro* digestion it is likely that the tempeh provides some antioxidant effect.

On day 1 and day 6 none of the treatments are significantly ($p > 0.05$) different in TBARS (Figure 37). The 10% tempeh patties digested at day 6 are not significantly different from patties digested at display day 1 whilst the other treatments at day 6 are significantly different.

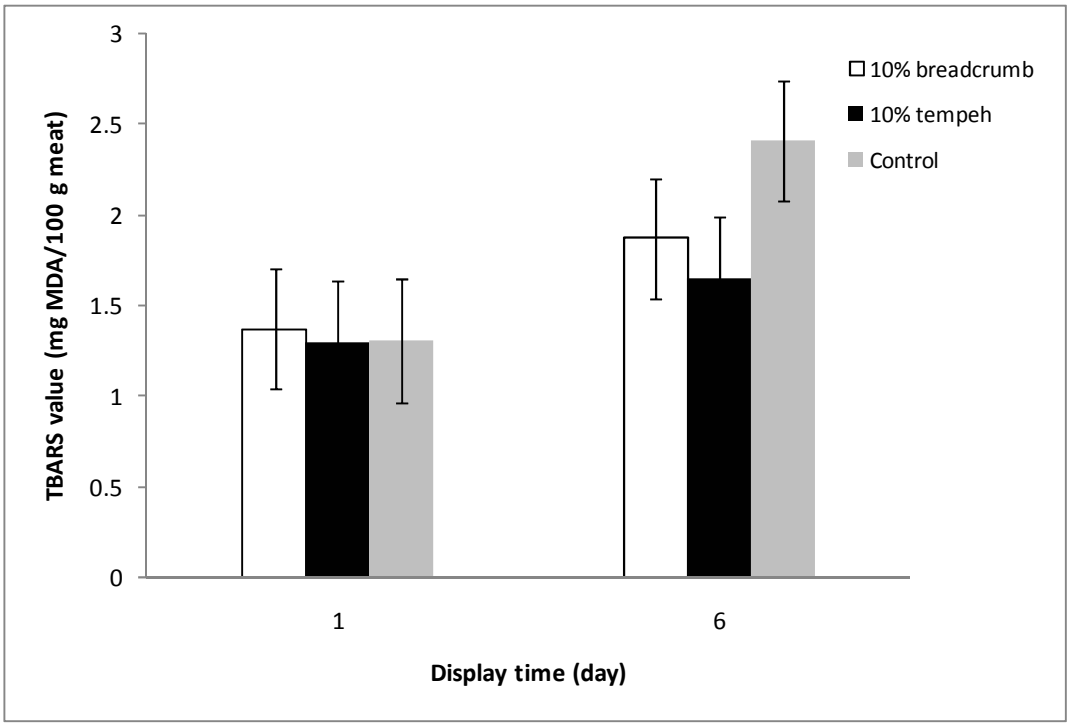

Figure 37: Effect of the addition of breadcrumb (10% w/w) and tempeh (10% w/w) on mean values of thiobarbituric acid reactive substances (TBARS) expressed as mg malondialdehyde MDA/kg in beef patties at days 1 and 6 of display at 4°C. Error bars are the standard error of the difference (SED).

The cause of this antioxidant effect may be due to the proteolysis of the tempeh in the model digestion as proteolysis of soy protein has been found to produce peptides with antioxidant activity (Fan et al., 2009; Park et al., 2010). Okara protein hydrolysates were demonstrated to

increase in radical scavenging activity (measured by ABTS assay) as degree of hydrolysis increased during *in vitro* digestion (Jimenez-Escrig, Alaiz, Vioque & Ruperez, 2009). This antioxidative effect occurs during *in vitro* digestion of seafood proteins (Sannaveerappa, Westlund, Sandberg & Undeland, 2007; Jensen, Abrahamsen, Maehre & Elvevoll, 2009). The *in vitro* digestion of saithe and shrimp was found to increase the antioxidative capacity as measured by ORAC due to the effect of proteolysis (Jensen et al., 2009). This effect also increased antioxidative capacity due to ORAC and inhibition of LDL oxidation in herring press juice (aqueous fraction) during in vitro digestion (Sannaveerappa et al., 2007).

The ORAC activity of the tempeh may also contribute to this effect. The lipid peroxyl ($LOO^{\bullet}$) radical is formed during myoglobin induced lipid peroxidation (Kanner & Lapidot, 2001; Lapidot et al., 2005b; Maiorino et al., 1994; Reeder & Wilson, 1998; Walters et al., 1983) and the tempeh in this study had relatively high ORAC (Section 3.3. 3). As the lipid peroxyl radical could oxidise more fatty acids the scavenging of this radical may also explain why the TBARS were lower than expected (Carlsen et al., 2005).

A significant reduction in TBARS over 180 minutes of simulated digestion occurs in the presence of dietary melanoidins incubated with turkey meat compared to a turkey meat control in the absence of melanoidins (Verzelloni, Tagliazucchi & Conte, 2010; Tagliazucchi, Verzelloni & Conte, 2010). Melanoidins from coffee, barley coffee or dark beer (Tagliazucchi et al., 2010) or from balsamic vinegar (Verzelloni et al., 2010) reduced the TBARS significantly during simulated digestion of turkey meat.

Addition of 0.5% chitosan, cellulose or pectin to beef patties significantly ($p<0.05$) reduced lipid oxidation by TBARS (Hur et al., 2009).

Phenolic compounds appear to play an important role in the inhibition of lipid oxidation by haem iron. A hydrophilic extract of caper containing 70μM GAE was able to inhibit TBARS formation and a 180 μM GAE extract was able to decrease the amount of ferryl myoglobin by reducing some to metmyoglobin (Tesoriere, Butera, Gentile & Livrea, 2007). In a model digestive system containing simulated or human gastric fluid (Gorelik et al., 2005) or during *in vivo* digestion for 90 minutes in a rat stomach (Gorelik et al., 2008a) consumption of red wine polyphenols with turkey meat lowered TBARS formation.

Although the *in vitro* digestion research previously mentioned has identified compounds with an antioxidant effect it is unlikely that these could be successfully incorporated into a burger patty without detrimental sensory changes. Further research could be done to investigate if lipid oxidation can be significantly reduced in the 10% tempeh patty, possibly with addition of a small amount of a synergistic antioxidant.

5.3.3 Effect of medical condition on TBARS

Patties for all treatments were higher than those in Section 4.3.4 as they are cooked and digested *in vitro* both of which increase lipid peroxidation (note than in section 4.3.4 the units are mgMDA/kg).

The TBARS in the model digestion for patients normally taking PPI medication was higher than those that did not normally take PPI medication (Figure 38). These patients produce more radical species as a result of their conditions and are not receiving the antioxidant effect provided by PPIs as they stopped using the medication for four weeks prior to their gastroscopies (Becker et al., 2006; Biswas et al., 2003; Kedika et al., 2009; Natale et al., 2004; Pozzoli et al., 2007; Simon et al., 2006).

In the stomach iron from meat is expected to increase TBARS by catalysing lipid peroxidation (Gorelik et al., 2005; Gorelik et al., 2008a).

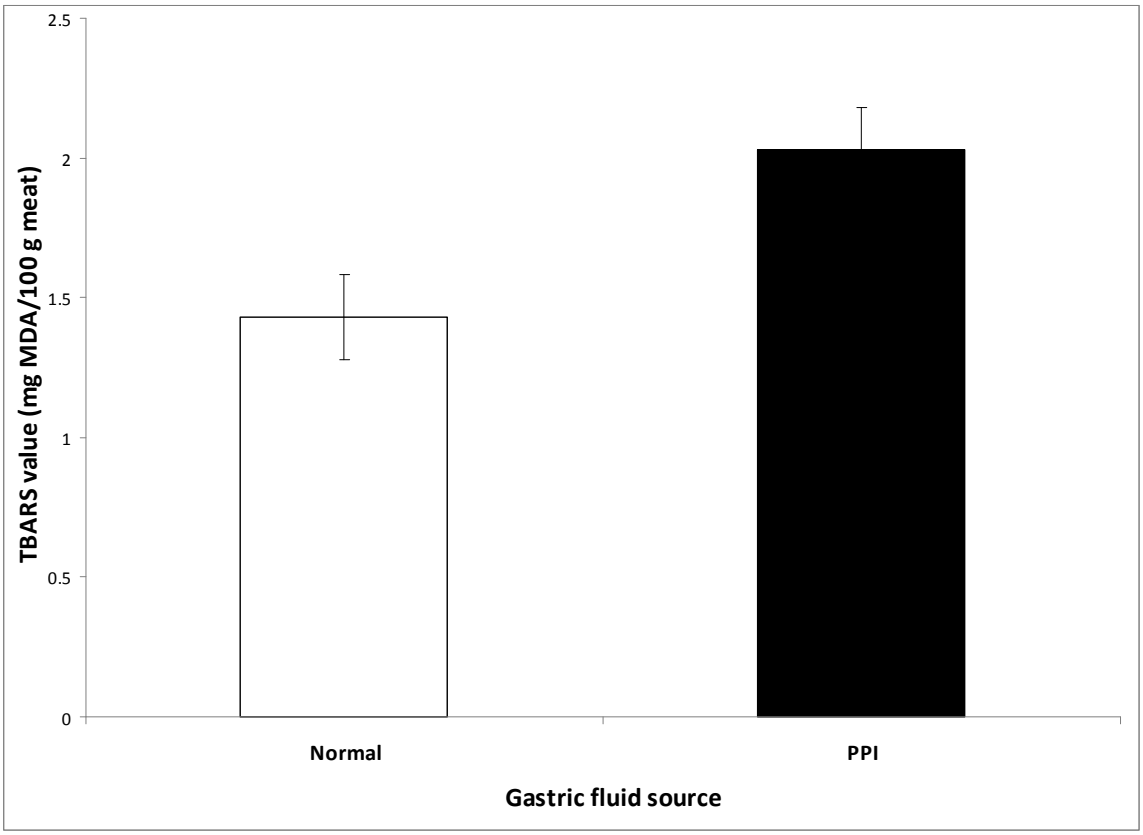

Figure 38: Effect of gastric fluid from healthy individuals and PPI patients on mean values of thiobarbituric acid reactive substances (TBARS) expressed as mg malondialdehyde MDA/100g in beef patties samples

The superoxide anion is involved in this pathogenesis as increased superoxide dismutase (SOD) activity has been observed in stress induced ulcers (Das & Banerjee, 1993; Das et al., 1997) and SOD was able to reduce oesophageal mucosal injury caused by acidified pepsin in a rabbit model (Naya et al., 1997). Increased SOD activity and the accompanied decrease in gastric peroxidase activity lead to the accumulation of hydrogen peroxide in the stomach (Das et al., 1997). This hydrogen peroxide can react with superoxide in a transition metal catalysed Haber –Weiss reaction to generate hydroxyl radicals (Naya et al., 1997). It can also react with ferrous (Fe^{2+}) iron to generate hydroxyl radicals in the Fenton reaction (Das & Bannerjee, 1993; Das et al., 1997; Bandyopadhyay et al., 1999).

The hydroxyl radical is the major cause of oxidative damage in gastric ulcers (Das et al., 1997) although it probably plays a smaller role in acid and pepsin induced damage in oesphagitis (Naya et al., 1997). The generation of this radical increases mucosal damage in several ways;

The hydroxyl radical is able to inactivate the gastric peroxidase enzyme especially when copper ions are present leading to the accumulation of more hydroxyl radicals and hydrogen peroxide (Das & Banerjee, 1993; Das et al., 1997; Das, Bandyopadhyay & Banerjee, 1998). The superoxide radicals do not take part in gastric peroxidase deactivation (Das et al., 1998).
Overall PPI patients had significantly higher ($p < 0.05$) TBARS than patients not on PPIs (Figure 38).

At day 1 there is no significant ($p > 0.05$) difference between lipid oxidation in gastric fluids of healthy or PPI patients (Figure 39). At day 6 however the PPI patients have significantly ($p < 0.05$) higher TBARS (Figure 39). There is no significant difference between the gastric fluids of PPI patients for patties displayed for one day and those of healthy patients for patties displayed for 6 days.

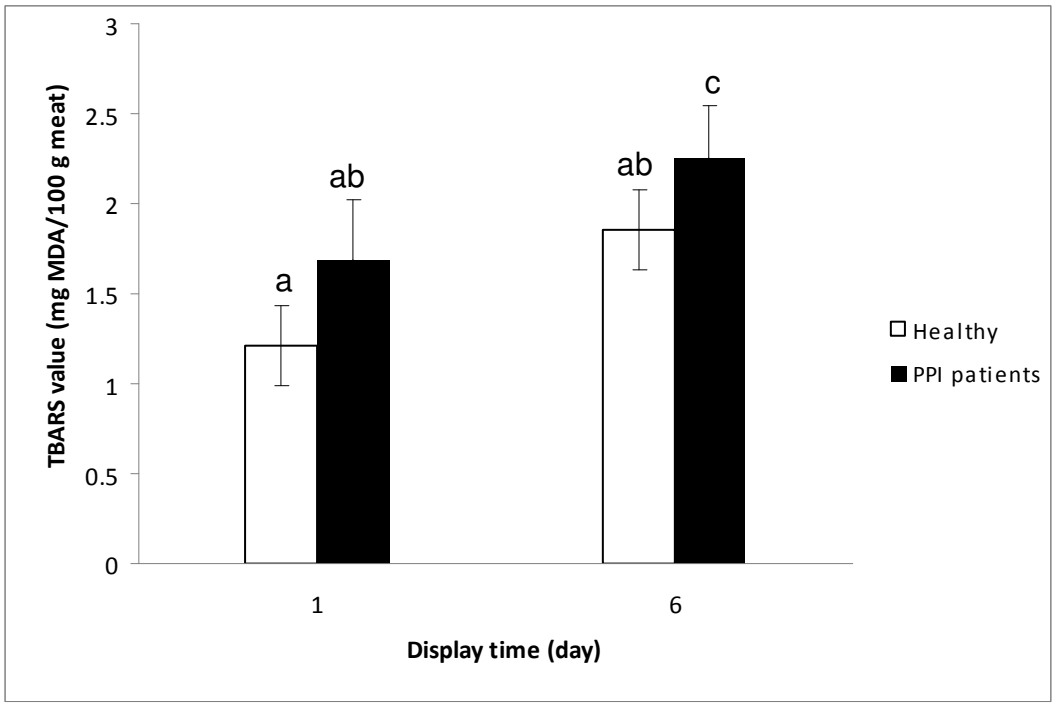

Figure 39: Effect of gastric fluid from healthy individuals and PPI patients on mean values of thiobarbituric acid reactive substances (TBARS) expressed as mg malondialdehyde MDA/100g in beef patties samples across the treatments outlined in section 5.2 at days 1 and 6 of display at 4°C. Error bars are the standard error of the difference (SED). Letters a-c denote significant differences between samples.

For the treatments there were no significant differences between healthy and PPI patients for TBARS with the exception of 10% breadcrumb which was significantly ($p < 0.05$) higher than the treatments (Figure 40). The lack of significant differences may be due to the large differences between display days which were pooled for the analysis. When compared against display day there were more significant ($p < 0.05$) differences (Figure 37).

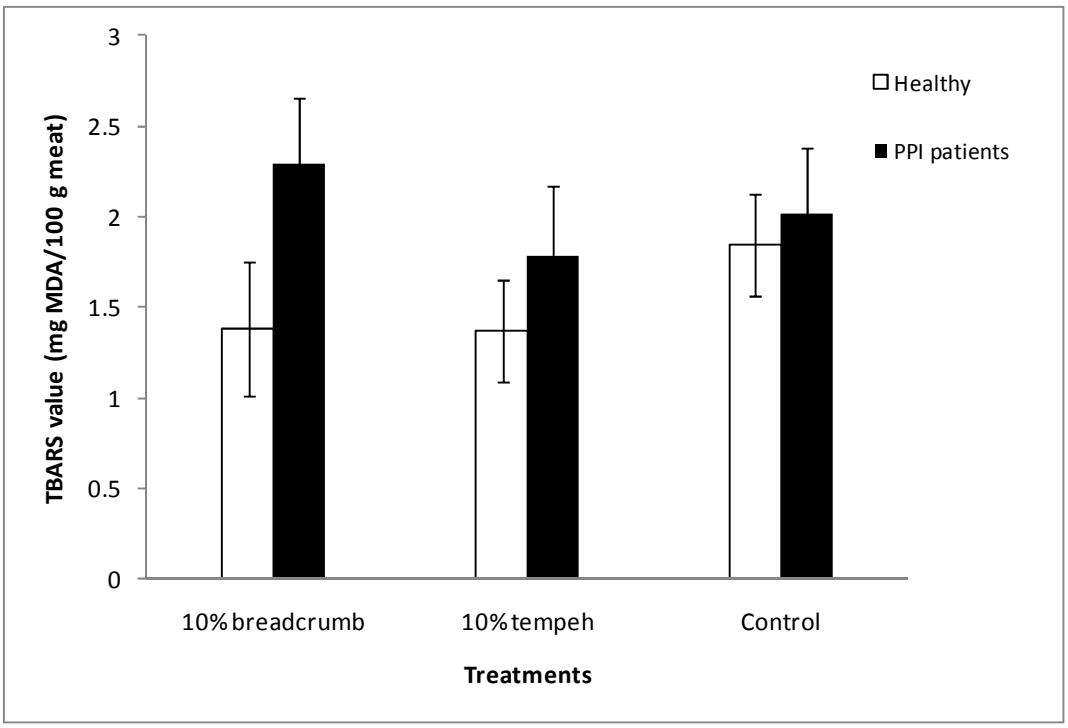

Figure 40: Effects of addition of breadcrumb (10% w/w) and tempeh (10% w/w) and gastric fluid on mean values of thiobarbituric acid reactive substances (TBARS) expressed as mg malondialdehyde MDA/kg in beef patties. Error bars are the standard error of the difference (SED).

As mentioned above, in the stomachs of unhealthy patients (which require PPI medication) it would be expected that there is an accumulation of hydrogen peroxide, hydroxyl radicals and catalytic transition metal ions (Das & Banerjee, 1993; Das et al., 1997; Das et al., 1998). Hydrogen peroxide is a substrate for myoglobin catalysed lipid peroxidation and participates in a reaction to form the ferryl myoglobin species (Baron and Anderson, 2002; Lapidot et al., 2005b; Reeder & Wilson, 2001; Vulcain, Goupy, Caris-Veyrat & Dangles 2005; Baron, Skibsted and Andersen, 2002; Kelman, DeGray & Mason, 1994). The activation of hydrogen

peroxide produces ferryl myoglobin by converting the Fe(III) centre of metmyoglobin to an oxo-ferryl complex (FeIV=O) and forms a protein radical on a tyrosine or a tryptophan radical by a one electron oxidation (Vulcain et al., 2005). The ferryl myoglobin could react with lipid hydroperoxides in the digested meat to produce the peroxyl radical, which in turn could produce more lipid oxidation (Reeder & Wilson, 2001; Kanner & Lapidot, 2001; Lapidot et al., 2005; Reeder & Wilson, 1998). The hydroxyl radicals and hydrogen peroxide could react to produce peroxyl radicals and increase lipid peroxidation further (Choe & Min, 2010).

5.4 Conclusions:

During digestion the stomach acts as a bioreactor where many oxidative and antioxidative components react under conditions of low pH. This oxidation is greater in patties stored for 6 days as these contain more lipid oxidation products.

There were no significant ($p < 0.05$) differences in TBARS between the treatments control, control + 10% breadcrumbs or control + 10% tempeh after digestion. The tempeh patties contain a high PUFA content and because of the content of linoleic acid and phospholipids are expected to release alkoxyl and peroxyl radicals when cooked and digested *in vitro*. The fact that tempeh patties did not have significantly higher TBARS suggests there may be an antioxidant effect due to proteolysed soy protein or the peroxyl radical scavenging activity of tempeh.

Beef patties digested in gastric fluid of patients normally taking PPI medication had significantly higher gastric TBARS than the healthy subjects. These patients have more pro-oxidative gastric fluid due to their medical conditions and did not receive the antioxidant effect provided by PPIs at the time of testing. The patients were expected to have accumulated hydrogen peroxide, peroxyl radicals and transition metal ions in their gastric fluid which would promote myoglobin induced lipid peroxidation.

Although some antioxidant effect of the tempeh was observed at the level of 10% there is not a decrease in TBARS compared to the two other treatments studied.

Addition of a synergistic antioxidant to the tempeh patties may be able to reduce the lipid oxidation further during gastric digestion.

Chapter 6: Conclusions

6.1 General Conclusions

Red meat consumption has been linked with the incidence of colorectal cancer. Meat contains iron which exists both as "free" ions and in the haem form as well as a significant content of fat. Therefore, significant catalysts and substrate for lipid peroxidation are found in meat and consumed together. This oxidation is expected to be greater in the pro-oxidative environment of the stomach which is regarded as a bioreactor.

The addition of antioxidant in the form of tempeh in beef patties was investigated as a potential solution to alleviate this problem. Soy and azuki beans (dehulled and hulled) were tested for total phenolic content, DPPH RSA and ORAC. Soybean had higher TPC than azuki tempeh, lower DPPH and similar ORAC. The tempeh produced from azuki beans was visually unappealing and much more labour intensive to produce. Thus it was decided to incorporate soy tempeh (the most commonly found at a commercial scale) into beef patties.

Addition of tempeh to beef patties produces a variety of physical, chemical and sensory changes and it was necessary to find an acceptable level of tempeh addition. Adding tempeh to the beef patties increased the water and carbohydrate contents. It had no significant ($p < 0.05$) effect on fat or ash contents but decreased protein content. The addition of tempeh did not produce a detrimental effect on the nutritional properties of the patties.

The fatty acid profile was significantly changed with the addition of tempeh. The PUFA content was increased mainly due to linoleic acid (18:2 n-6) which made up more than half the fatty acid composition of tempeh. Amounts of fatty acids associated with risk of coronary heart disease such as 12:0, 14:0, 16:0 and 18:0 were reduced (up to 35%, 36%, 19% and 26% respectively) with addition of tempeh. For these reasons tempeh addition improved the beef patties by reducing amounts of fatty acids which increase risk of heart disease and increase those which promote cardiovascular health, creating a healthier burger.

Tempeh containing patties generally had higher L*, a* and b* values over the storage period tested. Tempeh patties were not significantly different from control un-treated patties in C* or H* values but were higher in 630-580 and 630/580 nm ratios during storage indicating that they were slower to develop brown colour.

The higher PUFA content of tempeh patties (added at levels of 10, 20 and 30% of the patties) made them more susceptible to lipid peroxidation. There were no significant differences up to 2 days of storage, however after this time 20% and 30% tempeh patties were higher and after 4 days 10% tempeh patties were significantly higher. The 20% and 30% tempeh patties had surpassed a TBARS level of 2.0 deemed unacceptable by consumers at day 4 and 10% tempeh patties had reached this level on day 6.

A focus group study was conducted amongst predominantly students who were frequent consumers of takeaways. They often consumed processed meat and were not willing to change their consumption habits as processed meat products were cheaper. Some consumers were aware of the link between red meat and colorectal cancer, however they were sceptical about media reporting of cancer risk.

Many consumers were more willing to choose to balance their meals by consuming an antioxidant food separately to the burger patty. However several agreed that a health claim on the packaging would change this. The sensory results from two focus groups showed that the 10% tempeh patties were generally rated closer to the control than 20% and 30% tempeh patties.

A pilot sensory study was also carried out with the aim of choosing an acceptable level of tempeh for full size consumer sensory study and to aid in the design of this study. The general trend in this study was that the 10% tempeh patties were rated closest to the control.

Overall 10% inclusion of tempeh seemed to be the optimal amount that could be incorporated into a beef patty to produce maximum nutritional benefit without any negative effects in terms of sensory or keeping qualities. The 10% tempeh patty retained a lighter and redder colour than the control throughout storage and was slower to brown as measured by the 630-580 and 630/580 nm ratios. This patty also had a significantly improved fatty acid profile compared to the control patties. Although it had a reduced shelf life due to having higher TBARS than the control after 4 days storage its TBARS were not increased to the extent of the 20% and 30% tempeh patties.

The full size consumer sensory study was carried out with 10% tempeh patties compared to control and 10% breadcrumb patties. Under the conditions of the full sensory study tempeh was not significantly different in overall acceptability, flavour intensity or acceptance of flavour. This was important as these are determinants of consumer acceptance and for hedonic attributes tested there were no difference between tempeh patties and the conventional control.

The 10% tempeh patties were significantly ($p < 0.05$) softer than the control when measured by either compression or shear force. They were also less chewy and cohesive but not significantly ($p < 0.05$) different in springiness to the control.

The type of tempeh and level of addition had been chosen with the resulting product indicates that this approach of generating healthy beef patties could have commercial potential. It was important to investigate the antioxidant effect of the added tempeh (as a source of antioxidant) when it is consumed together with beef (pro-oxidant). Therefore, an *in vitro* digestion trial using human gastric fluid was conducted. The 10% tempeh patties stored for 6 days and digested *in vitro* were not significantly different from the patties digested on day 1, whilst 10% breadcrumb and control patties were on day 6. The patients normally prescribed PPI medication did not have different TBARS when consuming tempeh patties to those not prescribed this medication. As tempeh had a higher PUFA content and was expected to increase in lipid peroxidation when heated and digested *in vitro* to a higher extent than the control patties it was concluded that there was an antioxidant effect occurring during the digestion. The results are favourable to the development and introduction of a novel beef patty product containing 10% tempeh for consumers.

6.2 Limitations and recommendations for future research

The research undertaken involved many different experiments ranging from tests of antioxidant activity to a variety of chemical, physical and sensory tests to testing extent of lipid oxidation during *in vitro* digestion. Although this research contained topics which have been individually investigated before (antioxidant properties of beans, effect of vegetal extender addition on beef patties, *in vitro* digestion), the present work employed all these research skills collectively to target the production of a healthy beef patty product and it had very novel aspects for which no comparison in the literature could be found. Stemming from the content of this thesis is the potential to conduct more experiments and collect more data to obtain a fuller picture of the potential to limit oxidative processes in meat by tempeh addition.

Further antioxidant testing could be done on soy and soy tempeh. There are a variety of assays available to test antioxidant activity. One such assay is the Fe^{2+} chelating ability assay which would be interesting to test soy products to observe to what extent they bind ferrous iron. This

is because ferrous iron can cause lipid peroxidation thus testing tempeh's chelating ability would be a suitable antioxidant test for the beef patty.

For the proximate analysis of tempeh it would be interesting to study the amino acid content of tempeh used in these experiments. This would demonstrate how tempeh inclusion not only changes the protein content but would likely change the amino acid profile. Some antioxidative activity of tempeh has been attributed to peptides produced during the fermentation and thus it is important to investigate whether this tempeh does produce peptides with antioxidative activity. The length and sequences of peptides produced in this tempeh would also be advantageous to study.

The difference between tempeh and other soy meat extenders is its fat content. Whilst it has a favourable fatty acid composition, defatting the tempeh may be beneficial as it could increase the shelf life which is limited by lipid oxidation of PUFA. Adding 10% defatted tempeh would also greatly increase the protein content of the patties whilst also reducing the fat content. Testing a burger like this in a sensory trial would test whether the fat had a positive or negative effect on sensory properties.

The *in vitro* digestion experiments could also be modified further to gain more insights. It appeared that the oxidation occurring was lower than expected due to some antioxidant effect. Further testing could elucidate how this effect occurred. For example the effect may be due to peptides produced during hydrolysis of tempeh or during *in vitro digestion*. Peptides purified from the tempeh could be tested for an antioxidative effect in a model stomach. Also peptides produced from the *in vitro* digestion could be tested for their antioxidative effects. Testing defatted tempeh in this medium would demonstrate to what extent the PUFA content of the tempeh negates its positive antioxidant benefits. Use of iron chelators could be used to assess the affect of iron on this oxidation and the extent to which tempeh limits the effect of iron on oxidation. Also the ORAC assay could be adapted to measure antioxidative effects of tempeh. As the peroxyl radical is generated *in vivo* during lipid oxidation and can be generated by myoglobin induced lipid oxidation it would be used to measure the contribution of this radical to the increased oxidation from digestion and the effect of tempeh on this oxidation.

6.3 Implications of the research

The research demonstrated that soy tempeh can be successfully incorporated into beef patties to create a product with market potential. Results on a variety of changes occurring with addition of tempeh have been generated providing a range of information on this topic. The research is a foundation from which there is potential to investigate many aspects of the research in more detail.

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Appendices:

Appendix 1: Table of sample volumes used in DPPH· Assay

Volumes (µl)	Samples tested at these volumes
300, 200, 100, 50, 25, 0	RKB1- 2, RB1-3,
1000, 400, 200, 100, 50, 0	RKB3, SB1, SDRB2, SSB2, RT1-3, DRT2, ST1-3
100, 400, 200, 0	SB2
1000, 300, 200, 100, 50, 0	SB3, SRB1, SDRB1, SDRB3, SSB1, SSB3
500, 300, 200, 100, 50, 0	SRB3
400, 200, 100, 50, 0	DRT1
600, 400, 200, 100, 50, 0	DRT3

Codes for samples can be found in Table 4

Appendix 2: A typical vacuum sealed eye of the round (ST) used for preparation of burger patties



Appendix 3: Kenwood Blender used for mincing beef



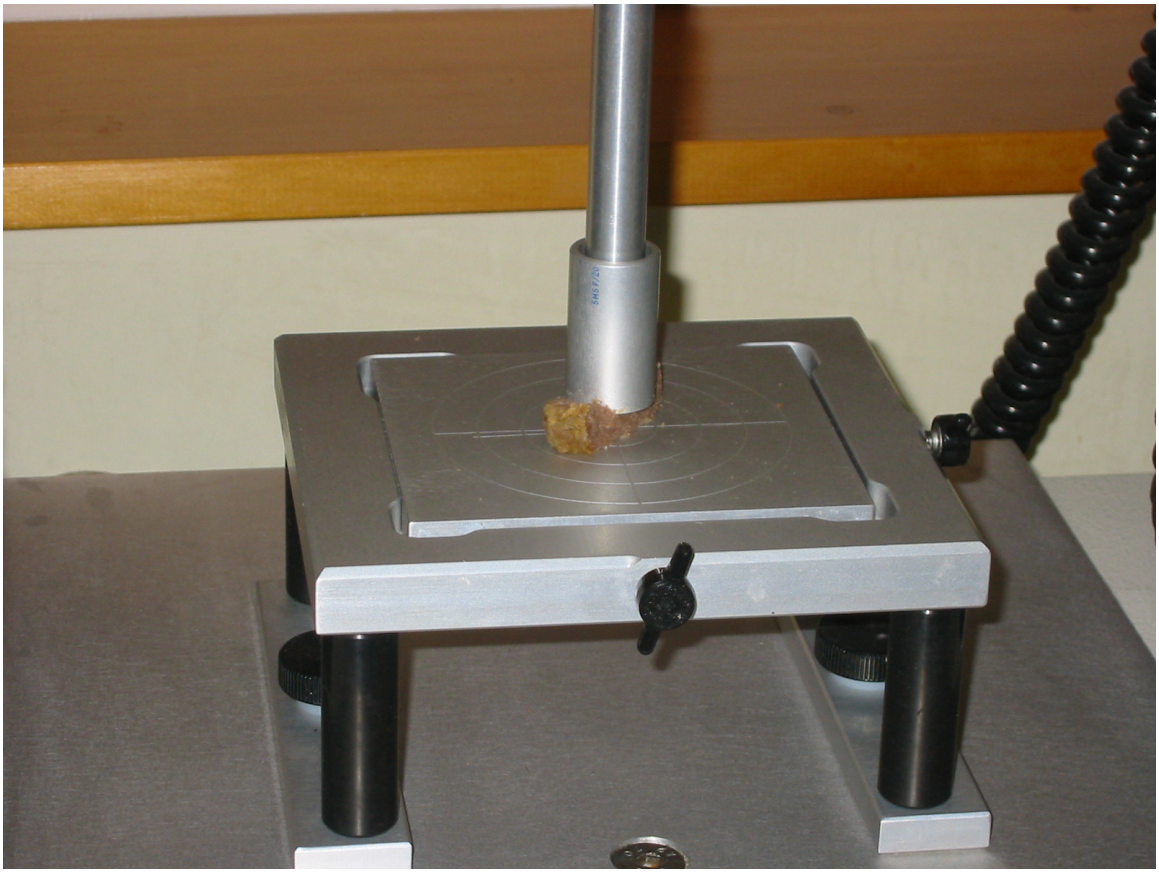
Appendix 4: Patty former used to press beef patties into shape. Shown holding five patties



Appendix 5: A 1 x 1 x 1 cm sample in the double scalpel used to cut it



Appendix 6: Sample compressed by cylindrical probe of TA Plus texture analyser during measurement



Appendix 7: Flyer used for recruitment of focus group participants



Participants needed

Do you like to eat hamburgers?

The Department of Food Science is looking for participants who normally consume hamburgers to take part in a discussion about a new burger product. Sessions will be held **Tuesday 27 October 2009** from **5:30-7:00pm**

Participants have a chance of winning a **$50 grocery voucher** which will be drawn at the end of the session

For more info:

Phone: Jordan on 03 479- 7661

Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz	Contact: Jordan Phone: 03 479- 7661 or 027 367 8679 Email: tayjo522@student.otago.ac.nz

or **email** tayjo522@student.otago.ac.nz

This project has been reviewed and approved by the University of Otago Human Ethics Committee.

Appendix 8: Information sheet for focus group participants

[Reference Number *as allocated upon approval by the Ethics Committee*]
[*Date*]

[*Burger patty sensory trial*]
INFORMATION SHEET FOR
[PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

This project is being undertaken as part of a student Master of Science degree. This project uses tempeh a fermented soy product to improve the nutritional qualities of beef patties.

What Type of Participants are being sought?

Anyone between the ages of 18-65 who normally consume beef and have no allergies to soy or gluten.

People who are in one or more of the categories listed below will not be able to participate in the project because, in the opinion of the researchers and the University of Otago Human Ethics Committee, it may involve an unacceptable risk to them:-

- People who do not consume beef or meat, people with allergies to soy or gluten

What will Participants be Asked to Do?

Should you agree to take part in this project, you will be asked to take part in a discussion based on a few questions answered by the facilitators. You will also be asked to taste burger patties and provide feedback on their flavour, texture and appearance.

Please be aware that you may decide not to take part in the project without any disadvantage to yourself of any kind.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

Data collected will be gender, age group and sensory data related to the project. There will be no way to relate the answers provided back to you. Information collected will be for the purposes of the study only, retained in secure storage for five years and disposed of after that.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:-

Jordan Taylor or Dr. Alaa El-Din Bekhit
Department of Food Science
University Telephone Number:- [*(03) 479-7661*] University Telephone Number:- [*(03)479-4994*]

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

[*Note: The above statement should <u>not</u> be included if the project has been considered and approved at departmental level]*

Appendix 9: Consent form for focus group participants

[Reference Number *as allocated upon approval by the Ethics Committee*]
[Date]

[*Burger Patty focus group*]

CONSENT FORM FOR

PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.
I know that:-

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. Personal identifying information [*audio-tapes*] will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for five years, after which they will be destroyed;

4. This project involves an open-questioning technique. The general line of questioning includes…**[How do you feel about adding a non meat ingredient to hamburgers?. Which one of these burgers is the preferred one to purchase?.]**. The precise nature of the questions which will be asked have not been determined in advance, but will depend on the way in which the interview develops. Consequently, although the University of Otago Human Ethics Committee is aware of the general areas to be explored in the interview, the Committee has not been able to review the precise questions to be used.

   In the event that the line of questioning does develop in such a way that you feel hesitant or uncomfortable you are reminded of your right to decline to answer any particular question(s) and also that you may withdraw from the project at any stage without any disadvantage to yourself of any kind.

5. During this focus group I will consume beef, soy and gluten and I do not have any objection to eating these.

6. At the end of the focus group I have the chance of winning a $50 grocery voucher which will be randomly drawn.

7. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve my anonymity. [*Note: only include the last part of this phrase if it is intended that anonymity will be preserved. For some kinds of research anonymity is inappropriate in which case this section should set out how and where the results will be published and whether it will be transferred to a public repository etc.]*

I agree to take part in this project.

....
(Signature of participant) (Date)

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Appendix 10: Focus group protocol for group moderation

27 October: Focus group

Recruitment Questions:

Question 1: Have you ever participated in a focus group before

Question 2: Are you willing to participate in a recorded discussion on this topic. The recorded data will be handled appropriately

Question 3: Do you have any ethical or religious objections to eating beef ?

Question 4: Are you allergic to gluten and/or soy?.

Question 5: Do you normally consume hamburgers?. Define normally consume as having consumed within the last 6 months.

The focus group will be divided into four parts. Prompting questions or suggestions for discussion are italicised for the moderators of the focus group.

Part 1: Introduction: (Around 5 minutes)

Introduce ourselves and meet the participants. Show the consent form and information sheet and get them to sign it. Offer drinks and snacks.
Explain what a focus group is, that the focus group will be recorded, that there are no right or wrong answers, the participants will not and cannot be contacted again. Briefly explain the

topic of my Masters thesis to a level of information that will not bias their answers for the first parts of the discussion.

Get participants to write their names down and put them somewhere to be drawn later. Ensure there is a form prepared that they can sign to accept the prize when it is drawn

Start the recording with the participants introducing themselves on tape. Serve drinks and talk a little bit.

A small questionnaire will be handed out to collect socio-demographic data. Data collected will be age, occupation

Questions: (10 minutes)

How often do you eat take aways ?

How often do you eat burgers?

What is the most important factor that they consider important for a burger?: Price, convenience, taste

Expand concept of burgers and takeaways

Where do you normally buy burgers from?

Do you normally consume burgers with vegetables such as lettuce and tomato. Probe into brand, point of purchase

Aim Q1: Determine how people feel about the addition of a non meat ingredient to a processed meat product.

Q1:

How do you feel about adding a non meat ingredient to hamburgers?

Introduction: (20 minutes)

Non meat ingredients are added to processed meat products for many reasons including adding value for the producer, improving the nutritional properties(expand this section, prepare cards),

for binding such as in hamburgers emulsifying etc. It is important to determine the attitude of the consumer if we are going to create a product which may contain as much as 30% tempeh.

Are you aware of how many non meat ingredients that you might eat in processed meat
Do you understand why a producer would do this?
Does it seem deceptive?
How do you feel about eating a meat product which is not entirely meat?
Explore how product should be made, meat vs non meat

Which one of these burgers is the preferred one to purchase?, and why?

Aim: Choose a formulation to use in sensory trials and gain information on the effects of increasing amount of tempeh on sensory properties of burger patties

Introduction: (30 minutes)
Five formulations of a burger patty have been prepared for participants to try. They will be coded and formulations served at the same time.
Attributes to assess:
Appearance: colour, volume, particulates
Flavour: meat flavour, beef flavour, foreign or unfamiliar flavours, dryness
Texture: crumbliness, firmness/tenderness

Health information: (5 minutes)

At this point provide information on potentially negative aspects of meat consumption and the nutritional benefits of tempeh. A referenced information sheet which can be taken from edited parts of the literature review will be prepared.

Link between meat and cancer, Recommendations of WHO and World Cancer research report
Positive benefits of soy and tempeh
Positive aspects of meat consumption and the need to balance with vegetable/ and or antioxidant source
The fact that a meat product often needs to be processed to improve its health benefits

Aim: To determine if information on health benefits influences the decision to purchase this type of product

Q: From the information which has been given how do you feel about the addition of the fermented soy product tempeh to hamburgers or other processed meat products (20 minutes).

Did you previously know of the link between meat and cancer
Does the link between meat and cancer concern you enough to modify your diet regarding meat?
If you were willing to change your diet would it be easier to modify it by consciously consuming more vegetables or would you prefer to purchase a product which already included other nutrients?.
How do you feel about consuming the product tempeh
How do you feel about consuming tempeh in the form of a ***burger***
Do you think it is useful to consume it in this form?
Would addition of tempeh increase your willingness to buy this product?
Would you prefer to buy a healthier burger above that which is already available?

Raw burger patty evaluation

Appendix 11: Information sheet on ingredients for processed meat extension

Much of the meat eaten is often processed in some way. Processed meat is often cheaper, makes use of cuts of meat which aren't always eaten and has different sensory properties. Producers will sometimes add ingredients to add value for the producer or consumer, extend the shelf life or improve the flavour.



Meat extenders are often incorporated into meat to improve flavour, colour or appearance. These are often ingredients such as soy protein or starches.

References:

Mills, E., 2004, Meat Additives: Extenders, In: Jensen, W.K., 2004, Encyclopedia of Meat Sciences,

Appendix 12: Response form for sensory evaluation of cooked patties

Focus group testing: Beef patties

Sex Male Female

Age range (yrs) <18 19-25 25-30 30-40 40-50 >50

Please evaluate the beef samples and answer the following questions with regard to the perceived smell, texture, flavour and acceptance.

1. **Smell**

   a. **Intensity** of beef odour

Sample Number	Not at all	Slight	Moderate	strong	Extreme

   b. **Other aromas** If you identified other aromas in the patties, what words would you use to describe them?

.....
..
.....
..
.....
..
.....
..

   c. **Indicating which of the meat samples contain these other odours and how strong they are by writing the odour in the appropriate box.**

Sample	Not at all	Slight	Moderate	strong	Extreme

number					

2. Texture

a. Tenderness (resistance of the meat to the first bite). Please bite into each meat sample and judge the force needed to bite a segment of meat of.

Sample number	Very tough	Moderately tough	Neither tough nor tender	Moderately tender	Very tender

b. Chewiness (is the resistance of the food to chewing with the molar teeth). Please put each samples between your molar (back teeth), chew and feel the resistance offered by the sample**.**

Sample number	Very chewy	Moderately chewy	Neither chewy nor soft	Moderately soft	Very soft

c. Juiciness when you chew each of these food samples, how moist (juicy) are they?

Sample number	Very dry	Moderately dry	Neither dry nor juicy	Moderately juicy	Very juicy

3. **Flavour**

   a. **Intensity of the flavour**

Sample number	Not at all	Slight	Moderate	strong	Extreme

   b. **Other flavours**

Sample number	Not at all	Slight	Moderate	strong	Extreme

   c. **If you identified other flavours in the meat, what words would you use to describe them**

.....
.....
.....

   d. **Acceptance of flavours** How do you feel about the overall flavour of these meat samples

Key:

1 Dislike strongly **2 Dislike moderately** **3 Dislike slightly** **4 Neither like or dislike**
5 Like slightly **6 Like moderately** **7 Like strongly**

Sample number	1	2	3	4	5	6	7

4. **Overall acceptance** Taking everything into account, how do you feel about these beef patties samples?

Key:

1 Dislike **2 Dislike** **3 Dislike slightly** **4 Neither like or**

strongly **moderately** **dislike**
5 Like slightly **6 Like moderately** **7 Like strongly**

Sample number	1	2	3	4	5	6	7

5. Which sample (s) do you think has (ve) been treated?.....

Thank you very much

Appendix 13: Response form for appearance evaluation of raw patties

Raw beef burgers assessement

sample No.

Overall Acceptability of the patties

Please indicate your overall acceptability.

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely — Neither like nor dislike — Like extremely

Overall appearance

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely — Neither like nor dislike — Like extremely

Colour

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely — Neither like nor dislike — Like extremely

Please indicate WHAT in particular you liked or disliked about this product.
(Please use words not sentences)

LIKED	DISLIKED
_____	_____
_____	_____
_____	_____

Evaluate the specific attributes:
Which ones you consider buying?

Comments: _____

Appendix 14: Health information for red meat and tempeh

There are many nutritional benefits gained from eating meat. Meat such as beef is rich in protein and is a good source of Bvitamins and minerals such as vitamin B12, iron and zinc (Williams, 2007, Mulvihill 2004). Although there are many health benefits gained from eating meat some studies have linked meat to negative health effects. Several studies have found a link between meat and colorectal cancer (Sinha and Rothman, 1999, Alaejos et al, 2008, Giovannuci et al, 1994).

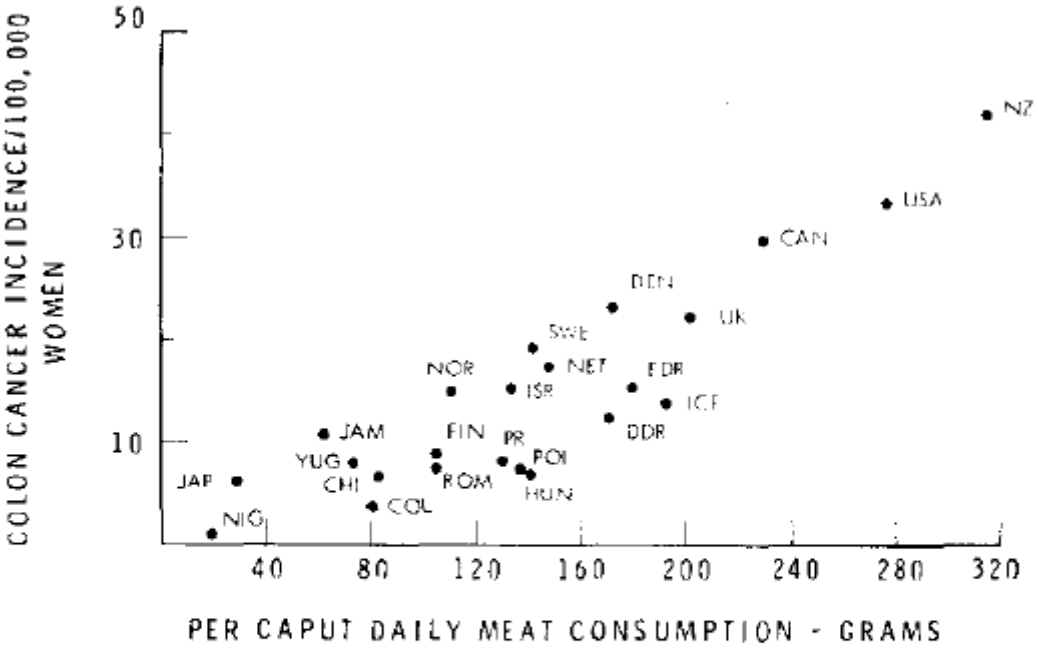


FIGURE 2

Correlation between incidence of colon cancer in women and *per caput* daily meat consumption in 23 countries.

From Armstrong and Doll, 1975

For this reason there is an interest in investigating whether consuming meat with an antioxidant could limit these negative effects. One of the few methods of improving the nutritional properties of meats is including them with a healthy ingredient in a processed meat product

Beans are very nutritious and are a good source of antioxidants. Soy is considered to be very healthy and a good source of antioxidants. Some research has linked soy to a reduced risk of cancer.

Tempeh is a soy food fermented with the mould Rhizoppus oligosporus traditionally from Indonesia. It is rich in protein and vitamins such as B12 (Nouts and Kiers, 2005). Soy products have often been used as meat replacers or meat extenders and the flavour and texture of tempeh make it a good candidate to partially replace the meat. Incorporating tempeh into a burger patty is a convenient way of improving its nutritional properties.

References:

Alaejos, M.S., Gonzalez, V. and Afonso, A.M., 2008, Exposure to heterocyclic aromatic amines from consumption of cooked red meat and its effect on human cancer risk: A review, Food Additives and Contaminants, Volume 25, Issue 1, p2-24

Armstrong, B. and Doll, R., 1975, Environmental factors and cancer incidence and mortality in different countries with special reference to dietary practices, International Journal of Cancer, Volume 15, Issue 4, p617-631

Giovannucci, E., Rimm, E.B., Stampfer, M.J., Colditz, G.A., Ascherio, A. and Willett, W.C., 1994, Intake of fat, meat and fiber in relation to risk of colon cancer in men, Cancer Research, Volume 54, Issue 9, p2390-2397

Mulvihill, B., 2004, Human Nutrition: Micronutrients in meat, In: Caballero, B., Allen, L. and Prentice, A (eds)., 2006, Encyclopedia of Human Nutrition, 2nd ed Elsevier Science, p618-623

Nouts, M.J.R and Kiers, J.L, 2005, Tempeh fermentation, innovation and functionality: Update into the third millennium, Journal of Applied Microbiology, 98, p789-905

Sinha, R. and Rothman, N., 1999, Role of well done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer, Cancer Letters, Volume 143, Issue 2, p189-194

Williams, P., 2007, Nutritional composition of red meat, Nutrition and Dietetics, Volume 64, Issue s4, ps113-119

Appendix 15: Focus Group 1 transcript

Panellistnumber	Name
P1G1	James
P2G1	Jacob
P3G1	Rachel
P4G1	Karen
P5G1	Tim
P6G1	Pippo
P7G1	Joe

Jordan:. . . . . And my project involves making a. . .a new hamburger product and the point of this focus group is one part of the research..the consumer part. And its to get you know peoples attitudes towards the product and to see how people like, like the taste and the flavour and the texture and things like that. And then that will um that will help me with writing my thesis and it also helps to plan um another part where I am going to get a lot more people in to taste the product. So I think I have a.,, there is a small summary on the information sheet so I will get you guys to read through those and then you can um sign the consent form if you are willing to participate.
1:29
P7G1: Whats the date?

Jordan:It's the 22nd of October

Aladin:Thank you

Unnamed: Thank you
1:49
Jordan: So umm during the session I will start I will just ask a few questions and hopefully it starts a discussion between everyone. And there will be two parts where you get to taste a set of five burgers, yeah. And some of them, they will all be different in some way but you have read the questions so you know some of them will contain soy and gluten and then you will end up rating them. And so the discussion should. . .there will be another set of discussion questions and we will probably finish around 7. . . . . .yep. So I am just going to start to warm up asking you guys how often do you eat takeaways..maybe going around the table.

P1G1: I eat takeaways quite a bit..I. . .this year probably not as much as I usually did, last year I used to pretty much eat them every week. In second year I was twice as bad..lived in Great King St round like McDOnalds and everything ..missing text..every single day. Um this year more um more subway more sandwiches but yeah still eat burgers at the flat quite a bit.

P2G1: Uh probably takeaways probably on average about once or once or twice a week, not sure yeah living in town there are quite a few options. Quite often intoxicated..you know..yeah.

P3G1: A couple of times a week depending on what a takeaway is. So yeah

Jordan: Oh ok, a takeaway being a food that you go and buy that's prepared that's prepared by someone else for you to eat quickly.

P3G1: So that

Jordan: Maybe not like ..missing text..

P3G1: Is that like subway? Just anything really, I mean about twice a week.

Jordan: Oh ok

P4G1: We probably at least once a week, once or twice a week.

P5G1: Yeah I would say once a week on average.
P6G1: Once a week

P7G1: Same

Aladin: Ah its very interesting what you mention that this year you tried to shift to subway what made this shift since you lived closer to McDonalds. Any reason for that?

P1G1: Well I live closer to subway now, that's why but ah

Aladin: Ok

P1G1: I am just lazy. The sub of the day as well you know its $3.90, a sandwich that fills you up.

Aladin: SO its value driven?.

P1G1: Mm yeah

Aladin: How often do you eat burgers, beef burgers?.

P1G1: Like in cooking them? Or in general

Aladin: In general, cooking buying, is it often?

P1G1: Um well second year it was a lot, I was pretty much eating a Big Mac a day almost (laughter) yeah now probably we have burgers pretty much once a week and I will probably get Mc DOnalds once a week.

P2G1: Umm, Oh yeah when we cook them in the flat the home made ones – but I don't eat at McDonalds. And yeah that about it

Aladin: So you use it at home more often.

P2G1: Yeah definitely, definitely. Oh they just taste nicer normally, home made patties and stuff

Aladin: So taste is the most important part?

P2G1: Yeah, well it's a , we all like burgers in the flat. So it's a pretty easy meal to prepare and make

P1G1: Quite filling as well

P3G1: About once a week from McDonalds

Aladin: Do you eat burgers Karen?

P4G1: We probably I would say so you (Tim) can pretend to I guess Um I don't really eat them, once a week?, once every other week?. Burgers?

P5G1: Yeah, I don't get takeaway burgers very often and probably in the summer we probably have more burgers maybe once a week in the summer. But every few weeks other times

Aladin: DO you use ah do you prepare it at home or do you prefer takeaway.

P4G1: No we prefer to eat them at home. We definitely make them, make the patties. ..missing text....so even I mean we use mince in other ways also other than as a burger

P6G1: Rarely you know, two , three times per year and I'm with kids forced to go to McDonalds. . .it would be really difficult for me to eat ah hamburger. But I prefer takeaway from Makimono Food like Japanese food

P7G1: I tend to cook them, make burgers more at home than takeaway ones.

Aladin: So what is the main reason for you that drive you to go and buy burgers. Is it special taste or the message that they normally say about special recipe or you know.

P1G1: Buying takeaway burgers now? Or yeah

Aladin: Is it the taste or is it the price is it..?

P1G1: Yeah I got pretty addicted to McDOnalds aye, I don't know why its just I don't know why, the sauces they use I suppose but yeah

P4G1: See I think for us if we do buy a takeaway burger the only place I can think of is the Queenstown Fergburger where it's a treat. And you are willing to pay a bit more for a really good burger but for us if we go to McDOnalds we wouldn't do that. But we would be willing to pay a bit more for a good burger.

7:39
Jordan: So how often when you when you eat your burgers do you often make an effort.. I mean I guess if you buy them that's different but to eat them along with a vegetable like along with lettuce or with tomato?

P7G1: Yeah

P2G1: Yeah, definitely makes a burger

P1G1: Pineapple usually

P2G1: Tomato

P1G1: Tomato, lettuce, yeah

Jordan: So you guys kind of .. when you include it is it just for flavour, is it an effort, is it an effort to make your burger healthy?

P1G1: Just for flavour I think

P3G1: Flavour mostly

P2G1: Yeah it's a nice bit of moisture in your burger, tomato is pretty important..its crucial

P1G1: Otherwise its just mince on toast pretty much.

Jordan: Ah ok. So when you are eating a hamburger meal, when you make it yourself theres not really a particular effort to eat it along with something healthy? Or is it just because maybe it's a hamburger you decide it doesn't need to be

P1G1: Yeah I don't think that healthy comes into it when I eat hamburgers personally.

Jordan: Oh, ok

P2G1: Yeah I put like tomato, lettuce..like I am making a tasty good burger, bit of crunchiness

Jordan: Yeah, so more for texture than for health?

P2G1: Yeah for texture, yeah I suppose like yeah definitely there is another benefit of health, its important for health and for taste and quality of burger

Jordan: What about you?

P3G1: Texture and nutrition wise

P4G1: I guess flavour

P5G1: Yeah I would say flavour, normally the more ingredients the better

P6G1: Flavour

P7G1: The nutrition

Jordan: Ok, do you want to move on to the next part?. So um that was just the first section and we will have one more and it will be broken up into a few sections but this particular one is about um adding non meating redients to the hamburgers. So we are going to give you an information sheet about processed meat and we are going to talk a bit about your attitudes towards that to see how people feel about processed meat and things like that

Handing sheets
11:32

Jordan: So you guys are all happy with the?, you all read the sheet?. Oh ok. So I was just going to ask you if you were aware of when you're when your buying processed meat are you aware of the fact that there are often non meating redients added and are you aware of what they are I am just going to ask around the table?

P1G1: Um not aware of what they are really but I presume that most of the meat I buy would be processed and have other stuff in it. I don't really mind

P2G1: Yeah, you kind of realise yeah when you are a student you become driven by cost as your main thing. But yeah and you sort of realise that they are just putting stuff into it but you don't really know what it is or what its effects on you are.

P3G1: Yep I just noticed that um I always thought that soy was added to other things and breads..I don't know.

Jordan: Oh ok

P4G1: Yeah I guess you know we accept it, we just buy what we like we don't really care whats in it..yeah

P5G1: Yeah like I notice on packets of sausages meat content is normally sort of 60-70% so yeah I guess the rest of it is soy or yeah something else

P7G1: Usually a lot of salt and fat as well

P3G1: A lot of sodium, salt

Jordan: Its one thing people are usually concerned about, how much fat and salt is in it sometimes. Do you understand why producers want to do it and yeah why they process it so much.

P1G1: To make it last longer I suppose, to make it go further. They can't sell the straight up stuff that they put in there maybe because some of it is shit so they mix it with other stuff that can make it go

P2G1: Yeah I suppose when I see that they are processing meat I am sort of I don't see them as doing it for our benefit I see especially with big companies like I can see them doing it in terms of it's a greed thing for them like its about them making money not so much as them caring about what we eat

Jordan: Yep

P3G1: Anything, adding salt always attracts people, the flavour. . . .its always very intoxicating in some way

Jordan: P4G1?

P4G1: Um I forgot the question

Jordan: I was going to ask about um do you kind of understand why producers would want to add a non meating redient to a processed meat product?.

P4G1: Yeah, yeah I mean make it go further and I think that makes it less expensive for consumers, more available and you know flavour, it tastes good.

P5G1: Yeah that's I would have thought, bring costs down for the product

P6G1: You know of course they are not adding ingredients because they want to make a consumer happy because they see that they can add value to the product

P7G1: The basic product has not been able to used for anything else so they just found a way to devise these products with some salt and fat and whatever else

Jordan: So, do you guys find that um, despite the fact that its labelled do you think you are kind of deceived by processed meat in any way or do you think you are quite aware of what you eat when you eat it?

P1G1: Ah I think I am quite aware, I don't really mind. It's cheap. I know that isn't they can't put the sizzlers with the other sausages because they don't have enough meat content in them or something, they put them over by the dairy products

P3G1: Oh
Jordan: Ok I have'nt really heard of that but I guess that's where they are in the supermarket. Yeah, yeah

P2G1: Your sort of aware that its processed. . .. . ..but I suppose if you wanted to make a choice to not buy it probably its going to cost you more money so. . ..

P3G1: Its always quite disturbing when you hear, because I used to belong to PETA hearing about what happens..what they are made of ..it's very disturbing

P4G1: I guess it doesn't matter, we will still eat it

P5G1: Normally pretty aware of whats in them, I mean they have got the ingredients on the box or on the packet or whatever..so

Aladin: Ah that's very interesting point, the ingredient. Do you read, do you read the ingredients when you buy processed meat.

P2G1: Yeah sometimes

P7G1: Sometimes I don't because my eyesight not very good and the print is so small unless I've got my glasses and the time it's not actually easy.

Aladin: Its interesting because nowadays you realise all the ingredients are coded into E numbers etc which probably wouldn't make much sense to lots of us

P7G1: I actually find the expiry date quite often quite hard to find on the product

P3G1: That might be on purpose

P7G1: You might find the ingredients but look for the expiry date

P4G1: I guess we tend to look at like if we are buying a meat product or I will I mean not that we take it into consideration but ..know what is the percent of meat in it you know relative to the other brands that are out there and take note. . ..pay for more meat..are we willing to pay a few cents more for the actual 2% of meat that's greater than the. . ..

P7G1: I don't eat sausages or whatever but

P3G1: But like

P7G1:. . .percentages. . .

P1G1: It tastes good

P6G1: You know generally I read the ingredients the first time, then you know I don't focus on percentage or numbers you know, its difficult

Jordan: Should we. . .do you want to, do you want to go more with this or should we move on to tasting

Aladin: What is the next question. . ..
Inaudible

JDogg: Ok I am going to go downstairs and get the um get all the samples

Aladin: Ok, so we weill start with the. . .. . .

Jordan: Yep

Aladin: We will be tasting five types of burgers and we would like to know what do you think of them. . ..etc

Conversation between participants. . .. . .inaudible

Aladin: Which do you think is more important, taste or price.. I mean I understand this is dependant on your. . .

P3G1: Taste

Aladin: On your, on your category but are you able to compromise the price for the sake of taste. I mean it happens in wine sometimes you would like to buy more expensive wine because its better, would it be the same case for meat.

P7G1: Yes

P3G1: Yes

Aladin: So you are prepared to pay more for more tender meat compared. . .

P1G1: I don't, no way

P2G1: I wish

P1G1: I'd like to

Aladin: Well, that's what I said it's probably have some sort of constraints on the purchase ability but if you , if you would. . .

P1G1: Yeah

P2G1: I definitely would

P6G1: But its difficult to get tender meat when you buy meat from shelves

Aladin: Uum, I think its not a problem in the states because they know that they have to age the meat for three weeks before they sell it so you have tender meat. I think here in New Zealand and in other places they just want the turnover so they don't age the meat appropriately, so I think here they just sell it to early it just that way. All different , of course different cuts will have different ah ability to become tenderised but ah most the meat potentially can be made tender, acceptable tenderness if its been left vacuum packed for extended time.

P6G1: Well if you don't buy a fillet it's a chewing gum. . .

Aladin: Well not

P6G1 it's a chewing gum. . . if you don't buy a fillet

P7G1: Some meat can be

P6G1: Yeah

Jordan: This one here
Inaudible

22:55

Aladin: The code number for this sample is 287

P6G1: Pardon. . .2?

Aladin: 287

P7G1: And we put it in the corner here.

Aladin: Yes

P3G1: 287

Aladin: So we will enter the number
P1G1: On each one?
Aladin: The sample number. We will have five so each characteristic we will evaluate for. . .

P1G1: I do that 287 in every. . . . . ..?

Aladin: Yes please

P3G1: Oh I see, 287 on the first one

P1G1: Why isn't it just one, how come its not number one

Aladin: Oh, we have to give them random codings

P1G1: Ah

Aladin: Ah it doesn't matter if you write on the first form its for all

P2G1: Oh yeah

P3G1:Oh well, done…

Aladin: So the first thing we would like you to do is to open the ah package and smell the flavour, odour

Inaudible, unwrapping

24:20
Aladin: And based on your perception of this flavour or this odour how much do you think the beef odour or the intensity of the beef ah flavour in this sample?.

Inaudible

Aladin: Is there any other flavour that you are able to detect?, any other flavours

P1G1: You mean smell?

Aladin: Mmm

P1G1: Smells like meat(loaf?)

P2G1: Yeah its like a vegetabley something

P1G1:Mmm

Aladin: Ok

Aladin: Would it be like everyday burger you buy?, different? Karen, what do you think?

P4G1:It just smells like warm pecans to me, haha it smells like

P7G1: I assume that means…supermarket aisle…not butcher

Aladin: Actually the chef, the head chef

P7G1: You can't tell me that..not allowed to

Jordan: Not yet, we can tell you at the end, what everything is

Aladin: Mm so…so if you detect any other flavour would you be able to tell us how strong the flavour is on the second page, number c, number c. Did you find any other flavour?, did you find any other flavour?,

P7G1 : Other than meat?

Aladin: Other than beef or smell of meat?, there is no wrong or correct answer

P3G1: Sort of a woody , smokey smell to me

P4G1: You mean a grilled, kind of?

P3G1: Yeah

Aladin: How strong this flavour or this smell is?

P3G1:I said quite strong, I said strong, yeah

Aladin: Would you like to try the texture and tell us. ……
Inaudible

28:59
P1G1: Do we keep going?

Aladin: Ah yes please

P3G1: So are we doing the other ones?

Aladin: Yeah, just indicate how much the juiciness scale is….. The perceived juiciness.

P3G1: Ok
Inaudible
31:00

Aladin: If you think this sample is different from what normally you have or it has been treated you might like to put the number in the back on the last page

P3G1: Oh

Aladin: If you think this has been slightly treated or different for the pattie that you might

P7G1: When you say treated what do you sort of mean, like what?

P3G1: Yeah

Aladin: If you think there is any additive or its not ..its different from what you normally consume probably could be better to indicate

P7G1: You just want the code then?

Aladin: Yes
P6G1: It is no different

Inaudible

Aladin: maybe? Next number please 602
Inaudible
Aladin: 602
P7G1: What did you say?
Aladin: 602
Inaudible

3604
Aladin: Any comment regarding the two samples, any comment at all, James what do you think?

P1G1: I reckon that one was real mean

Aladin: Mean?

P1G1: Yeah like heaps better than the first one. The first one tasted weird, that one tasted real good. . . I like that one

Aladin: In terms of colour, differences in colour which one do you think is better

P6G1: That one was more dark

Aladin: This one is darker?
P6G1: No ,. . ..

Aladin: The first one?, the first one is darker?

P1G1: Yeah

P3G1: I thought the first one looked more appealing

P1G1: Yeah, this one looks yuck

Aladin: Ok, ok

P4G1: Has sort of a squeaky texture

P1G1: Yeah, kind of squeaks in your mouth aye?

P3G1: Very dry

P1G1: Tasted like beef or, the other one tasted like other stuff

P7G1: I detect a taste of offal in that

Aladin: offf?

P7G1: Offal

Aladin: Offal?

P3G1: Ok, I will stop eating
Laughter

P2G1: In both of them I kind of taste like another meat like a kidney or something

P7G1: No, no, liver

P2G2: Liver, yeah

P3G1: It's a bit liverous

P2G1: Yeah…

P2G1: Yeah I wrote liver aye
Laughter
Inaudible
P6G1: Liver, mmm

P2G1: I will taste that…

P7G1: and when you look at it

P6G1: Mm

P4G1: And when you cut into it

P7G1: It actually looks like it too

P3G1: Mmm

P4G1: If you actually make a fresh cut its pink vs the outside
P7G1: You're not meant to eat that though are you?

P6G1: Probably
Laughter
P4G1: Yuck

P1G1: I'll eat it

P7G1: So you know they might not have told the truth on that one, will you put that down. . ..
Aladin: Ah there is, at the end here there is other flavour. . ..write down, you can write down of there is

P7G1: The number

Aladin: Yeah, just the number ..or what is flavour if you want to describe it

P5G1: Question number five

Aladin: Tell us if you think there is something this burger is different from what you normally have or if you think that has been treated you just write the number down

P5G1: Oh ok

P6G1: If I think that the hamburger is very different from

Aladin: If you think it's different from what you expect…

P6G1: Positive or negative?

Aladin: Ah, you can write down

P6G1: Mm
Inaudible

Jordan: Are you done with this?

P7G1: No, no I don't want any more, sorry
Laughter
Inaudible

P6G1: You look terrified

P7G1: It's a real taste like you get indigestion with it as well
Inaudible
P7G1: Doesn't it

P6G1: Yeah

P6G1: This is a new one

P7G1: You actually don't leave any room for anything more on five so what should does one do?

Jordan: Oh, you can um add more to the side or down the page if you want, I'll fix that one for next time

P3G1: What numbers this one?

Aladin: This numbers 085. 085

P7G1: 085, yes
Inaudible
41:30

P6G1: Can you smell liver as well?
Laughter

P7G1: Yeah I can

P6G1: Oh, I think that

P7G1: I come from a farm so I

P6G1: Mm, ,,. . ..don't think its liver but egg

P7G1: Breadcrumbs

P4G1: I find it to be very salty

Aladin: This one is salty?

P6G1: Not really

P7G1: Can that be counted as an aroma?

Aladin: Salt

P7G1: Salt doesn't really smell does it

Aladin: Ah taste, its mostly taste and you can put that down in the overall acceptability ah

P7G1: Doesn't actually say where we , you can't actually write the word can we?

Aladin: Ah the overall acceptability. . . write if its saltier
Inaudible

P6G1: Its salty
Mm

P6G1: Salty

Aladin: Saltier

P3G1: Taste saltyness

Aladin: Interesting, because they all have exactly the same amount of salt

P4G1: I thought 602 was also salty but now tasting 085 and whoo aw
Laughter
P7G1: It might not actually be salt, its preservative isn't it

P3G1: We don't know do we?

Aladin: I don't think its preservative, but we will tell you everything in

Inaudible

Aladin: Another two samples, everything will be revealed

Inaudible
43:52
P4G1: So is the flavour as in beef flavour?, or overall?

Aladin: Overall flavour
Inaudible
44:10
P7G1: So I assume they come from the same company

Aladin: They all, they all

P7G1: Produced by the same company

Aladin: They all from the same batch but different treatments

P3G1: Ah

P2G1: Your going to crack the mystery soon

P7G1: You can tell can't you
Laughter
Inaudible
P7G1: You've just drunk more you two guys after you've eaten these

P2G1: Whats that?

P7G1: You've just drunk more you after you've eaten these

P2G1: Yeah

P3G1: . . . salty as

Aladin: Good recipe for the pubs

P2G1: Haha yeah, salty burger
Inaudible

P7G1: I assume they want to try and improve their product and their sales. . . is that the object of the exercise?. You can't tell me that?

Aladin: Two more samples and I will tell you everything
Inaudible

P7G1: I think I can almost identify what product they are now

P3G1: As long as we are not being fed. . . brewers .thats for sure. . . . . . . haha I remember years ago my mother got these BB brewers and they were so disgusting they were so greasy they are really cheap burgers really cheap burgers and my brother

END OF SIDE A

. . . .recording starts 0:30
Inaudible

Aladin: This sample is 447

P7G1: . . . .did you say? 4?

Aladin: 447

P3G1: Missing text

Inaudible

P6G1: Disgusting

Unidentified panellist: Hmmmmm?

P6G1: Disgusting

Aladin: Why you don't like it?

Laughter

P6G1: Its ah. . .

Aladin: Describe. . .why you don't like it

P6G1: A strong odour

Aladin: How do you describe this odour?, what is the flavour

P3G1: So hard to describe

Inaudible

Aladin: Jacob what do you think?

P2G1: Ummm. . .give me a chance

P1G1: Smells like raw, raw beef, its like

P4G1 : Yeah

P1G1: I don't know

P4G1: . . ..missing text. . .just a tiny, tiny slight I don't know missing text it was very slight like a grassy. . .missing text

P7G1 : Like what
P4G1: Like a grassiness, just like

P3G1: Don't eat it

P4G1: It was going kind of like. . . .missing text
Inaudible

P1G1: This is not you guys, you guys haven't made these aye?, so if we say harsh stuff you're not crying inside?

Laughter

Aladin: No, no, no

Laughter

Aladin: We want your honest opinion

Inaudible, many people talking
P6G1: I could not..

P3G1: Just a bit dry
Inaudible
Aladin: Anything different, this one from the previous three samples?

P2G1: I didn't like the texture of it
Aladin: The texture?
P2G1: Dry

P3G1: Very dry

Aladin: Ah so the texture is drier?

P2G1: Yeah dry and just um. . .

Aladin: Anything with the flavour?

P1G1: I like the flavour

Unidentified Panellist: Mmm?

P1G1: I like this one, it's like the second one

P7G1 : You're a professional takeaway boy
Laughter

P2G1: It's disgusting

P1G1: You reckon?

P2G1: I can't eat

P1G1: I think it's got a good flavour but a bad texture
Inaudible

P7G1: Do you ever eat anything else other than take aways?
Laughter

P1G1: It's why I'm so fat, definitely need some water after that though
Inaudible

P7G1: Do we have any laws as to what um what how much they are allowed to feed them

Aladin: Um definitely, any product there is a set of regulations in terms of if its meat any product should have minimum amount of meat and its good digestable meat not like some type of material like grisle they call it they call it number or protein content, this needs to be excluded and there is lots of ah methods to determine which one is good for myofibrillar protein or chemical ah grisle etc

P2G1: When they test ah test food like this do they go to the supermarket and buy them or do they get go to the

Aladin: Ah the company need to report to MAF before they have to have separate samples to send away for analyis

P2G1: Independent sort of stuff

Inaudible

P7G1: In other words they can really put whatever they like in it as long as they say on the packet what percentage of those items are

Aladin: It needs to be approved ingredient first of all
Inaudible

P7G1: What was that number we've just done
Laughter
P3G1: And what number is this one

Jordan: This is 572

P3G1: Thank you

P6G1: 572

P7G1: They've been produced by the same company but not meat

P6G1: I don't know

P7G1: The same

P6G1: I don't think so

P3G1: . . . that smell

P6G1: How long was the meat cooked?

Aladin: It was cooked until the centre of the meat was 75 that's why we will find its not pink, it needs to be well done, so the internal temperature is 75, that's missing text
Inaudible

P6G1: Its ok

Inaudible

P6G1: Very salty

P3G1 : Horrendously horrible

P6G1: They're salty?
Laughter
P6G1: Ooh

P3G1: Very artificial tasting

P6G1: I think that we are eating chips

P4G1: Smells a beaniness to it.

P7G1: Its one of those patties with . . . missing text either something dye or something iron.. isn't it

P6G1: Very salty

P7G1: What do you think?

P3G1: Yes

Aladin: How about the texture, is there any differences?

P7G1 : It's 572 did you say?

P6G1: Well..

Aladin: 572 yes

P6G1: The texture I think is more or less the same among the samples that we have tasted in terms of tenderness and chewiness ah I

Aladin: Any observed differences between the different patties in terms of texture?

P6G1: Very close

P4G1: I think the first one was actually quite soft

Panellists: Mmm

P4G1: I thought that missing text

P7G1: You mean 287

P4G1: Sorry, 287

P3G1: The second one I think, that was the really tough one, I remember

P2G1: Quite juicy I thought

P1G1: That one, mmm

Aladin: So if you have the choice to go and buy one which one will you select out of
Inaudible

Aladin: Is there any sample that left a good feeling like ah that probably the best one or. . .

P1G1: I like 602 aye, that's my favourite

P3G1: 287 was my favourite

P7G1 : Which one

P3G1: 287, the first one

P2G1: 085 was a nice one

P3G1: We had two for 287

P2G1: No we didn't

Inaudible

P2G1: You're not meant to be genuine
Laughter

P2G1: Nourishing

Aladin: Tim, which one do you think you prefer taste?

P5G1: Ah, 085

Aladin: 085, the third one. Any particular attribute that make you achieve this decision?

P5G1: Ah I didn't like the kind of sort of, I described it as a sort of irony, blood kind of taste

Aladin: Mmm

P5G1: So that one didn't have much at all and it was a bit salty but not too salty

Aladin: Mmm. Ok than you very much for this if you finished your questionnaire I would like to collect them. Thank you

P6G1: Oh can you. . . write something
Inaudible
P2G1: Like I could eat like a home made patty eat home made patties I would eat them by myself

P1G1: Yeah I did eat them by myself

P2G1: They are way tasty man

P1G1: Yeah they

Aladin: Missing text
Inaudible

Many panellists talking at once, inaudible

Jordan: Ah, ok. So in this part now that you've taste it there is two short parts left the first part is a short discussion part and then the next part is you are going to rate the samples again, the same samples. Theres going to be an extra set for you so in this part we are going to give you an information sheet to read first as well and then we are going to have a short discussion.

P1G1: Haha, colorectal

P3G1: Wow, That's depressing

P2G1: So is New Zealand one of the biggest family meat consumers?

Aladin: Because the abundance of meat,

P3G1: Yeah, huge industry

Aladin: Actually meat here in New Zealand is very, very cheap compared with other places overseas

Jordan: Its kind of older information but I did see one that was a paper that said we were the 6th largest per capita meat consumers

Aladin: Ok, we all know that there is, there is good nutrition in meat which has been widely publicised. Ah protein, vitamins, minerals etc. What do you think is the main reason for you to eat meat?. Is it because its something you like to eat?, the taste? Or you are already aware that its very important?

P3G1: Iron. Missing text. . . and protein are the reasons to eat meat.

Aladin: Ok

P3G1: Laughter, the nutritional benefits

P5G1: Yeah, its fill you up

Aladin: Ok, so the fullness. Anything in terms of taste compared with we know that the meatness, the taste itself has unique characteristics thats not existing in other different types of food so taste is or texture is an attribute that you like in meat products?

P2G1: Yeah

P1G1: I like the taste of meat

P2G1: Bacon. . .

Aladin: So there is lots of research actually that's been done trying to not only focus on the good aspect of meat but also if there is link to any negative impact and as you can see there is a list of publications at the back and just a figure tell us that there is demographic correlation between amount of meat that has been consumed with the incidence of ah colon cancer for example. If you go and try to check more, more recent ones there is every few months there is a propaganda, something in the media telling us there is some sort of link between cancer and increased consumption of meat or meat products, especially the processed ones. So Jordan's project basically is trying to identify some of the mechanisms that might explain why this incidence might be occuring so we are interested in examining free radical generation during digestion of meat. Meat contains certain compounds that can cause generation of free radicals which can contribute to disease etc. Part of our project is to try to reduce these free radicals. We all know that free radicals or these certain compounds can be inhibited by antioxidants so if we add antioxidants its less likely the free radicals will be causing problems so part of our project is to add product that can increase the antioxidant in meat and reduce any potential negative impact for consuming meat. One of the good sources for antioxidant and it has been used earlier is to add soybean products. And in this case we used

traditional product that is commonly used in Asia as a substitute for meat because it has this "meatness" this desired flavour, its called tempeh which is fermented soybean. Anybody know about tempeh?, do you know about tempeh Joe? Have you heard about tempeh?. So it's a fermented product very commonly used in Indonesia, in Vietnam and its consumed in different ways. It can be fried, basically it looks like a white cheese and its made of soybean.

P3G1: SO its like tofu?

P6G1: Tempeh

Aladin: Its not tofu it's ..yeah

P3G1 : Its similar

Aladin: Similar but you can see the whole grain just bound by ah type of mould which is nutritionally good. So it can increase the antioxidant content and at the same time other vitamins like vitamin B12 etc. So we added tempeh in our burgers at different levels with the aim of reducing. . ...

P6G1: Sorry, but tempeh is protein.

Aladin: Tempeh is protein

P6G1: Protein

Aladin: Yeah but its not as the protein content as not as high as in beef, or in the meat. The main source, the main concept is that when we are adding tempeh we are adding antioxidant to the beef. So when we digest these burgers or these patties we will have less likely generation of free radicals. So it's more or less a healthier product. So we wanted to see what this will be affecting the taste or not. And whether the product will be acceptable and what level of adding tempeh can be the threshold where you are happy with the burger or not.

P1G1: So you added this tem-pah to the beef

Aladin: Yes, at different levels

P1G1: At different levels and that helped the antioxidants?, what did it do all the other, all the other sides of things.

Aladin: That's, that's, theres nothing else, just

P1G1: I mean level wise, just protein..

Aladin: Ah, the tempeh after its been made could contain protein, could contain a little bit carbohydrates but the most important thing we believe its contributing to the burger is antioxidant.

P1G1: Antioxidants, oh yeah

Aladin: So this antioxidant is very important later on when you digest the meat

P1G1: I don't mind

Jordan: So do you want to ask the other questions?

Aladin: Yes please

Jordan: SO I guess, I take it um that the information sheet , was it the first you ever hear in the media or somewhere else that you've ever heard of a link between red meat and cancer. Or have you heard of that before?

P1G1: I have heard that before

Jordan : You've heard that before?

P2G1: I think so

P1G1 : Everything causes cancer these days

Jordan: Yeah, so I, did that information that you hear did it influence your decision on buying red meat or did it not change anything?

P2G1: Not change anything

Jordan: Anyone?

P4G1: We toyed with the idea of being vegetarians but I don't think we can do it
Laughter

Jordan: It's a difficult thing to do

P4G1: We end up at the supermarket buying more meat. So I think . . .missing text

P7G1: We go through phases of trying to reduce meat consumption, red meat consumption

P3G1: I went from vegan to meat and stuff

P7G1: But it doesn't last very long

Jordan: So um for those of you who are wanting to change the diet would it be um would it be easier to modify it by consuming more vegetables or would you prefer a product that would have a healthy ingredient added where you didn't have to think so much about buying extra vegetables and consuming them?

P7G1: I would be going for the first option, vegetables

Jordan: Vegetables

P2G1: Yeah..vegetables

P1G1: Yeah, I suppose

P2G1: You kind of yeah, I think I would factor in my cost and dates..when making a decision as well
Jordan: Oh, ok. So um you think the vegetables would probably be cheaper than a mixed product?

P2G1: Yeah, well maybe if I if the mixed product just tasted a bit like chicken but didn't taste like chicken

Jordan: Oh, ok

P1G1: I would probably stick with chicken and try to eat more vegetables

Jordan: Yep, alright. How do you, how do you guys feel about consuming the product tempeh I guess you weren't familiar with it before we mentioned what it was. Do you like the idea of a traditional Asian fermented soybean product, do you eat maybe tofu or miso or something like that

P7G1: Not especially

P3G1: Yep. I do because I really like tofu and miso soup

P2G1: Yeah it doesn't worry me

Jordan: Oh, ok

P1G1: Yeah, I suppose I would like to know that it's in the meat but then I probably wouldn't mind

P5G1: I'm ok with it

Jordan : Yeah

P4G1: I guess its like any other thing you eat a processed thing you're not sure whats in it anyhow

P2G1: Yeah

P4G1: They . . ..percent so if it's in there

P2G1: You would rather you would know about this and it was like a natural product and

P1G1: Its actually helping you

P2G1: Than just stuff to help the people producing it

P1G1: Rather than just, yeah to make them make more money.

P2G1: It's a pretty good thing

Jordan: So you are happy if, you would be happy to consume it in the form of a burger or do you think you would just avoid this tempeh containing burger altogether?

P1G1: Yeah, I always make my own burger patties but if I was buying burger patties then I would

P2G1: Definitely yeah, if the taste was similar probably try to do something that was good for you

P1G1: Its pretty hard to tell you know if it was good or not because I have never bought patties before and eaten them by myself. If you hooked us up with some tomatoes and lettuce laughter, nah. You would taste it

P2G1: Yeah it would be interesting to eat a meat patty after that, beef patty to see if you ..missing text

P1G1: Or are you going to tell us that they are all beef products or something afterwards, laughter

Jordan: Maybe
Laughter

P4G1: I guess that's the other thing and you made that point. For us we would make patties at home would these potentially come pre-made? Or would they, would it be a mince that you actually go through the making of yourselves?

Jordan: I guess a product like that would be pre-made unless you gave the consumer the idea of putting them together but probably if you wanted people to do that selling it in a pre-made product would probably be the best way.

Aladin: The most important point is that when you go and buy burger from McDonalds or Wendy's this small piece of tomato and lettuce that they put on the meat is not enough to inhibit the free radicals that will be generated in your stomach. So our aim was to produce something less harmful whether they put this tomato or not you will get some sort of protection. So that that's the health, the idea of producing healthier burger. But of course taste is very important because you can make the most healthiest product ever and if it doesn't taste good the consumer will never eat it. Consume. So any any particular interest, I mean would you prefer to have product that provide you with food protection or as you indicated before you probably will get the burger and have it with salad, somewhere?

P4G1: What kind of establishment is this thing sold at?, I mean would it be like, are you at the McDOnalds window and you're trying to decide to order just a burger or the burger that has this benefit, is that what you mean?.
P6G1: But you know

P4G1: I mean I'm not sure of the situation

Aladin: Ah, would it be more interesting to have just regular burger, that's what I know and I will do more effort eating green material with it or I prefer the easy way out

P4G1: Probably the greens

P6G1: But you know. What are the health benefits of eating hamburger in McDOnalds or Burger King

P3G1: Yeah you're not thinking of that

P6G1: The health benefits are really zero, probably its more interesting to produce ah such hamburger for retail you know probably consumer might be interested in the fact that the hamburger contains a certain ingredient that's good for health. But you know personally I don't believe that you know it's a good idea to go to eat to McDonalds to have a healthy hamburger, it seems a bit paradoxal, you know what I mean. You know you go there you buy chips and you know

P4G1: And then you buy the diet coke
Laughter

P6G1: Well you know probably if you find your hamburger containing this ingredient in bakery deli probably that will be a healthy choice because you go back home, you cook that hamburger and you eat the hamburger you know . . .missing text

Aladin: Joe, what do you think?

P7G1: Much the same really
P5G1: I think its not a bad idea for fast food places because I think people who go to the fast food places are not going to bother getting extra vegetables or a salad to go on the side so if it actually makes a difference for health reasons think its quite a good idea

P1G1: Yeah they are doing enough damage , maybe they should chuck the old soy in there help people a little bit, just without them knowing.

P3G1: Mmm

P1G1: Because they probably go there a lot, they are probably doing enough, them, those people

P4G1: I mean if you rock, if you went up to the window and you had two choices and said this is beef burger this is the burger same flavour not compromising flavour or taste you're going to get this added benefit of

P3G1: Yeah

P4G1: Producing your antioxidants, you know giving you some antioxidants

P3G1: Most

P4G1: Same price, same everything which would you choose?

P1G1: Mmm

P4G1: You just went up to the window and just like

P1G1: Yeah, if its if its just for not getting cancer then you would obviously choose that one

P3G1: But if it had like a fun name or something just like ..missing text.. or something you know like you get these power shakes with various names

P1G1: Same price, same taste, get that one

P3G1: Some people would get the other one because they would think it was unhealthy so it might taste better, I just know that

P2G1: Yeah, I if you're going there you probably be one just a treat meal or something like that, like you're going to eat greasy fast food so you wouldn't

P1G1: You don't really think about health aye, when you go get fast food anyway

P2G1: No you just need this you just satisfy this lust for just like greasy crap

Jordan: Should we um serve them again

Aladin: Ah, who would like to try this again?, who is willing to try this again

P1G1: How much are we talking?

P2G1: Is it the same patties?

Aladin: Yeah, will you be interested in trying this again?

P1G1: What, are you not going to tell us what they are or anything before?

Aladin: Ah, we will be able to tell you what is differences while you are consuming them

P2G1: Oh yeah

P7G1: We have to, you want us to try each of the five of them again?

Jordan: Yeah, I mean you don't have to if you are full but if you can

P3G1: SO as we try them you will tell us what they are?

P5G1: Are we, are we going to fill out another form?

P7G1 : Are they going to be in the same order as what we did them in?
Laughter

P2G1: I will eat some tempeh

Jordan: So should we tell everyone what they were and then we will get them to

P1G1: Alright, we will eat your soybean

Jordan: So we had, you were served five different burgers. Five different burger patties, unfortunately some of them with the mistake I made did have more salt. There was one that was a control and all it had was 1% salt and some beef. Another one had 1% salt, 10% breadcrumbs and beef, the next one had um 1% salt, 10% tempeh and the beef. The other one had 20% tempeh and it actually had 2% salt and beef. And the next one had, there was another one with 30% tempeh that had more than 2% salt and beef. So the control was 447, the 10% breadcrumbs was 287, the 10% tempeh was 602, the 20% tempeh was 085 and the 30% was 572.

Aladin: So the last one was the was the one with the highest amount of tempeh.

Jordan: Yeah

P2G1: Yeah, my favourite two were the ones with the most tempeh
Jordan: Oh, ok

P2G1: I like tempeh, I just found out
Laughter

P4G1: So you're going to go for that one

P1G1: Love that tempeh

Aladin: We have, last more thing we do is ah we want to show you the product as it is and we want to see which one you will like best now you know what is the treatment. Because um, it will be just visual assessment there is no eating, there is nothing, but if you are going to the supermarket which one you would like taste. We are mixing the treatments so we are changing the treatment a little bit.

P2G1: What one um, what one was your favourite

P1G1: I forgot, I don't have my sheet anymore. 602, what was 602 do you know?

Aladin: Mmm

P1G1: Do you know what 602 was?

Aladin: 602

P1G1: Have you got the notes?

Aladin: Ah

P7G1: So you are going to show them to us again before?

P6G1: Aladin, we don't have to. . .?

Aladin: But we changed the coding for this one

P1G1: I was right

P2G1: What do you mean you were right

Pippo: Aladin..male or female. . ..

Aladin: Ah it doesn't matter, only the sequence

Inaudible, many people talking

P4G1: See this for me I keep thinking about, there's this place back home this place called Jungle Juices and it's a juice shop, it's a natural chain and you walk in , you order a juice, you have mango, orange something and then you can say I want the energy boost, I want the immunity boost, I want the calcium boost

P3G1: Energy boost is more sugar

P4G1: And they put these extra things in your juice and you actually order these immunity whatever. In some respects I can see that when you walk into the burger shop and say ok, I want the antioxidant boost

P1G1: Yeah

P2G1: The no cancer

P4G1: Yeah

P1G1: Give me some of that no cancer

P3G1: Like those vitamin

P2G1: Yeah I always used to yeah. I always joke about people in America like when they are ordering like a tub of diabetes or something like that. When you see big fat people at Maccas. And so Type II diabetes

P3G1: Diabetes, yeah

P4G1: Those are the ones who said they wanted diet coke

P3G1: And the fruit juice, because its healthy, it has fruit so, even though its all sugar

P5G1: 5% fruit

P2G1: So whats the difference? Are the tempeh and the tofu made out of the same thing or

Aladin: The tempeh and?

P2G1: Tofu, are they made form the same?

Aladin: They are made from the same material but they are processed differently. One is ah fermented with type of mould which naturally grows in Asian countries. And they basically what the mould do is binding the grain together by a network of hyphae so it becomes very firm, so you can cut it and slice it like cheese. The other one is just curd so they mash the whole soybeans, they make it like a curd, like a white cheese if you like

P2G1: And do they sell tempeh in New Zealand now, is it used?

Aladin: Tempeh, tempeh is very very expensive and its available in New World. 225 grams will cost you almost $8

P7G1 : And what, is it in the cheese section

Aladin: No, no its in the chilled, chilled product section

P7G1: Chilled , that's what I mean

P2G1: So how, how this can almost be a p. . . .can it

Aladin: Ah

Inaudible

P3G1: Looks awful

Aladin: Sorry the light probably is not

P1G1: That ones got a black hole in it

Aladin: SO based on colour which one do you think the colour

P6G1: 10% of tempeh for me

P3G1: Yeah that one in the corner

P6G1: This one

Aladin: So you like this colour is better?

P6G1: Yeah

P3G1: I like that one

P1G1: That ones got more chunks and shit in it

P6G1: I like that one

P7G1: That looks more like real meat doesn't it

P4G1: Which one?

P6G1: I don't like oh whats that ah?

P2G1: Cancer. . ...

Aladin: Basically, basically you are correct because free radical when its generated it can actually cause auto-oxidation, this is browning is just oxidation, oxidising something and its caused by free radical. In your stomach free radical will be targeting something, so the easiest target is lining of the stomach. It doesn't happen instantly because we have immune system but on the long term that's where things might happen..um

P2G1: So is this just red meat? Is this problem with antioxidants or are you saying white meats? . . .

Aladin: Its all 100% red meats

P2G1: White meats are better for you?

Aladin: Ah white, red will contain well some to a lesser extent the catalyst that can cause free radical generation
P1G1: Negative. . .

P6G1: So when you add the tempeh, it becomes less dark

Jordan: Actually the control was stored for a little bit longer but the tempeh does make it lighter

P6G1: Its white

Jordan: It's a white colour and when its mixed in its mixed in more like a flour so when its mixed through it gives it a paler colour

Aladin: So if you, if you can write the sample number and describe the overall acceptability, just for the one you like best, we will appreciate that. Thank you

P3G1: I don't know which sample number

P7G1: So

P3G1: Top right

Aladin: So you have here , ah

P7G1: So it hasn't got a number

Aladin: Ah its just the sample

P1G1: Oh the one you're looking at

P6G1: Aladin, do we have to specify 10% of tempeh or…

Aladin: Just, that will be the number of the sample

P6G1: Yeah, you know but we have to specify what we like more

Aladin: Yes, only the one you like
Inaudible

Jordan: 10% breadcrumbs?

P1G1: Oh, breadcrumbs

Inaudible, long pause

P1G1: Acceptability?, aren't we just going off visual

Aladin: Yeah, which one if you are going to buy

P1G1: Ah

Aladin: Ah this is acceptable product for me

P3G1: Says here we taste

P1G1: What does that say?

P3G1: What's that?, we taste the infusion. Are we not doing that

Aladin: Ah. No

P3G1: Ok, That's cool
Inaudible, long pause
Aladin: Thank you very much for that

P7G1: Thank you

Jordan: Thanks
Inaudible
Aladin: Cheers, thank you very much for your help. Would you like someone to draw?

Jordan: Should we draw it out of a hat?
Laughter

Aladin: I am the only one that got the hat

Jordan: What do you want to draw it out of?

Aladin: Ahh

Jordan: We could do it out of this box

P6G1: Can we go?

Aladin: Hmm?. You are not participating in the hat?

P6G1: No, not participating, one less

Aladin: So we have to take more chances

P6G1: Thanks, goodbye

Aladin: Thank you Pippo, cheers
Inaudible
Aladin: Who would like to draw? Jacob

P2G1: Ok

P7G1: Salt leaves a strong taste doesn't it?

P3G1: Yep

P5G1: This will clear the salt up

P1G1: Da da da

Jordan: P4G1

Aladin: Thank you very much for that

P7G1: Thank you

Jordan: Thanks

P1G1: Sweet as, yeah sorry

Aladin: Thank you very much for your participation

P7G1: Thanks for that

Jordan: There you go

Aladin: Appreciate

P7G1: You're about to go and buy your burgers
P4G1

P2G1: $50 worth

P7G1: Are you going to tell us what brand they are?

Jordan: Actually this is um,, there is no-one funding this just pure research. Maybe someone will want to turn this into a product, I'm not sure but there is nothing like this out there with tempeh

P7G1: Oh, ok

Jordan: I think this might have been done before but not very often so, its just for the sake of writing the Masters thesis and hopefully will learn something from it

P2G1: What other ah products are being put in these patties normally?

Jordan: Into hamburgers?

P2G1: Mmmm

Jordan: People do put other soy products in there like soy protein and things like that but normally hamburgers apart from spices and maybe onions do not have that much added to them

Aladin: Do you want to draw someone else?

P1G1: Ah

P2G1: Do we want to?

P1G1: Ah wicked

P7G1: Oh well good luck with your

P1G1: Ah thank you

P7G1: Good luck with your

P1G1: Thanks

Aladin: Thank you

Jordan: Thanks for coming

P1G1: Ah wicked, thank you very much

Aladin: Ok, thanks Karen, cheers. Thank you

Inaudible

Jordan: Thanks

Aladin: Cheers
Goodbyes

Jordan: Hey sorry can I get you to um to write your name and sign that you took the voucher, like I don't know an official thing

P2G1: Have you eaten tempeh straight before by itself?

Aladin: Ah, fantastic
P2G1: Huh

Aladin: I eat it so often ah. Yeah if you are going to New World look it up in the chilled product section it's quite expensive but and um yeah just give it a go if you can, its quite nice but probably first time it will be a bit weird

P1G1: Yeah, bit weird. Yeah I am keen for it

P2G1: What, what
RECORDING ENDS

Appendix 16: Focus Group 2 transcript

Panellist number	Panellist Name
P8G2	Maurice
P9G2	Rachel
P10G2	Anna
P11G2	Will
P12G2	Powell
P13G2	Jim
P14G2	DOnny
P15G2	Amalina

Jordan

….little bit of chemistry and some tests in the .. they have a lab downstairs that's not for food and um all the food for this was prepared in a kitchen and this is just to see um , just the discussion that's gonna come out of the focus group will give me lots of good information to put in my thesis. And later on you will be trying the burgers and the feedback from that will help me design some of my studies. So first off now that everyone has introduced themselves we are just going to like ask a few questions and hopefully the discussion from that will give us good answers. . .and later on you'll get to try the burgers and you will be rating them on forms for how much you like them and then we talk a little bit more and I will give out some information sheets during the talks and aswell at the end you are going to look at the raw product and see what you like you are not going to taste it, just look at it. Yeah

Panellists

Cool
Jordan
So I was just going now that all the introduction has been done I was going to ask you some questions some general questions about burgers but first of all how often do you guys eat takeaways

Unidentified panellist
Three times a week
P9G2: I am going to go with three times a week, it varies

P15G2: Probably once a week to twice or once a month

P10G2: Sometimes more than three times a week
Laughter
Jordan: And do you eat burgers often?

All: yes

P10G2: Bit of a cheeseburger fan

Jordan: Oh, ok so you do you normally get them when, from like a fast food outlet or do you buy them premade or

P8G2: From a fast food outlet

P10G2: Yeah fast food outlet

P9G2: But, I love homemade hamburgers the best

Aladin: Why?

P10G2: Oh just because you get to make it yourself and it tastes way better, and good quality stuff
P8G2: Yeah, good quality yeah

P9G2: Yeah, velvet burger is really yummy

P10G2: Yeah, more realistic I guess. . . .plasticy kind of
Laughter
P8G2: Cheeseburger

P10G2 : And we get them nearly once a week at the hall aswell

P9G2: Oh yeah, right

P8G2: Yeah, that's right

P9G2: Yeah, every Friday we have burgers. I forget about that

P15G2: Oh I usually make my own

Aladin: Ah you make your own

P15G2: Yeah, I don't really like buying burgers outside

Aladin: So why?

P15G2: Oh because I know whats inside, I'm quite um

P9G2: Yeah, I used to work at a McDOnalds so I'm just like mmm

P15G2: Yeah

P9G2: You know, how long the meat sits there and all that and everything

Jordan: So you are a little bit put off by fast food burgers, but you two really like to make them yourselves
P9G2: Oh I still eat fast food, I don't really care, it all tastes good, you just don't want to think about. . .

Aladin: So what do you think a good burger should be you know

P10G2: It should be filling and it should taste good and be healthy

Aladin: Healthy

P9G2: Yeah, you feel better when there is healthy stuff combined with it

P8G2: I agree with that, like cheeseburgers at McDOnalds, I always have to eat at least two before I feel fill, feel full

P10G2: Yeah

P8G2: So I agree with ..missing text

Aladin: And in your opinion, what makes a good burger?

P15G2: I definitely think that all meat kind of burgers not like probably 30% meat and the rest is other things

Aladin: Ok

P15G2: I probably prefer most of the things to be meat

P10G2: Like the consistency and the texture of it like counts
P15G2: Yeah

P10G2: If I like. . .. good with mince

P8G2: It needs cheese

P10G2: With mince you know it is mince

P8G2: Definitely with cheese

P10G2: rather than I don't know like in chicken burgers and you are like is it actually chicken

P9G2: Oh I love chicken burgers I. . ..with chicken burgers

Jordan: So you like to make them, you were saying that you like to know what's inside the burger when you make it yourself

2 panellists: Yeah

P9G2: Yeah and like getting the right amount of sauce like if its just like if you've got a cheeseburger its like oh I don't have enough tomato sauce or like yeah you get to choose a quantity

P10G2: Yeah having more than one sauce like tomato sauce and mayonnaise and barbeque sauce

P9G2: Yeah or aioli

P10G2: Yeah, aioli. . ..And when , when you like make your own ones at home and you like can put spices and stuff in the mince meat rather than just bland
Jordan: So you guys, oh sorry what were you going to say?

P9G2: Oh I was just going to say tasty burger is good

Jordan: So you guys like to season them and add a lot of ingredients other than mince?

Many panellists: Yeah, in agreement

Jordan: Do you ever try putting maybe vegetables like onions and carrots and stuff

P8G2: Yeah, yeah carrots, like when you make rissoles(?) and stuff

Jordan: Oh, ok

P9G2: Carrots are yum

Aladin: So you add this ingredient as part of prospecting a better nutrition

P8G2: Yeah

Aladin: Ok, alright

P10G2: I remember at McDOnalds, I think for a limited time they had a burger and it had carrot in the patty..maybe I don't know. And it was square shaped.

Jordan: Oh, ok

P10G2: You guys, you don't remember that?

P8G2: No, I don't remember

P10G2: Crap, it was there for a bit. Oh, it was like the burger with no name maybe

Jordan: The one that they wanted people to name?
Unidentified
Yeah
Jordan
I don't remember that one being square

P9G2: I don't know what you are up to

P10G2: Neither do I, don't worry

Jordan: Do you know I know you put vegetables in the burger but are you really conscious about the nutrition like try to add maybe some lettuce and some tomato aswell

All panellists: Yeah

P9G2: Then you don't feel so bad

P8G2: And cheese

P10G2: Definitely

Jordan: Oh, ok. So when you are buying them, where do you normally buy them from?

P8G2: Do you mean fast food ones or. . . . . ?

Jordan: Fast food or maybe the pre-made ones aswell

Many panellists: Premade ones?

Inaudible, many panellists talking
P8G2: Like from the supermarket?

Jordan: Like do you ever buy like a frozen premade or

P8G2: Patty?

Jordan: Yeah just the patty

P8G2: Yeah

P9G2: Yeah, quite often

P10G2: I have tried vegetarian premade ones but I haven't, I can't remember the last time I had them

P9G2I, I don't think I have ever bought the frozen patties because I just think they look so yuck, but I would buy the premade patties that are like in the butchery

Jordan: Yeah

P9G2: Like where, because if we have dinner parties we won't buy the frozen stuff we buy the actual fresh stuff because

Aladin: Fresh

P9G2: Like it just looks so processed and its frozen too

P10G2: Otherwise McDonalds and Burger King

P9G2: Burger King, yeah
P10G2: Velvet Burger

Jordan: Ok. So you guys like to get like kind of like the high end of the takeaway burgers

P8G2: Yeah. Like Burger Wisconsin aswell is really nice

Jordan: Yeah, they don't have that in Dunedin do they?, is it just the North Island?

P8G2: I think so yeah

P15G2: We have one in Christchurch I think, Burger Wisconsin

Jordan: Ok, I think we have exhausted the takeaway part, so now we are going to talk a little bit about processed meat and first of all I am going to give you an information sheet just to read through

P9G2: Do I hand it back to you

Jordan: Oh you can leave it there while we are talking if you want, if you prefer. So I hear you just from talking before that you have already talked about kind of like having an aversion to eating the processed meat from the or burgers looking too processed.

P9G2: Yeah, I guess we are quite naive about it, like you still would eat it and stuff but then someone is like "oh that's really processed". Its like oooh no but like usually

P8G2: In a perfect world it would be great not to eat processed stuff but its also a lot cheaper

P9G2: Yeah

Jordan: Yeah
P9G2: That's just the way it is

P8G2: I don't know about you Anna because we both grew up on farms like having home kill and stuff you always made your mince meat out of whatever was like killed on the farm so like I guess it gave you an aversion to buying stuff that was like so processed

P10G2: Yeah

P8G2: When you are eating stuff that you know came straight out of your paddock

P9G2: I guess.. that if you have got knowledge about it then you will make decisions based on the process otherwise, you won't care

Jordan: So there is a little bit of a small kind of lack of trust about where the meat has come from and how much is actually meat

P8G2: Yeah

Jordan: And

P8G2: It never looks like what you make at home

Unidentified
Mmmmm

P11G2: But then it tastes so good sometimes you just can't resist

P8G2: Yeah, because you get used to it
Unidentified
..missing text
P10G2: I mean yeah, McDonalds is a lot cheaper than velvet burger
P9G2: Yeah, like maybe sometimes you do want processed food, just like oh "I just want fatty food"

P10G2: Yeah

Jordan: Oh there is someone, yeah there is someone downstairs. Yeah

Jordan: So you guys I mean you have done nutrition Amalina you understand do you

P9G2: Oh, he just text me and he is like "we are coming"

Jordan: Oh, ok , how many people do I have?

P9G2: I am not sure. It will be like Lockie and his friends
Inaudible
Jordan: So you know um, did you learn a little bit about why producers process meat and do you guys

P15G2: No, not really

Jordan: Never?

P9G2: Is that the reasons why like shelf life and all that

Jordan: Yeah, some of the reasons. Did you know that before or ever really considered why?

P8G2: We always know that processed stuff has heaps of additives in it

P9G2: Yeah. Had a fair idea

P10G2: Yeah

Jordan: Are there any of them that you really try to avoid?, like maybe the salt or something else

P9G2: I never really read what's on the back

P10G2: No me neither
Laughter,
Panellists
Hey boys
New panellists come in, hellos

Aladin : Do you have some more information sheets

Jordan: Yeah we have some spares

Aladin: These ones?

Jordan : Yeah

Aladin: Ok

Aladin: Please read the information sheet, you can sign it..ah the consent form

P12G2: Benny you're a vegetarian

P10G2: I am not vegetarian, I was vegetarian, I started eating meat
Inaudible

Aladin : Looks like this has been signed already
Unidentified: Yeah

Unidentified: Yeah so has this one

Unidentified: So has this one

P10G2: Oh, whoops I thought I gave you the information sheet.

Unidentifed: Thanks Beenie

Jordan: Oh these are the, these are the consent forms that haven't been used.
Inaudible

P14G2: This is like real dangerous bro
Inaudible
Conversation not relevant to focus group

P11G2: $50 grocery voucher

P12G2: Whats that for?

P9G2: We go in the draw

P11G2: Is that just out of us?

P9G2: If you guys win and you

P13G2: How do we win?

Jordan: Well um at the end we will just get everyones name down and just draw the winner, so you have got one chance in 9, no one in 8
P14G2: We have got a 4 in 9 chance

P13G2: So whats the date today

Jordan: It's the 27th

P9G2: We've got a 3 in 9 chance
Inaudible

Jordan: SO, um now that everyone is here shall we just , again we will just introduce ourselves around the table just so everyone knows who is who. So I will just start first, I'm Jordan and I'm kind of, I advertised the project, I am running this as part of my Masters degree in food science so I needed to do this focus group as part of the consumer testing and

P8G2: We already know. . .missing text

Jordan: Oh just for the sake of..just for the record

P8G2: Hi, I'm Maurice and I'm taking part in this project

P9G2: Hi I'm Rachel and I

P10G2: I'm Anna Parsons

P11G2: I'm Willie

P12G2: I'm Powel and I'm part in this project

P13G2: I'm Jimmie

P14G2: I'm Donny, or Donald and I'm taking part in this project

Aladin: And my name is ALadin

Jordan: Ok, so we have already done the first part.

Aladin: So we just go ahead with this

Jordan: They have got the information sheet?. Yeah we will just um,
Inaudible

Jordan: SO I will just ask if you guys are aware of how many non meat products you find in processed meat

P13G2: Wouldn't have a clue

P12G2: No, not really

Jordan: Never kind of look at the label when you buy processed meat?

P14G2: Oh, don't sausages have like bread in them?

Jordan: I think, do some producers add bread?

Aladin: Ah not really bread but they add other additives just bulking agents, make it go a little bit farther.

P11G2: Haven't known any figures or anything

Aladin: There is regulations, they have to comply with certain percentage by law, otherwise they can be penalised for that
P11G2: Is that all type of meats?

Aladin: All meat products

P14G2: Isn't it different types though?

Aladin: Mmm

Jordan: Processed meats have different rules and regulations

P14G2: Yeah, that's right

Jordan: but there is nothing that you ever, I don't know if you are allergic to something or you just try to avoid salt, too much salt or..?

P14G2: Nah, personally I don't

Jordan : So it's mainly the price that just you eat the processed meat for or is it the taste as well?

P11G2: Mainly, whats cheap and available

P12G2: Lasts quite a while outside the fridge. I bought some sizzlers today, they are quite processed

P14G2: Were they double cheese?

P12G2: No, just normal cheese
Laughter

P12G2: They were on special actually, that's why I got them aye

P13G2: Yeah, exactly

P11G2: Pretty much the main factor

Aladin: Any idea why we, actually the processor put any ingredient in the product, any idea why do you think the producer has to do that?

P9G2: Because its cheaper for them?, is it?

Aladin: And does it make you happy?, does it make you happy at all

P15G2: It doesn't bother me

Aladin: I mean if you go and buy sausage and you realise that there is at least 40% other ingredient other than meat

P9G2: If it was like really pointed out to you I would be like oh maybe I will get that one, but you don't take much notice

Aladin: Ok

Jordan: You, so you guys don't find it deceptive at all that um, that you buy meat products and not all that you are eating is meat, because I know that some people have said that they kind of look at the meat content and what they are eating and they are kind of disappointed

P13G2: Oh to an extent Is' pose it is deceptive cos you think you are buying meat Is' pose but

P9G2: Yeah, well we just don't take much notice so we wouldn't feel deceived, only if someone pointed it out, you are like oh

Jordan : Oh, ok. So you don't mind at all eating a meat product that's not entirely meat?

P13G2: I prefer to eat a fully meat product but I am not sure about how to go about finding the products that are fully meat

Jordan: So do you, you don't, do you guys all live together?

P12G2: Yeah

Jordan: And cook together?

P12G2: Ah, nah not this year, next year

P9G2: Like hall food

Jordan: Oh it's, you're all in Cumberland courts apart from Amalina

P9G2: And Anna

P10G2: And me

Jordan: Ok

P14G2: She is gonna win the voucher aye
Laughter

Jordan: So when, when um just generally for everyone when you are buying meats do you go for the whole cuts or do you just go purely sausages, burgers, stuff like that?

P11G2: Are we talking about sausages?
Jordan : Just um, you know any

P14G2: Just meat in general

P11G2: I think it depends like when you are at home and like obviously flatting and stuff it would be like whats cheap and stuff but like at home we have full meats

P13G2: If money was no object then of course I would like yeah, I mean just for me anyway I like to get better quality meat like not just pre-cooked sausages, like actual beef and pork ones

P8G2: It's no biggy

P15G2: I don't really buy sausages or any processed meat I usually buy like proper meat and make whatever I am going to make at home

Jordan: Is that like your study in Nutrition that encouraged you to do that or. . .. . .

P15G2: Um yeah, I think. It makes me more aware of whats inside processed meats but I don't really usually look at the back of the packet and actually see what's inside it

Jordan: Do you want to move on to the next part?

Aladin: Yes please

Jordan: Ok

Aladin: So what will happen next is that we will be serving you different types of burgers and we would like you to tell us what do you think of it. We have evaluation sheet for each sample and I will be collecting them after. So please if you can write down the sample number on the side
P14G2: Is this sample number 1?

Aladin: Ah we will give it these are all coded and we will like

P10G2: I am Anna Parsons. I am still Anna Parsons

Aladin: To keep them the same please
Inaudible conversation

Aladin: So basically what we would like you to tell us any flavour unique to the sample, ah how do you like it, the texture, the flavour etc. And at the end if this is something you are willing to buy or not. Ah there is more forms here

P13G2: Ah thank you

P14G2: Thank you
22:44
This part of the tape is hard to hear but is most likely irrelevant
23:30
P12G2: Maurice how did you find out about this?

P9G2: We just saw a poster and had nothing to do
Inaudible

P9G2: Ok so we smell it

P10G2: Oh dear

P10G2: It smells a lot more meaty

P12G2: Why are you eating meat Bean?
Aladin: So what is the sample number

P11G2: I have been eating meat for the last two months you just haven't noticed

Jordan: That one is 447

P11G2: You are a vegetarian

Aladin: The sample number for this sample is 447

P14G2: I think it's quite intense. . ..

P14G2: Number 447?

Aladin: Yes

P9G2: Do you eat the tinfoil?

P13G2: Chewy

P12G2: I reckon it's pretty dry

Inaudible, recording may need to be enhanced

P13G2: What do you mean by which samples do you think have been treated?

Aladin: Do you think this is 100% beef or do you think there is something that is not

P14G2: Do we just tick it if we think it has been treated?

Aladin: If you think there is anything other than beef please write down the name or say yes. We will explain everything to you at the end.

Jordan: Can I take your plates?. Thanks, you are all done eating?

P9G2: Sure

P14G2: Are we meant to keep our forks?

Jordan: Um, yeah

P9G2: I will keep my forks

Aladin: We will have the second sample and we will repeat exactly the same, please.

P11G2: I didn't mind really, the first bite was like. . .

P12G2: I mean I have definitely had worse

P14G2: I have had a lot better

P9G2: It reminded me of Maccas that one

P11G2: I liked it

P11G2: But you are vegetarian, I thought she was vegetarian but shes actually not

P13G2: What was that thing in the critic that abuses the shit out of the vegetarians

P9G2: Oh yeah I saw that

P13G2: Says they are just self absorbed people, they voice opinion that are not even considered in normal society

P12G2: Bro, all things aside these chocolate fingers are so mean bro

Jordan: Have you guys got cutlery?

P8G2: I need some cutlery please

P9G2: Yeah, please, sorry

P8G2: Thank you

P10G2: I feel crappy as

Aladin: This sample is 085

P11G2: They're the same

P13G2: No it's not

P12G2: Its not

P11G2: How do you know bro?

P12G2: Cos

P11G2: . . T sauce. . . . . that's the variable

P13G2: It is actually

P9G2: Oh my god, it actually does

P8G2: Sorry

P11G2: What?

P9G2: No,no,no, I won't influence your thoughts

34:11
P10G2: I am easily influenced

Jordan: Are you guys done with your samples?

P10G2: Oh yeah, I am done

Jordan: You're done

P14G2:So many dishes. I don't know who. . . . . .I would just boost it. . . . . .things have taken on a whole new perspective

P9G2: It actually did

P11G2: It actually did, I couldn't taste what that smell was

P9G2:Do you guys reckon that smelled like this one?

P11G2: The first one was cat

Aladin: DO you guys have any preference to any type of meat?, do you like any type of meat in particular?

Jimmy: Are you talking about red meat or?

Aladin: Red meat yes

P13G2: Um

Aladin: Or meat in general, which you eat most?

P12G2: I like chicken

P8G2: Yeah I'm a.. I quite like chicken

Aladin: Chicken

P13G2:Um lamb

P11G2:Quite like beef aye

P14G2:I like venison

P11G2: I like lamb flavour

P8G2:Venison's better

P11G2:I reckon its better too

Aladin: Which part do you prefer?

P14G2:Wag, wag its so wag like

Aladin:Do you?
P11G2:Whats the number for this one?

Aladin: Ah, whats the number for this?

Jordan: This one is six zero two

Aladin: Six zero two

P9G2: Six zero two

P11G2:Six Oh two

P11G2: Is that a number?

P14G2:This ones the Wag

P12G2:Not moderate, theres a slight
38:12

P11G2:Its not your type of eating, it's not your type of eating

P11G2: Who's into this eating with a fork

P11G2:Its not your type of eating

P13G2:I have got a good feeling about

P14G2: Bro, how many cans of baked beans can we buy?, so many bro

P13G2: Bro, baked beans are a dollar a can

P12G2: No but you can only buy Watties

P11G2: Baked beans are shit

P11G2: There is a reason they are that cheap bro

P12G2: Bro there is a thing called price/quality association and I studied it at university

P11G2: Its got a sort of rubbery texture anyway
40:01

P12G2: Hard to say, real hard to say

P11G2: How many samples are there?

Aladin: Five samples and we will do evaluation for raw sample at the end, just the colour, see how you like it if something you will be willing to buy. So you guys you said majority eat chicken, why?. Do you think, perceive chicken as more healthy option

P13G2: No, I just like the taste

P9G2: I think only if it's a nice cut of chicken though. Like fresh, it can't be burnt. . .. .I reckon beef

P13G2: I'd rather have a good steak I reckon

Aladin: I mean chicken sorry, red meat

Jordan: There is still one, still one to come

Aladin: What?

Jordan: There is one more sample still to come

Aladin: Ah we will. . .. .

Jordan : Ah ok

P9G2: I. . ..

P13G2: I prefer if the meats cooked right ah beefs the best, steaks and things

P9G2: This is probably naïve but I perceive chicken to be less processed than beef

P11G2: Why?

P11G2: I just do, I just think its better, yeah

P9G2: Better and tastes less like mince

P11G2: What about those chicken patties and stuff, chicken nuggets

P10G2: Still, I know that but like

P11G2: I like chicken nuggets, I don't care whats in them aye

P14G2: When you deep fry them, that stuff is so much better though
42:01

Aladin: Can you remember how much it cost you the most expensive piece of meat you eat?. Probably nothing because you are broke and . . .probably you got it for free. Just when it comes to meat the price range can be very, very wide. Have you heard about Kobe beef?

P11G2: Yes

P13G2: Is that like

P8G2: What beef?

P13G2: When they feed the cows on like really nice things like beer and oh

P11G2: Nah that's wagyu

Aladin: Well the rumour has it they give them special feed and they give them beer to drink and listen to classic music. One kilogram Probably it costs about $900, New Zealand dollars

P9G2: Wow

P10G2: For meat?

P11G2: Whats this

P13G2: Like Kobe beef they like treat their cows like kings like feed them real nice, they give them beer to drink, they play classical music

P9G2: Beer?

P13G2: Beer

P11G2: And what?
P13G2: And the meats really good

P11G2: Its probably not even that good

P10G2: Its probably the propaganda, yeah

Aladin: This number is five seven two

P11G2: Is this the one where they uh, is Kobe the one where they massage the cows

Aladin: Well they

P13G2: Massage the cows?

Aladin: Well they do massage the cows but I doubt they give them beer. It would be very expensive

P8G2: I liked the middle one, I didn't like the other two

Aladin: This is number 572

P12G2: This one is laced with P. This one is really tender

P11G2: It is quite soft

P13G2: It does have that aftertaste, yeah

P12G2: Nah I can't

P13G2: . . .. kind of chemically, this ones like. . . .been cut on this one

P11G2: I like this one

P12G2: Tastes like Pen

P14G2: Tastes like what?

P12G2: Nothing

P14G2: Everything is so moderate though bro, nothings really intense

P11G2: Whats the number?
46:34
47:14 END OF SIDE A
BEGINNING OF SIDE B
Aladin: And the last sample

P11G2: I haven't put all my details on the last couple

P10G2: Oh, yeah, same

P12G2: Male, 19-25?

P13G2: Ah, whats the sample number for this one?

P9G2: "Anna"

Aladin: Ah, what is the code number?

Jordan: Two eight seven

Aladin: Two eight seven
P9G2: Its disgusting

P10G2: Two eight seven?

Aladin: Yes please

P10G2: Its making me want my pasta

P9G2: Did you say its making you want your pasta?

P10G2: Yeuuck

P9G2: Is there any courgettes or mushrooms left?

P8G2: Nah

Jordan: Which papers which?

P11G2: Ah.., mine was that one

P12G2: Cheers

P9G2: Thanks

P12G2: That's different, you can tell just by the look of it, its dark

P8G2: Oh, that's chewy

P11G2: Tenderness Stage. . ...definitely moderate

P12G2: I am thinking not so much
P13G2: Not really

P12G2: You like that one do you?

P13G2: I do, I like that ones flavour. That ones the processed one aye?

P12G2: Settle down Jimmy

P13G2: Oh man I just cut into it

P11G2: I don't know I couldn't pick it

P9G2: Oh, bad smell man

P11G2: I liked that one

P13G2: Its not bad. . .. .I liked that last one

P9G2: Thank you

P13G2: Thank you

Jordan: Are you finished?

P11G2: Yeah, you're good man, you're good

P13G2: . . ..theres forks
5:00

P11G2: I know theres forks

P13G2: Don't use your fingers, its not going to be counted
Inaudible

P11G2: Nah shes got four

P9G2: Just fingers

P13G2: Nah shes got more than four

P9G2: Just fingers!
Irrelevant conversation

Aladin: We have here some information for you regarding the consumption of meat and its impact on health if you can spend a few minutes please to read it and we have a five minute discussion about that

P9G2: Whats colorectal (whispered). Whats colorectal cancer?. Oh, oh
Laughter
P14G2: Huuuuuuh

P11G2: What was that?

P8G2: Rachel. Laughter, oh not that on to it today

P8G2: Sorry, sorry, sorry, at least I didn't have to ask myself

P13G2: Oh, this is only in women, its ok

P9G2: I am humiliated

P8G2: We're kiwi as
Jordan: Its not really something to be proud of

P12G2: Colorectal as
Laughter
P10G2: Wow, I did not know that

P9G2: There goes another example of my word vomit

P13G2: Where abouts is the Netherlands on this

Jordan: Do you want to use this or?

Aladin: I don't know

P14G2: Tiny

P9G2: Japan's really low

P13G2: They don't get any meat aye

P11G2: Right in the middle though

P11G2: That's another 10 g of meat a day

Jordan: Have you guys all had a good read of the sheet?

Aladin: So were you aware about this information about the potential relationship between consumption and any negative impact on health

P9G2: No

P10G2: No

P8G2: No

P9G2: I really wasn't

P13G2: Not particularly, no

P11G2: I heard something but I never really looked too much into it

P9G2: I've never thought about it aye

P8G2:Neither

Jordan : Did you hear it in the news or did you just hear it?

P11G2: Yeah, just through the media

Jordan: Though media?

P11G2: You hear like oh I don't know

P13G2:Some new study, report

P11G2:Meat, colorectal cancer, but you hear things about food and cancer all the time

P10G2: But they link everything to cancer

P11G2: Yeah that's kind of my

Jordan: Yeah

Aladin: Amalina what do you think?. You were aware of this connection?

P15G2:Mm just a little bit, I've heard about how the meats cooked and the relationship with cancer, especially with barbequing like carcinogenic..beef and

Jordan: But you have never heard of red meat and cancer, specifically red meat and cancer or?

P15G2:Processed meat and cancer

Jordan:Processed meat and cancer. Does that, does hearing about it like make you think at all about maybe trying to modify your diet or include.., reduce the amount of red meat

P9G2:Maybe

P11G2:Well I just want to know what part specifically of the meat, like is it consumption of things like processed meats or natural meat like.

Jordan: Well theres a high rate of processed meat compared to meat in general but its more noticed in red meat and overcooked meat as Amalina was saying.

P9G2:Overcooked

P10G2:Overcooked meat?

Jordan: Yeah sometimes um overcooking they think it produces carcinogenic compounds—compounds which cause cancer

Aladin: In barbequed or grilled

P9G2:Whats carcinogenic?

Jordan: It means that something causes cancer

P9G2:Oh

Aladin: So if there is potential, can add something to reduce the potential of carcinogenicity of the cooked meat will you be able to compromise that for taste or you prefer

P9G2:Eerr I just think its another thing that causes cancer so I wouldn't worry about it, like just another thing

P12G2: Yeah, I would be leaning to I'd rather keep the taste and not worry too much about cancer

P8G2: Yeah, its more dangerous to dye your hair, but maybe its not cancer, maybe its because we are all fat countries and we don't exercise as well and its not just because

P11G2:Wheres Australia

P8G2: I mean I am sure that we eat just as other things that much, during the day anyway you can't say just because you eat that much meat you are going to get cancer

P14G2:True

P8G2:If you are healthy and you have got a balanced lifestyle then you can eat red meat and not worry about it

Jordan: So would you try to include maybe a lot of vegetables along with the meat to balance it

P8G2: Yeah,mm, yeah

P13G2:Brocolli is meant to be good to stop cancer apparently

Jordan: So you would try to eat, would you ever try to eat broccoli along with meat like consciously to be healthy

P13G2: Nah

P13G2: I don't know aye, I like a bit of broccoli

P9G2: ..stir fry aye

P13G2: Don't get me wrong, broccoli is quite nice

Jordan: What about you Amalina?, do you try to eat a lot of vegetables with meat?

P15G2: Yeah, but I don't really consciously eat thinking oh I should eat vegetables because of my worries about cancer

P11G2: Yeah you can't just, you don't just want to have meat for dinner, you want something to have with it

Jordan: So in a burger if you had like lettuce or tomato as well it would be like for flavour and texture and not for health reasons

P9G2: Flavour yeah

P11G2: Bit of both

P10G2: Ah, I reckon its health reasons. Because you can't taste lettuce, tomatoes disgusting

P13G2: I think the flavours complement each other well. I like the tomato

P11G2: Tried and true

Jordan: So would you um, if you were given a processed meat product that already had an antioxidant source in it how would you feel about eating that would you choose that over a straight meat product or something you would make yourself?

P11G2: Mmm, nah, I wouldn't personally

P11G2: Not if I knew

P13G2: Nah, probably not

P11G2: Because you can get antioxidants from other things can't you, like broccoli

Jordan: Yeah
Laughter

P8G2: I think it would depend on the taste, if it tastes good, yeah..it depends

Aladin: If there is no difference in taste will you prefer that like over the normal one. I mean if they both taste the same and one is just have better chances

P9G2: If one had more benefits then yeah

P14G2: Normally I would rather I think something more natural

P13G2: Yeah I think something natural

P11G2: I don't want people chopping and changing my food

Jordan: So do you, do you like maybe the convenience of having your antioxidant source right there or something to just balance the meal right there in the burger or do you prefer to balance it yourself

P11G2: Balance it myself

P9G2: Because then like you are balancing it twice if you add vegetables on top anyway

P13G2: You know what you are balancing it with, so

P11G2: Yeah, this is true

Jordan: How do you feel about consuming the soy product tempeh

P10G2: I like that

Jordan: You have tried it before?

P12G2:Its because she is actually a vegetarian

P10G2:No, I'm not vegetarian

P13G2:Um, whats this tempeh thing?

P10G2:The guys have never had it

Jordan: Oh, ok. It's a traditional Indonesian soy product. Its all it is is soybeans and the skins are removed and its fermented. And its just um kept at a warm temperature and fermented and then it just, the fermentation helps it stick together and it also makes it more digestable.

P11G2:I don't like that

P14G2:That doesn't sound good

P9G2:That doesn't sound appealing

P11G2:Is that like similar to tofu

P10G2:Its really nice fried

What is that like a meat subsitute

Jordan:You can, yeah some people do substitute it with meat, vegetarians like to substitute it with meat because its got like kind of, they like the flavour and its considered quite healthy for a vegetarian because it has vitamin B12 which they don't often get as much

Aladin:Are you aware of that Amalina?

P15G2:Oh yeah, I eat tempeh quite a lot back home. Yeah, its one of my favourite foods

Jordan:So you are happy, you would be happy to eat it. Would you normally eat it along with meat?. You would prepare it in stir fry or?

P15G2:No, I eat it fried

P9G2:Yeah

P8G2:Does it taste like tofu?

P10G2No

P11G2:What does it taste like?

P15G2:The taste was quite strong in the beef, I could actually taste it

Jordan: Oh ok

P15G2:In two of them

Jordan:So do you think um, that having the tempeh in the burger would make you more willing to buy it or would it put you off?

P9G2:I think it would put me off

P11G2:Put me off

P14G2:Depends if I liked it or not, I've never had it before

P13G2:Try it once I spose

P14G2:Yeah, I don't know

P9G2:Try it but I would always pick real meat over it

Jordan:But if it was on a labelled you know a product where its labelled that its in the ingredients and it tasted really similar to what you were used to do you think it would maybe influence your decision to buy that.

P11G2:Yeah

P13G2:I spose so

P11G2:Yeah

P13G2:Probably

P8G2:If you outlined the ingredients its all like, you need to have that in it maybe, otherwise it would be like why change

Jordan: So you probably need it on the label or probably need to be told in advertising you think?

P12G2: Yeah if you knew, but if you didn't know and it just said contains you would be like whats that and probably wouldn't eat it

P8G2: Mm, yeah, if they said why it was good for you, you would be like ooh I would like

P12G2: Yeah if you knew, but if you didn't you would be like whats that

P8G2: Yeah

Jordan: So what if you had say a tempeh burger and you had a normal burger but the tempeh burger was cheaper, would that change the decision at all. Considering that you said you like the low price of processed meat?
P15G2: Would it taste as nice?

P8G2: Yeah, do they taste the same

Jordan: Yeah, they taste the same

P15G1: Then definitely

P9G2: I don't know

P11G2: Yeah

P9G2: If things taste the same I would probably always go with the cheaper one, but I'm a student

Aladin: So basically our project..ahh Jordan's project red meat actually contain ah lots of compounds, chemical compounds that can cause catalysis in the stomach during you eat it and that's why it cause free radical generation in the stomach, that's why it is potentially can cause cancer. What we are trying to do we are trying to add material that counteract that reduce free radical in the stomach. And a good source of antioxidant and has been used for long time to complement the meat is tempeh or soybean substitute. So tempeh is basically we add it as a source of antioxidant and we expect that it will improve the digestion and reduce the free radical generation. Ah one part is to see how consumer will be accepting that new product and basically you are helping us to identify which level of tempeh we can add. So we had four different types of burgers; one with 10% tempeh, one with 20% and one with 30% and one is we used bread crumb at 10%, and one is 100% beef. So your opinion, we will analyse your feedback and that will help us to make bigger, well choose one of this levels and try to get 120 people to tell us if they can find any differences between the control-100% beef and the one you said it taste better or taste similar

P9G2: I think that also like if you want to get to the inner hamburger it depends how it complements the rest of the ingredients like you could maybe not notice so much when you are having an actual burger

P15G2: Yeah

P9G2: When you are just trying it like that

ALadin: Mm, well we tried to eliminate any contribution of the side things like sauce, lettuce, which probably in an ideal world we should probably have it in an outlet and get the people to try you know. But what we found before that lots of people actually don't know the taste of 100% beef as it is. So we got , we had another focus group and we got confusing messages about, they preferred our product more. But just because they thought its 100% beef, which is not

P14G2: And you can be influenced by like how it looks, or I was

P8G2: Yeah

P14G2: Because

Aladin: It could be

Jordan: The cooked one or the raw one?

P14G2: Yeah the ones that were like, I don't know, some of them just seemed more meat like or the texture did anyway

Aladin: So the last thing we will do is we want to evaluate the raw burgers if which one you like. We have the five formulations and we want to see which one you will pick up best, if you went to the supermarket which one you probably will buy, which one looks better etc

P8G2: This is the like just looking at it one?

Aladin: Yeah, just the raw one. So we have the sheets for the evaluation here. And. . .

Aladin: So we are actually doing this study with compromise, actually what Jordan is doing processing for the development, also he will be working closely with the Dunedin hospital getting ah material that we can ..stimulate the digestion and doing sensory studies as well. So we will have the five samples put up, we try to keep them chilled so the colour doesn't change much, exactly as will happen in supermarket.

Inaudible conversation

Aladin: So we have the five samples here and. . .. .. . .you are able to have a look tell us which one you like most. . ..it will be really good. If you just put the sample and how do you like it. would be really good.

P11G2: Are we allowed to touch them?, just tricking

P13G2: Do we have to do one for each of them?

Aladin: Yes please

P11G2: After the second page mine goes into one of these, is that meant to do that?, is it different for every person?

Jordan: Did you have the five pages for the five burgers?

P11G2: Um
Aladin: AH we had one page for each sample

P1G2: Yeah my thing has got like

Aladin: Ah, ah, sorry

Jordan: Finished with your form?. Cool, thanks

Aladin: Thank you very much for your help. Who would like to do the draw?. You wanna do that?, ah you wanna do it?

P11G2: Nah I don't want to do it

Jordan: Which raw burger did you guys like?, which one of the raw ones? Did you think

P9G2: The reddest one.

P11G2: The reddest one yeah

P13G2: 447

P12G2: 447

Jordan: You liked this one did you?

P10G2: I liked that one

P8G2: I liked both of them

P11G2: The one that's got the breadcrumbs in it

P13G2: Is that one the breadcrumb one? 287?

Jordan: Ah its over so um 287, yup that's the breadcrumbs

P13G2: Is 447 the natural one?

P9G2: What was the second one we tasted cos that was my favourite?

Jordan: The ah, which number?

P11G2: What was the fourth one?

P12G2: What was the last one?

Aladin: Second was 085

Jordan: Should I just

P12G2: What was the last one?

P11G2: How much did that have in it?, 085?

Jordan: Should I just get um, I will get the forms and I will tell them what each one they tasted was

Jordan: The evaluation forms

P14G2:This could be make or break

Jordan:The first one was 287, that was the

P11G2:Oh, ok, is that aahh breadcrumbs

P11G2:That might have been a bad call

P13G2:What was 287

P10G2:The first one

P11G2:That breadcrumb one

P10G2:The breadcrumb one

P13G2:Is that breadcrumbs?

P10G2:I liked the second one best

P8G2:I didn't like any of them

P8G2:The fourth one

P11G2:Nah, they always try to trick you bro

Jordan:The first one was 447 that was all beef and 1% salt

P10G2:Oh the first one was all beef?

P13G2:The first one

P11G2:I thought the 447 was the all beef one

Jordan:Yeah, yeah, 447 was all beef
P11G2:Yeah

Jordan:085 was the second one and that was 20% tempeh

P10G2:That was my favourite

Aladin:602, the third one

Jordan:That was 10% tempeh

Aladin:And 572

Jordan:That was 30% tempeh

P8G2:That one tasted like chemicals

Jordan:Chemicals

Aladin:287

Jordan:That was 10% bread

P10G2:Oh yeah, yeah

P12G2:I remember I didn't like number fours so that was…

Jordan:So you guys really didn't like 30%

P10G2:No, I think 20% was the best one, 10% was not like it was like

P13G2:287, ah 287
Jordan:287?, oh yeah the breadcrumb

P12G2:But that that kind of proves that like the look of it versus taste of it because you wouldn't buy these ones on look but when you actually taste them they seem just the same almost

Jordan:The thing is people when they make the decision to buy it they are going to look at it first

P10G2:Yeah

Jordan:Unless they buy it through takeaways or something like that

P13G2:Add colouring to it

Aladin:So winner draws one, can you read the name

P13G2:Please be me

Jordan:Which ones could you taste the tempeh in Amalina?

Amalina:This, I think it was this two that I could taste, I couldn't taste any

Jordan:You couldn't taste 10%

P9G2:Thank you

Jordan:Thanks for coming

P13G2:Thank you

P11G2:Thank you

Aladin:Cheers

P8G2:Thank you

P12G2:Cheers

P10G2:Feel gross now

Jordan:Oh Amalina, I will just get you to um sign here. Can I just get you to write your name on that line and then sign there. Thanks guys. Cheers

ALadin:Thank you

Jordan:You guys can take the chips if you want

P12G2:Oh, nah

Jordan:Thanks

P15G2:Thank you so much

Aladin:Ok, so
END OF TAPE

Appendix 17: Sensory Trial recruitment flier

Participants needed

Do you like to eat hamburgers?

Participants are needed to taste different types of burger patties for a Masters project in the department of Food Science. The tasting will take around 10-15 minutes of your time and you get a tasty treat afterwards as well as the chance to taste these burger patties. To arrange a time to come in for tasting contact Jordan at

Email: sensory@stonebow.otago.ac.nz



This project has been reviewed and approved by the University of Otago Human Ethics Committee.

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Appendix 18: Sensory testing response ballot

Gender

1. Male
2. Female

Age

1. 18-24
2. 25-30
3. 31-40
4. 41-50
5. <50

Sample Number:

Overall Acceptance:

1. Dislike Extremely
2. Dislike Strongly
3. Dislike moderately
4. Dislike slightly
5. Neither like nor dislike
6. Like slightly
7. Like moderately
8. Like strongly

9. Like extremely

Intensity of beef odour

1. Not at all
2. Very Slight
3. Slight
4. Moderate
5. Strong
6. Very strong
7. Extreme

Tenderness

1. Very tough
2. Moderately Tough
3. Slightly tough
4. Neither tough nor tender
5. Moderately tender
6. Very tender
7. Extremely tender

Chewiness

1. Very chewy
2. Slightly chewy
3. Moderately chewy
4. Neither chewy nor soft
5. Moderately soft
6. Very soft
7. Extremely soft

Juiciness

1. Very dry
2. Slighty dry
3. Moderately dry
4. Neither juicy nor dry
5. Moderately juicy
6. Very juicy
7. Extremely juicy

Flavour: Intensity of flavour

1. Not at all
2. Very slight
3. Slight

4. Moderate
5. Strong
6. Very strong
7. Extreme

Other flavours: Non meat flavours

1. Not at all
2. Very slight
3. Slight
4. Moderate
5. Strong
6. Very strong
7. Extreme

Acceptance of Flavour

1. Dislike Extremely
2. Dislike strongly
3. Dislike moderately
4. Dislike slightly
5. Neither like nor dislike
6. Like slightly
7. Like moderately
8. Like strongly
9. Like Extremely

Sample Number:

Overall Acceptance:

1. Dislike Extremely
2. Dislike Strongly
3. Dislike moderately
4. Dislike slightly
5. Neither like nor dislike
6. Like slightly
7. Like moderately
8. Like strongly
9. Like extremely

Intensity of beef odour

1. Not at all

2. Very Slight
3. Slight
4. Moderate
5. Strong
6. Very strong
7. Extreme

Tenderness

1. Very tough
2. Moderately Tough
3. Slightly tough
4. Neither tough nor tender
5. Moderately tender
6. Very tender
7. Extremely tender

Chewiness

1. Very chewy
2. Slightly chewy
3. Moderately chewy
4. Neither chewy nor soft
5. Moderately soft
6. Very soft
7. Extremely soft

Juiciness

1. Very dry
2. Slighty dry
3. Moderately dry
4. Neither juicy nor dry
5. Moderately juicy
6. Very juicy
7. Extremely juicy

Flavour: Intensity of flavour

1. Not at all
2. Very slight
3. Slight
4. Moderate
5. Strong
6. Very strong
7. Extreme

Other flavours: Non meat flavours

1. Not at all
2. Very slight
3. Slight
4. Moderate
5. Strong
6. Very strong
7. Extreme

Acceptance of Flavour

1. Dislike Extremely
2. Dislike strongly
3. Dislike moderately
4. Dislike slightly
5. Neither like nor dislike
6. Like slightly
7. Like moderately
8. Like strongly
9. Like Extremely

Sample Number:

Overall Acceptance:

3. Dislike Extremely
4. Dislike Strongly
5. Dislike moderately
6. Dislike slightly
7. Neither like nor dislike
8. Like slightly
9. Like moderately
10. Like strongly
11. Like extremely

Intensity of beef odour

1. Not at all
2. Very Slight
3. Slight
4. Moderate
5. Strong
6. Very strong
7. Extreme

Tenderness

1. Very tough
2. Moderately Tough
3. Slightly tough
4. Neither tough nor tender
5. Moderately tender
6. Very tender
7. Extremely tender

Chewiness

1. Very chewy
2. Slightly chewy
3. Moderately chewy
4. Neither chewy nor soft
5. Moderately soft
6. Very soft
7. Extremely soft

Juiciness

1. Very dry
2. Slighty dry
3. Moderately dry
4. Neither juicy nor dry
5. Moderately juicy
6. Very juicy
7. Extremely juicy

Flavour: Intensity of flavour

1. Not at all
2. Very slight
3. Slight
4. Moderate
5. Strong
6. Very strong
7. Extreme

Other flavours: Non meat flavours

1. Not at all
2. Very slight
3. Slight
4. Moderate
5. Strong

6. Very strong
7. Extreme

Acceptance of Flavour

1. Dislike Extremely
2. Dislike strongly
3. Dislike moderately
4. Dislike slightly
5. Neither like nor dislike
6. Like slightly
7. Like moderately
8. Like strongly
9. Like Extremely

Consumption:

How often do you consume beef and beef products e.g. hamburgers

1. Once every fortnight
2. Once a week
3. Twice a week
4. More than twice a week

How often do you consume products where soy is the main ingredient

1. Never
2. Less than once a month
3. Once a month
4. Less than once a week
5. Once a week

Appendix 19: Front view of serving doors of sensory booths in the kitchen of the sensory science research centre



Appendix 20: Rear view of sensory booths



Appendix 21: Individual sensory booth



Appendix 22: Dry weight composition of soy tempeh compared to that of other studies

Author	Carbohydrate	Ash	Fat	Protein
Current study	17.36	3.7	32.7	46.21
Van der Riet et al, 1987	1.9	2.8	27.9	48.6
Zamora and Veum, 1978	-	4.4	22.0	45.9

Appendix 23: Information sheet for patients supplying gastric fluid during endoscopy

UNIVERSITY of OTAGO

Te Whare Wānanga o Otāgo

INFORMATION SHEET

Information for potential volunteers

Principal Investigator: Dr Michael Schultz - Senior Lecturer in Gastroenterology

Department of Gastroenterology, Dunedin Hospital and the Department of Medical and Surgical Sciences, Dunedin Medical School

Telephone contact: 03 474 0999 (Dr M Schultz)

Improvement of oxidative processes during the digestion of meat by addition of tempeh

Introduction

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide not to take part there will be no

disadvantage to you of any kind and we thank you for considering our request. The aim of the project is to discover the oxidative role of meat during digestion in healthy and patients on medication for stomach problems. Another aim for this project is to investigate if the incorporation of antioxidant source will be successful in improving the oxidative processes during digestion.

This leaflet will tell you about the project and how you would be involved. When you have read it carefully, you will be given an opportunity to discuss the study with one of the investigators. Please feel free to ask questions if there is anything you do not understand.

Your participation is entirely voluntary (your choice). You do not have to take part in the study, and if you choose not to take part it will not affect your care in any way.

If you do agree to take part you are free to withdraw from the study at any time, without having to give a reason and this will in no way affect your future health care**.**

If you need time to consider whether you wish to take part, just explain this to the investigator.

If you agree to take part you will be asked to complete a written consent form.

Outline of the project

A few studies reported the generation of free radicals (harmful compounds that can lead to some health problems) during the digestion of meat due to the catalytic effect of myoglobin and its derivatives in propagating lipid oxidation. The proposed current study is looking into ways to reduce the oxidative processes in simulated digestion of red meat and to inhibit free radical generation.

What is involved?

The digestive fluid is routinely sucked up from patients booked for gastroscopy as part of the procedure to improve visibility during the investigation of the gastric system. This fluid is normally collected in a waste basket for later disposal. For this project, gastric fluid will be collected in a separate container and it will be used for the further work on the digestion of meat. No extra harm, discomfort or extra effort will result from the collection of the fluid.

Where can I get more information about the study?

Further information can be obtained from the principal researcher (Dr Michael Schultz) whose contact details are given at the beginning of this information sheet.

How will the study affect my care?

The fact that you are in a study will not influence your care in any way. You may withdraw from participation in the project at any time and without any disadvantage to you of any kind.

Outcome of the study

It is intended that the results of the study will be published in a scientific journal. Copies of the article will be available to those participants who request it. There is often a considerable delay between data collection and publication. All the data will be stored for 10 years and then it will be destroyed according to the University regulations.

Privacy and Confidentiality

Once you have agreed to take part in the trial, some basic information regarding your age, ethnicity will be collected. The data and any information collected will be only accessible by Dr Michael Schultz - Senior Lecturer in Gastroenterology, Department of Gastroenterology and Dr Aladin Bekhit- Senior Lecturer in Food Science, Department of Food Science, University of Otago.

Any personal information (age and gender) that you provide will only be used for statistical analysis. It will be held privately, and will not be published or presented in any format that will allow you to be identified. Only the researchers directly involved in data collection will have access to the data.

The data collected will be securely stored in such a way that only those mentioned above will be able to gain access to it. At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for ten years, after which it will be destroyed.

Cultural Issues

As samples of gastric acid will be taken during this study, there may be cultural issues associated with the collection and storage of gastric acid that need to be discussed with your family/whanau. Some Iwi disagree with storage of human tissue citing whakapapa and advise their people to consult prior to participation in research where this occurs. To avoid problems at a later stage, we suggest your family/whanau is involved with you at all stages of this research. However, we also acknowledge that individuals have the right to choose to participate.

Compensation Arrangements

In the unlikely event of a physical injury as a result of your participation in this study, you will be covered by the accident compensation legislations with its limits. If you have any questions about ACC please feel free to ask the researcher for more information before you agree to take part in this trial.

If you have any queries or concerns about your rights as a participant in this study you may wish to contact a Health and Disability Services Consumer Advocate, telephone: (03) 479 0265 or 0800 37 77 66.

If there is a specific Maori issue/concern please contact Linda Grennell at 0800 377 766

Thank you for taking the time to consider this study.

This study has received ethical approval from the Lower South Regional Ethics Committee (LRS/09/08/033).

What if you have any Questions?
If you have any questions about our project, either now or in the future, please feel free to contact either:

Dr Michael Schultz - Senior Lecturer in Gastroenterology

Department of Gastroenterology,

Dunedin Hospital and the Department of Medical and Surgical Sciences,

Dunedin Medical School

Telephone contact: 03 474 0999

Or

Aladin Bekhit
Department of Food Science
University of Otago
PO Box 56, Dunedin
University Telephone Number: 03 479 4994
Email: aladin.bekhit@otago.ac.nz
Or

Jordan Taylor
Department of Food Science
University of Otago
PO Box 56, Dunedin
University Telephone Number: 03 479 7661
Email: tayjo522@student.otago.ac.nz

Appendix 24: Consent form for patients supplying gastric fluid during endoscopy



CONSENT TO PARTICIPATE IN MEDICAL RESEARCH

Improvement of oxidative processes during the digestion of meat by addition of tempeh

Principal Investigator: Dr Michael Schultz. Senior Lecturer in Rheumatology

Department of Gastroenterology, Dunedin Hospital and the Department of Medicine, Dunedin School of Medicine

Participant's Name:

_____

I have read and I understand the information sheet for people taking part in the study designed to the oxidative processes during the digestion of meat. I am satisfied with the answers I have been given.

I consent to participate in this study. I understand that taking part in this study is voluntary and that I may withdraw from the study at any time. This will not affect my continuing health care.

I understand that participation in this study is confidential and that no material which could identify me will be used in any reports on this study. Any data collected will be stored securely and anonymously.

I understand the compensation provisions for this study.

I know whom to contact if I have any concerns regarding the study, or wish to withdraw from the study.

This study has been given ethical approval by the Lower South Regional Ethics Committee.

This means that the Committee may check at any time that the study is following appropriate

ethical procedures.

I would like to be informed of the results of the study. **YES/NO**

Signed: _____ Date _____

Printed Name: _____

Address : _____

Researcher statement; I have explained the nature of the study to the participant and confirm his/her agreement to take part.

Signed: _____ Date _____

Printed name:_____

Version 18 August 2009