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**Faculty of Natural Resourses and Agricultural Sciences**

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# Abstract

Meat is an excellent source of many essential nutrients and makes an important contribution to a balanced diet. Vacuum frying is the technique of deep frying foods under vacuum, and is essentially a dehydration process that develops the desirable sensory properties of traditional fried foods. Vacuum frying has been demonstrated to great effect on fruits and vegetables, but has been used only rarely with animal products and, until now, not with beef. In this study, strips of marinated beef were vacuum fried and stored in two different packaging materials in order to determine the effects on lipid quality of packaging and storage. The packaging types were polyethylene terephthalate (PET) and aluminium foil laminate. Vacuum fried beef samples were stored at 11°C, 15°C, 25°C, 35°C and 39°C for 3, 5, 10, 15 and 17 weeks and the samples and extracted oil was analysed. Response surface methodology (RSM) was used to analyse the responses and the equations generated were also used to predict responses during storage up to 32 weeks at 15, 20 and 25°C.

Moisture content and water activity ($a_w$) were only affected following storage in PET packaging. Lipid decomposition was evidenced by significant increases in free fatty acids (FFAs) for both packaging types and Totox value with PET packaging, though these changes were considered to be minor with FFAs reaching only 4.0 (% oleic acid) during 17 weeks of storage and predicted to increase to over 8% after 32 weeks of storage and Totox value not exceeding 32 even after 32 weeks of storage. There were no significant changes in the unsaturated fatty acid content but the levels of polyunsaturated fatty acids, linoleic and linolenic acids decreased during 17 weeks of storage, and average levels were higher in the aluminium foil packaging, indicating that less oxidation had occurred. The tocopherol content decreased significantly from initial concentrations and mean levels were higher in the oil from vacuum fried beef strips stored in aluminium foil laminate packaging, but the response could not be satisfactorily modelled by RSM. Sterol oxidation products (SOPs) were also measured but were unable to be modelled, although higher average levels were found in the oil from vacuum fried beef strips stored in PET packaging.

The results of this experiment suggest that vacuum fried beef strips can be stored for up to 32 weeks at temperatures 15-25°C without significant lipid deterioration. Aluminium foil laminate

packaging reduced most of the changes that occurred due to lower oxygen and water vapour transmission rates. Future research into the shelf-life of vacuum fried beef strips should concentrate on the use of antioxidants to reduce oxidative changes, SOPs formation and a sensory analysis to determine the acceptability of the products after storage.

Keywords: Vacuum frying, packaging, PET, aluminium foil laminate, lipids, free fatty acids, tocopherols, Totox, sterol oxidation products

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# 1.0 Introduction

Meat is an excellent source of many essential nutrients, including haem iron, protein, B vitamins and zinc, and makes an important contribution to a balanced diet. As meat does not store well for extended periods of time, processing it into a form with an extended shelf life is desirable. Dehydration is one of the oldest forms of food preservation and can be achieved through many different processes. Currently the main type of dehydrated meat is jerky, which is air dried thin strips of meat often treated with preservatives such as sodium nitrite or benzoate. Jerky has a very chewy texture and is dark in colour, and can be smoked or marinated to improve the flavour. Pszczola (2002) identified innovations in 'meat snacks' as an area with high potential.

Food fried in fat and oil develops unique sensory characteristics that have proven to be desirable to consumers (Stier, 2000). Vacuum frying is the technique of deep-fat frying foods under a vacuum (5-150 mbar), which causes a reduction in the boiling point of the oil and the moisture in the food. Frying under reduced pressure serves to reduce oil content, discolouration and losses of vitamins and other compounds in the fried food product, as well as reducing the production of potentially harmful compounds normally associated with oxidation and high temperature processing (Dobarganes & Marquez-Ruiz, 2003; Fan, Mujumdar & Zhang, 2005; Shyu, Hau & Hwang, 1998). It is a technology that can be used to process foods into products with the necessary degree of dehydration without excessive darkening or scorching (Shyu, Hau & Hwang, 1998).

In response to the increasing health concerns of consumers (reviewed by Dueik & Bouchon, 2011), vacuum frying of fruit and vegetables has been studied as a potential alternative processing method for high quality snack foods. However, there are no recorded studies about vacuum frying meat as a snack source of protein.

Preliminary studies indicate that vacuum fried meat contains desirable organoleptic properties, being crunchy in texture and pleasant tasting. Vacuum fried meat has potential as a high value snack food that was tasty and has enhanced nutritional properties, such as no chemical preservatives, higher vitamin contents and fewer harmful lipid oxidation products (Dobarganes & Marquez-Ruiz, 2003) than its traditional counterparts. Through the use of marinades, a range of different flavours could be developed to further increase appeal to consumers. Recently, consumers have become more interested in purchasing foods

containing less saturated fat and more nutritional benefits from food manufacturers while requiring a level of convenience suitable for a modern lifestyle. Dehydrated foods that retain the physical and nutritional characteristics of the original product are desirable because they represent a convenient, nutritious food.

Previously reported quality changes in meat and dried meat products include changes in pH, colour, moisture content, water activity ($a_w$), lipids and cholesterol (Gök, Obuz & Akkaya, 2008; Kesava Rao, Kowale, Babu & Bisht, 1996; Lin & Lin, 2002; Modi, Sachindra, Nagegowda, Mahendrakar & Narasimha Rao, 2007; Rhee, Cho & Pradahn, 1998). Moisture content and $A_w$ can change during storage as moisture is obtained from the atmosphere and can affect texture, food safety and storage stability. Lipid stability is a major factor affecting meat product quality, as lipid oxidation products can be detected sensorially. Oil oxidation products such as oxidised monomeric, dimeric and oligomeric triacylglycerols formed from native triacylglycerols are reported to have negative effects on human health (Dobarganes & Marquez-Ruiz, 2003; 2006), and cholesterol can undergo oxidation to produce many compounds known collectively as cholesterol oxidation products (COPs) which may also be detrimental to health. Thus, minimising levels in foods is desirable. Temperature and packaging method have an important effect on meat product quality during storage, with high temperatures and aerobic packaging accelerating deterioration (Gök, Obuz & Akkaya, 2008; Kesava Rao *et al.,* 1996; Lin & Lin, 2002).

The main aim of this study was to investigate the effects of packaging type, storage temperature and storage time on the quality of vacuum fried beef. The moisture content, $a_w$, fatty acid (FA) profile, free fatty acid (FFA) content, tocopherol content, peroxide value (PV), *p*-anisidine value (*p*-AV), Totox value and sterol oxide levels were monitored to observe the changes occurring during storage of vacuum fried meat under a range of conditions. The ultimate objective is to develop vacuum fried beef strips as a high-value, nutritious and healthy snack food.

# 2.0 Literature Review

## 2.1 Deep Fat Frying of Foods

Food fried in fat and oil develops unique sensory characteristics that have shown to be desirable to consumers (Stier, 2000). Fats are able to carry and enhance other flavours and

create a desirable mouth-feel (Giese, 1996). The degradation of certain lipids in frying fats and oils generate the characteristic flavour of fried foods (Pokorny, 1999); however, oil deterioration as a result of oxidation, hydrolysis and thermal alteration reactions can negatively affect the quality of fried foods, and lipid oxidation has been identified as the single most important factor affecting oil quality (Fujisaki *et al.,* 2000; Saguy & Dana, 2003). Processing conditions can have a marked effect on the reactions that take place during frying; therefore, novel approaches to deep frying have been developed, including deep frying under vacuum.

Vacuum frying is the technique of deep fat frying foods under vacuum (5-150 mbar), which serves to reduce the oil content, discolouration and losses of vitamins and other compounds in the fried food product, as well as reduce the production of potentially harmful compounds normally associated with oxidation and high temperature processing (Dobarganes & Marquez-Ruiz, 2003; Fan, Mujumdar & Zhang, 2005; Shyu, Hau & Hwang, 1998). Frying under reduced pressure causes a reduction in the boiling point of both the oil and the moisture in the food. It is a technology that can be used to produce fruits and vegetables with the necessary degree of dehydration without excessive darkening or scorching of the products (Shyu, Hau & Hwang, 1998).

## 2.2 Health Concerns

Increasing consumer awareness of the links between food, nutrition and health have resulted in trends to limit the consumption of saturated fats, calories, and potentially toxic compounds produced during heat processing of foods.

Oil oxidation products such as oxidised monomeric, dimeric and oligomeric triacylglycerols formed from native triacylglycerols are reputed to have negative effects on human health (Dobarganes & Marquez-Ruiz, 2003; 2006), and thermal treatment of oils such as its use for deep-fat frying of foods is well known to accelerate oxidative reactions. In addition, cholesterol and other sterols such as phytosterols can undergo oxidation to produce numerous products, known as cholesterol oxidation products (COPs) and phytosterol oxidation products (POPs) and collectively referred to as sterol oxidation products (SOPs). COPs are produced in cholesterol-containing foods in conditions of light exposure, high temperatures, in the presence of oxygen and during storage (Savage, Dutta & Rodriguez-Estrada, 2002), and are a health concern due to their purported ability to change cell

membrane morphology and function (Vejux & Lizard, 2009), giving rise to atherogenic, cytotoxic, mutagenic and carcinogenic effects (Dutta *et al.,* 2006; Guardiola *et al.,* 1996; Savage, Dutta & Rodriguez-Estrada, 2002; Sevanian & Peterson, 1986). It is unclear what health risk, if any, POPs represent to humans (Hovenkamp *et al.,* 2008). While the risk of these compounds to humans consuming a normal diet remains unclear, chronic consumption of lipid oxidation products in large amounts is generally regarded as being detrimental to health (Dobarganes & Marquez-Ruiz, 2003).

Many of the benefits of vacuum frying arise from the lower cooking temperatures used and reduced oxygen exposure (Dueik, Robert & Bouchon, 2010). Vacuum frying has been shown to decrease acrylamide production in potato chips, as well as preserve vitamin C (normally reduced substantially during heat processing) and decrease the fat content of foods when compared to atmospheric frying (Da Silva & Moreira, 2008; Garayo & Moreira, 2002; Granda, Moreira & Tichy, 2004; Perez-Tinoco *et al.,* 2008). Therefore it appears that vacuum fried food products could be introduced as a healthier alternative to traditional fried snack products.

## 2.3 Vacuum Frying of Meat

Studies have been published on vacuum fried fruits and vegetables such as jackfruit, kiwifruit, apples, carrots, potatoes and others (Da Silva & Moreira, 2008; Diamante, 2008; Diamante, Presswood, Savage & Vanhanen, 2011; Garayo & Moreira, 2002; Granda, Moreira & Tichy, 2004; Mariscal & Bouchon, 2008; Shyu, Hau & Hwang, 2005). However, few studies have addressed vacuum frying of animal products and none have used beef. Andres-Bello, Garcia-Segonia and Martinez-Monzo (2010) investigated the use of vacuum frying technology to produce fried gilthead sea bream fillets with reduced oil content. The fish fillets fried under vacuum had lower oil content than their atmospheric counterparts, but some treatments appeared to absorb no oil due to variability in the initial fat content of the samples, and therefore the effects on oil absorption were less pronounced than in previous studies on fruits and vegetables with minimal fat content. Vacuum frying also retained the natural colour of the fish and reduced shrinkage.

As a dehydration process vacuum frying removes moisture from food, and the final moisture content appears to be inversely related to uptake of the surrounding cooking oil (Fan *et al.,*

2005). The stability of this oil is likely to be the main factor affecting the shelf life of vacuum fried foods.

## 2.4 Stability of Lipids in Food

During heat processing and in the presence of oxygen and moisture, fats and oils undergo three main types of reactions: hydrolysis, oxidation and thermal alteration. Hydrolysis of ester bonds within lipids results in the formation of FFA, mono-and di-acylglycerols and glycerols (Perkins, 2006). Oxidation produces rancid flavours and is the major cause of food spoilage for lipid-containing foods. Major primary oxidation products are hydroperoxides, which are not stable and decompose rapidly to secondary oxidation products such as aldehydes and ketones (Nawar, 1996). Peroxide value and *p*-AV can be used to measure primary and secondary oxidation products, respectively, and thus infer the extent of oxidation. Thermal alteration reactions are those such as isomerisation, cyclisation and polymerisation (Saguy & Dana, 2003).

While many studies have investigated changes during storage and oxidative stability of oils such as olive oil, nut oils, soybean oil and various types of fish oil, few have concentrated on the stability of oils in food. Masson *et al.* (2002) stored potato chips that had been fried in canola oil, sunflower oil and palm olein at 60°C for 16 days and found that the degree of unsaturation of the oils used appeared to determine its stability in the resulting potato chips. Kristensen *et al.* (2006) used diacylglycerol from rapeseed and sunflower oils in butter blends and evaluated the hydrolytic and oxidative stability over 12 weeks at 5°C. It was found that oxidation (as indicated by PV and volatiles) increased with time and was correlated with a decrease in α-tocopherol levels. Hexanal was the major volatile produced during storage, which is in agreement with it being the main oxidation product from linoleic acid, the major acid in both types of oil tested. The blends with higher degrees of unsaturation were again more susceptible to deterioration. There was no significant increase in FFA levels over time.

Hexanal levels in pecans during a storage experiment of 37 weeks at 25°C reached threshold levels for objectionable rancidity by sensory evaluation at week 22 of storage, corresponding to concentrations of 7 to 11 mg/kg pecans (Kanamangala *et al.,* 1999). FFA levels increased with storage in this experiment. Vanhanen and Savage (2006) found that the PV of walnut flour (walnut press cake) with 20% oil content increased with storage over 26 weeks at five

different temperatures. Walnut flour stored in brown paper bags increased in moisture content and PV more than flour stored in sealed polypropylene plastic containers and 11 µm high density polyethylene-lined paper bags, drawing consideration to the type of packaging that should be used during storage of foods. Jensen *et al.* (2003) also included different packaging types in a walnut storage experiment that used hexanal content as an indicator of rancidity. Polyethylene, polyethylene terephthalate (PET) and ethylene vinyl alcohol (EVOH) packaging were combined with an oxygen absorber, nitrogen ($N_2$) flushing or atmospheric air, and it was concluded that in the absence of cooled storage conditions rancidity could best be avoided using $N_2$ flushing and packaging with a low oxygen transmission rate such as PET or EVOH.

It appears that the amount of polyunsaturated fatty acids (PUFA), the degree of unsaturation of the lipids and the presence of oxygen and/or antioxidants are critical determinants of lipid deterioration in foods.

## 2.5 Storage of Meat Products and Packaging Effects

Some groups have directly investigated quality changes occurring in meat and meat products during storage and in response to different packaging conditions; however, few studies have looked in particular at packaging material, with the majority focused rather on modifying food atmospheric conditions.

Rao *et al.* (1996) investigated the effects of cooking and storage on water buffalo meat in terms of lipid deterioration and cholesterol oxides. It was found that FFA levels increased during both refrigerated and frozen storage of both raw and cooked meat. COPs increased in the meat after cooking and during storage, and TBARS values increased during storage, indicating an increase in rancidity. TBARS values were directly related to changes in storage temperature, underlining the importance of temperature effects on food quality during storage. The same group also looked at the quality of buffalo burger meat with different legume flour binders and during frozen storage (-16 ± 2°C) for four months. FFAs and TBARS both increased during storage, but the products were still acceptable after the storage period (Modi *et al.,* 2004a). Chicken nuggets stored frozen (-18 ± 2°C) for six months were also found acceptable, despite increases in FFAs and TBARS values (Modi *et al.,* 2004b).

Rhee, Cho and Pradahn (1998) found that the PV of expanded extrudates at 26.5% moisture content containing different meats increased during aerobic storage at 37°C for 120 days.

Extrudates containing beef or chicken had higher PVs than those containing goat, lamb or mutton, and this was attributed partly to differences in unsaturated fat content and partly to the presence of alkylphenols and thiophenol in goat, lamb and mutton causing an antioxidant effect. Meat-containing chicken snacks stored at 30°C for 30 days and sampled every six days were found to have significantly increased TBARS values after 12 days and significant changes in texture after six days of storage (Singh, Sanyal, Dubey & Mendirtta, 2011). The snacks were vacuum packed and the products were found to be still acceptable after the 30 day storage period. In another study on chicken products, chicken breast rolls were stored for up to seven days at 4°C, with or without vacuum, in oxygen permeable bags and analysed for lipid and protein oxidation at one, four and seven days (Xiao, Zhang, Lee, Ma & Ahn, 2011). The hens from which the meat was sourced had been fed a diet containing either oxidised oil (5% oxidised oil, PV=100) or antioxidants (500 IU vitamin E and 200 ppm butylated hydroxyanisole [BHA]). It appeared that antioxidant-supplemented chicken breast rolls were less susceptible to oxidation as measured by carbonyls (proteins), TBARS value and hexanal and pentanal production. Modi *et al.* (2007) used Box-Behnken RSM experimental design to optimise the levels of binders in ready-to-eat dehydrated chicken kebab mix, and investigated quality changes during storage in terms of pH, $a_w$, colour, FFA content and TBARS values. The Box-Behnken design was found to be useful in correctly optimising binder levels with respect to sensory quality, generating very high regression coefficients ($R^2$=0.950-0.998). During a six month storage experiment at room temperature (27 ± 2°C) it was found that pH, $a_w$, FFAs and TBARS values increased, with an accompanying slight colour change. After six months of storage, the chicken kebabs prepared from this mix were deemed acceptable to a sensory panel, although quality differences could be detected especially with regard to TBARS values and FFA content.

Pelser *et al.* (2007) substituted 10%, 15% and 20% of pork backfat with canola, flaxseed and fish oils in Dutch style fermented sausages in order to improve the nutritional quality with respect to essential fatty acids. It was found that the sausages with canola oil had comparable lipid oxidation characteristics to control sausages without any oil substitution, as indicated by measurement of PV, TBARS and hexanal. This was attributed to the relatively high concentration of tocopherols in canola as compared to flaxseed and fish oils. Similarly, Delgado-Pando *et al.* (2011) evaluated lipid stability (among others) in frankfurters with pork backfat substituted with healthier oil-in-water emulsions and stored at 2°C for 41 days.

TBARS values were increased from unsubstituted frankfurters and the degree of unsaturation of the oil used influenced the degree of oil oxidation. Kim, Jin, Mandal and Kang (2011) investigated the effects on quality of low-fat pork sausages using tomato powder as a food additive instead of nitrite to impart colour and functional effects. The sausages were stored at 4°C for one, 15 and 30 days under aerobic conditions and it was found that pH initially increased, then decreased after 15 and 30 days of storage respectively, and TBARS values were significantly decreased in tomato powder-containing sausages as compared to the control sausages. Colour was most stable during storage in sausages with the two lower concentrations of tomato powder, and texture did not noticeably change. Lin and Lin (2002) investigated the effects of packaging methods (vacuum packaging, $N_2$ gas flushing and flushing with varying concentrations of carbon dioxide [$CO_2$] gas) on the quality of reduced-fat Chinese-style sausage. There was a decrease in pH during 10 weeks of storage at 4°C, but no considerable changes in $a_w$ or colour. Interestingly, vacuum packing of sausages resulted in lower TBARS values than sausages packaged with $CO_2$ or $N_2$. Gök, Obuz and Akkaya (2008) also investigated packaging methods on the quality of a meat product during storage at 4°C for 120 days. In this study the product was Turkish pastirma, a dry cured beef product. Again, TBARS values increased with storage, as did hexanal content and colour change. The pH of the pastirma also increased with storage, which is in agreement with Modi *et al.* (2007) but not with Lin and Lin (2002). Modified atmosphere packaging (MAP) using 65% $N_2$ and 35% $CO_2$ was determined to be the optimum packaging method over aerobic packaging (AP) and vacuum packaging (VP). A very high correlation between subjective and objective quality parameters was obtained following a sensory analysis of the stored pastirma.

Taken together, these studies suggest that important quality parameters on which changes occur during storage are pH, colour, moisture content and $a_w$, lipids and cholesterol, and that the physico-chemical and sensory quality of dried and processed meat deteriorates over time. Aerobic packaging appears to cause the greatest deterioration of stored meat and meat products and antioxidants offer a protective effect, indicating that the majority of the changes are oxidative, and storage temperature also has a marked effect.

# 3.0 Objectives

The overall aim of this work was to develop vacuum fried beef as a high-value snack food. The present study evaluated lipid stability in vacuum fried beef during storage. Specific objectives were to:

- identify the effects of storage temperature on lipid quality in vacuum fried beef by comparing samples stored at different temperatures
- identify the effects of storage time on lipid quality in vacuum fried beef by comparing samples stored for different lengths of time
- identify the effects of packaging material on lipid quality in vacuum fried beef stored at various time-temperature combinations by comparing two packaging materials.

# 4.0 Materials and Methods

## 4.1 Sample Preparation

Meat (topside beef) was sourced from Independent Meat Processors Ltd. (Hornby, Christchurch) and sliced into approximately 3mm thick slices with a mechanical slicer (Bizerba GmbH & Co.Kg., Balingen, Germany). The meat slices were marinated in a 'Tapa' marinade sauce for 24 hours at 4°C and stored at -18°C until frying could commence. The marinated beef slices were then vacuum fried and approximately 50 g packaged in either aluminium laminate foil pouches or PET bags (Contour Sales & Packaging Systems, Tauranga, New Zealand). The thickness of the aluminium foil laminate and PET was 81 µm and 90 µm, respectively. The oxygen transmission rates (OTR) and water vapour transmission rates (WVTR) are presented in Table 1. The packages were flushed with nitrogen gas at 220 kPa pressure before being heat sealed to exclude atmospheric oxygen.

## 4.2 Experimental Design

The experiment used a central composite rotatable design (CCRD) consisting of a two-factor-three-level pattern with 12 design points (eight combinations with four replications of the centre point) (Myers, Montgomery & Anderson-Cook, 2009). This meant that for each type of packaging, two samples were stored at each temperature (15°C, 25°C and 35°C) with two

additional samples stored at 11°C and 39°C and four additional samples stored at the median temperature of 25°C. In terms of storage time, two samples for each packaging type were stored for 5, 10 and 15 weeks with two additional samples stored for 3 and 17 weeks, plus two replications of the middle storage time of 10 weeks. Therefore, 24 samples in total were incubated at the prescribed temperatures for varying lengths of time and then analysed. The temperature and time combinations are described in Table 2.

**Table 1: Oxygen and water vapour transmission rates of packaging materials**

| | **Polyethylene terephthalate (PET)*** | **Aluminium foil laminate** |
|---|---|---|
| **Oxygen transmission rate (OTR)** | 14.7 cc/$m^2$.day (23°C, dry) | 3.0 cc/$m^2$.day (23°C, dry) |
| **Water vapour transmission rate (WVTR)** | 2.0 g/$m^2$.day (23°C, 50% RH) | 2.0 g/$m^2$.day (25°C, 50% RH) |

*Lange and Wyser, 2003

## 4.3 Analyses

### 4.3.1 Moisture Content

The moisture content of the fried meat was measured by hot air drying the sample at 105 °C until a constant temperature was reached (AOAC, 2000). The moisture content was calculated from the weight difference between the original and dried samples and expressed as a percentage of the dried sample on a dry weight basis.

### 4.3.2 Water activity

Water activity of ground vacuum fried meat strips was measured in triplicate using an Aqua Lab water activity meter CX-2 (Decagon Devices, Inc., Washington, USA). Results were expressed as mean $a_w$ at the mean temperature of 21.7 ± 1.7°C.

### 4.3.3 Oil extraction

Oil was extracted from the vacuum fried beef samples using a modified method of Savage *et al.* (1997). In brief, approximately 40 g of ground vacuum fried beef was extracted in 60 ml of hexane:isopropanol (HIP) (3:2 v/v) at room temperature in 250 ml screw cap conical flasks on a rotary agitator (speed setting 9) for one hour. The homogenate was then allowed to settle overnight at 4°C, and the supernatant transferred to 50 ml screw cap test tubes and

washed with 30 ml of 6.7% w/v sodium sulphate. The supernatant was then transferred into 100 mL round-bottom flasks and evaporated to dryness using a Büchi Rotovapor-R (Postfach, Switzerland) set at 37.5°C, and any residual HIP evaporated under $N_2$ gas. The oil was stored in 4 ml glass screw cap bottles at -18°C and the headspace flushed with $N_2$ prior to analysis. The homogenate was re-extracted using the same procedure and the additional oil combined with the oil from the first extraction.

**Table 2: Storage conditions of vacuum fried beef strips with corresponding coded values**

| Storage Temperature | | Storage Time | |
|---|---|---|---|
| **Coded** | **Uncoded** | **Coded** | **Uncoded** |
| -1.00 | 15 °C | -1.00 | 5 weeks |
| 1.00 | 35 °C | -1.00 | 5 weeks |
| -1.00 | 15 °C | 1.00 | 15 weeks |
| 1.00 | 35 °C | 1.00 | 15 weeks |
| -1.41 | 11 °C | 0.00 | 10 weeks |
| 1.41 | 39 °C | 0.00 | 10 weeks |
| 0.00 | 25 °C | -1.41 | 3 weeks |
| 0.00 | 25 °C | 1.41 | 17 weeks |
| 0.00 | 25 °C | 0.00 | 10 weeks |
| 0.00 | 25 °C | 0.00 | 10 weeks |
| 0.00 | 25 °C | 0.00 | 10 weeks |
| 0.00 | 25 °C | 0.00 | 10 weeks |

### 4.3.4 Fatty Acid Profile

The FA profile of the oil in each vacuum fried meat sample was determined to monitor any changes occurring during storage. The method was that described by Appelqvist (1968). In brief, approximately 10 mg of the extracted oil was dissolved in 0.5 ml of hexane and transmethylated using 2 ml of 0.01 M NaOH in dry methanol in a shaking water bath at 60°C for 10 minutes. Three ml of boron trifluoride ($BF_3$) reagent was added and the solution incubated in a water bath at 60°C for 10 minutes. The tubes were cooled under running water, then 2 ml of 25% NaCl and 1 ml hexane were added. The tubes were shaken vigorously and centrifuged for one minute at 3000 rpm and the hexane layer collected. This

was followed by gas-liquid capillary column chromatography (GC) analysis of the resulting FA methyl esters (FAME), as described in Pickova *et al.* (1997).

A fused silica capillary column BPX 70 (SGE, Austin, Texas, USA) (length 60 m, i.d. 0.22 mm and thickness 0.25 µm) was used to separate the FAME. This was fitted in a Chromapack CP 9001 gas chromatograph with a flame ionisation detector, split/splitless injector with an auto-sampler CP 9050 and Maestro 2 version 2.4 software (Chromapack, Middelburg, Netherlands). The samples were injected in split mode at a ratio of 1:30. The oven temperature started at 150°C for seven minutes and then increased to 220°C at a rate of 2°C/minute. Helium was used as the carrier gas and nitrogen was the make-up gas, at a pressure of 220 kPa and a flow rate of 30 ml/minute, respectively. The detector and injector temperatures were 250°C and 230°C, respectively. Peaks were identified by comparing the retention times of known FAME single standards and reference sample mixtures 20A and 68A (Nu-Chek Prep, Elysian, MN). All samples for GC analysis were carried out in duplicate. The preparation of FAME was checked by thin-layer chromatography (TLC). Approximately 10 µl of the FAME sample was spotted onto pre-coated TLC plates (silica gel 60, 20 cm x 20 cm, 0.25 mm thick) and the plate developed in a mobile phase of hexane:diethyl ether:acetic acid (85:15:1, v/v/v). The plate was then transferred to an iodine chamber to view the results.

#### 4.3.5 Free Fatty Acid Content

The amount of FFA in the oil in each stored meat sample was determined according to the method described by Kwon and Rhee (1986). Approximately 100 µl of oil sample was dissolved in 5 ml of isooctane. One ml of cupric acetate-pyridine reagent (5% [w/v] aqueous solution of cupric acetate, adjusted to pH 6.1 with pyridine) was added and the contents mixed vigorously on a vortex mixer for 90 seconds. The tubes were allowed to stand for 10-20 seconds for good sedimentation of the aqueous phase, and the absorbance of the upper fatty acid-containing layer was read at 715 nm. The FFA % in the oil samples was calculated using a standard curve constructed using oleic acid as the standard solution and Equation 1 below:

$$y = kx + m \qquad \textit{(Equation 1)}$$

where:

y = absorbance

x = amount of oleic acid (µg)

### 4.3.6 Tocopherols

Tocopherols were analysed by HPLC according the method described by Dutta *et al.* (1994). Approximately 10 mg of oil sample was weighed accurately and diluted 1:100 with 1 ml of n-heptane (BDH, LiChrosolv). The diluted oil sample was then injected into a Waters 7725 Rheodyne injector with a 10 µl sample loop and 510 HPLC pump (Waters, Milford, USA). A Varian LC 9070 fluorescence detector (Varian, Walnut Creek, USA) was used for the detection of tocopherols at a wavelength of 294 nm for excitation and 320 nm for emission. A Lichrospher 100 $NH_2$ (250 x 4 mm, particle size 5 µ) coupled with a LiChroCART 4-4 guard column was used to analyse the samples. An isocratic elution was carried out using a mixture of heptane:tert-butylmethylether:tetrahydrofuran:methanol (79:20:0.98:0.02 v/v/v/v) at a flow rate of 1.2 ml/minute. Identification of peaks was performed by comparing the retention times to a standard reference mixture of α, β, γ and δ tocopherols. Integration of peaks was accomplished by a HP 3396A integrator (Hewlett-Packard, Avondale, USA). External standard curves were used for the quantification of tocopherols using standard solutions of α, β, γ and δ tocopherols at concentrations of 5, 25, 50, 75 and 100 ng/10 µl, and the amount of tocopherols (ng/g) in the oil samples was calculated using Equation 1, where *y = absorbance* and *x = amount of tocopherol* (ng). Tocopherol analysis was carried out in duplicate for all samples.

### 4.3.7 Peroxide Value

PVs were determined using the IDF 74A standard method (IDF, 1991). Briefly, approximately 200 mg of oil and 9.8 ml of chloroform:methanol (70:30 v/v) were combined in a test tube with 50 µl of ammonium thiocyanate (30% w/v) and then mixed on a vortex mixer. Fifty µl of iron (II) chloride solution (0.35 g of iron(II) chloride 4-hydrate [$FeCl_2.4H_2O$, ferrous chloride] made up to 100 ml volume with distilled water plus 2 ml of 10 M HCl) was added and the contents incubated at room temperature for five minutes. The absorbance was measured within 10 minutes at 500 nm against a blank containing all the reagents except the oil sample, and the concentration of iron(II) was determined by comparison to a standard curve and using Equation 1, where *y = absorbance* and *x = concentration of iron(II)* (µg). PV (meq. $O_2$/kg oil) was then calculated based on Equation 2:

$$PV = \frac{m}{55.84\ m_0} \qquad \textit{(Equation 2)}$$

where:

m = concentration of iron(II) (μg)

$m_0$ = weight of oil sample (g).

All oil samples were tested in duplicate.

#### 4.3.8 *p*-Anisidine Value

The *p*-AV was determined using the IUPAC standard method 2.504 (Paqout, 1987). Two hundred mg of oil sample was diluted to 10 ml with isooctane in a volumetric flask and the absorbance read at 350 nm. Five ml of this fat solution was then combined with 1 ml of 0.25% *p*-anisidine in glacial acetic acid and the absorbance at 350 nm was read after 10 minutes. The *p*-AV was given by Equation 3:

$$p\text{-AV} = \frac{10\,(1.2A_s - A_b)}{m} \qquad \textit{(Equation 3)}$$

where:

$A_s$ = absorbance of fat solution at 350 nm after reaction with p-anisidine reagent

$A_b$ = absorbance of fat solution alone at 350 nm

m = mass of oil sample (g).

#### 4.3.9 Totox Value

PV and *p*-AV values were combined to give the 'Totox' value, which describes the total of peroxides and secondary oxidation products in oxidising oil. The Totox value is given by Equation 4:

$$\text{Totox Value} = (2 \times \text{PV}) + \text{p-AV} \qquad \textit{(Equation 4)}$$

#### 4.3.10 Sterol Analysis

Total sterols were analysed following the method described by Ubhayasekera and Dutta (2009). Approximately 200 mg of oil samples (in duplicate) and 1 ml of 2 M KOH in 95% ethanol were combined in a glass test tube and hot saponified in a water bath at 100°C for 10 minutes. The reaction was stopped by cooling the tubes, then 1 ml of water, 2 ml of hexane containing 10-30 μg/ml of internal standard (5α-cholestane) and 0.2 ml of absolute ethanol were added. The tubes were mixed thoroughly and centrifuged at 3000 rpm for three minutes after which the hexane layer was transferred to a smaller glass tube and the solvent evaporated under $N_2$ gas. One hundred μl of Tri-Sil reagent was added and the tubes incubated at 60°C for 45 minutes to form trimethylsilyl (TMS)-ethers. The solvent was

then evaporated again under $N_2$ gas and the TMS-ether derivatives dissolved in 500 µl of hexane. The tubes were centrifuged at 3000 rpm for three minutes and then analysed by GC and GC-MS.

The analysis of total sterols was performed on a Chromapack CP 9001 gas chromatograph (Chromapack, Middelburg, The Netherlands) with a flame ionisation detector and a split/splitless injector. Separation of sterols was carried out on a non-polar capillary column, DB-5MS (J&W Scientific, Folsom, CA, USA) (20 m x 0.18 mm x 0.18 µm). The samples were injected using an auto-sampler CP 9050 with a split mode of injection, and the split ratio was 1:30. The oven temperature was programmed to 60°C for one minute, followed by gradual increments of 40°C per minute until 280°C at which point it was held for two minutes and then further increased to 320°C at a rate of 2°C per minute. Detector and injector temperatures were 325°C and 260°C, respectively. Helium was used as the carrier gas at 70 kPa pressure and $N_2$ was the make-up gas at a flow rate of 30 ml per minute. Sterols were identified by comparing the retention times with those of sterol standards, and confirmed using GC-MS. The peak areas were integrated using the software Maestro 2 version 2.4 (Chromapack, Middelburg, The Netherlands). The internal standard (5α-cholestane) was used for the quantification of individual sterols and the concentration of sterols (mg/g) in the oil samples was calculated using Equation 5:

$$x = \frac{\text{Internal standard (µg)} \times Y}{\text{Weight of oil sample (g)} \times Z} \qquad \textit{(Equation 5)}$$

where:

x = concentration of identified sterols (mg/g)

*Y* = area of identified sterol peak

*Z* = Area of internal standard peak.

The sterols in the unsaponifiable fraction were identified by the GC-MS mass spectra of standard samples of sterols using a gas chromatograph (ThermoQuest Italia S. p. A., Rodano, Italy) coupled with a Voyager mass spectrometer with the MassLab data system version 1.4 V (Finnigan, Manchester, England). The sterols were separated on the same column as used for the GC analysis. Helium was used as the carrier gas at an inlet pressure of 180 kPa. The injector temperature was 260°C and the samples were injected in splitless mode. The oven temperature programme was 60°C for one minute, then increased to 280°C at a rate of 50°C

per minute and held for five minutes, and then finally increased to 300°C at a rate of 1°C per minute and held for seven minutes.

#### 4.3.11 Sterol Oxidation Product Analysis

Cholesterol and phytosterol oxidation products were analysed using a GC and GC-MS method, as reported by Azadmard-Damirchi and Dutta (2008), with some minor modifications. A combined method was used as the vacuum fried meat contained both cholesterol from the meat and phytosterols from the cooking oil.

For purification of SOPs by transesterification, approximately 100 mg of oil sample was dissolved in 2.5 ml of sodium methylate (30% in methanol) diluted with tert-butylmethyl ether (TBME) (4:6, v/v), mixed thoroughly for 30 seconds and incubated at room temperature for one hour with additional mixing after 30 minutes. Two ml of water and 5 ml of dichloromethane were added to the tubes and shaken vigorously; the tubes were then centrifuged at 3000 rpm for three minutes. The water phase was removed and 2 ml of citric acid (1% aqueous) was added to neutralise excess alkali, the tubes shaken and centrifuged again. The aqueous layer was again removed, the dichloromethane evaporated under $N_2$ gas, and the unsaponifiable fraction dissolved in 1 mL hexane:diethyl ether (9:1, v/v). Enrichment of sterol oxides by solid phase extraction (SPE) was performed by loading the transesterified samples onto a 1.0 g silica cartridge solvated with 5 ml of hexane and with a small amount of dry sodium sulphate on the top. The tube was washed with 15 ml hexane:diethyl ether (9:1, v/v) and 10 ml of hexane:diethyl ether (1:1, v/v) to elute the non-SOP compounds. The SOPs and the remaining unoxidised sterols were eluted with 10 ml of acetone and collected, then dried under $N_2$ gas and the residue again dissolved in 1 ml of hexane:diethyl ether (9:1, v/v). Another 1.0 g silica column was then prepared as previously and a second SPE performed following the same procedure. Approximately 5 µg of 5α-cholestane in hexane was then added to the acetone fraction, the solvent evaporated under $N_2$ gas and the SOPs derivatised to TMS-ethers, as described above, for GC and GC–MS analysis.

For GC analysis of SOPs, a DB-5MS (10 m x 0.18 µm x 0.18 µm) (J & W Scientific, Folsom, CA, USA) silica capillary column was fitted in a Chromapack CP 9001 gas chromatograph (Varian, Palo Alto, CA, USA) and used to separate the TMS-ethers. The oven temperature was set at 60°C for one minute, then increased to 280°C at a rate of 50°C per minute, maintained for

eight minutes then increased to 300°C at a rate of 1°C per minute. Helium was used as the carrier gas at a pressure of 80 kPa and $N_2$ gas was the make-up gas at a flow rate of 30 ml/minute. The samples were injected in splitless mode and the injector temperature was 260°C. The SOPs were identified by comparing the retention times obtained from the standard COPs and authentic POPs samples. SOPs were quantified using an internal standard (5α-cholestane) and Equation 5 where *x = concentration of identified SOPs* (μg/g), *Y = area of identified SOPs peak* and *Z = area of internal standard peak.* The results were presented as the mean of duplicate analyses.

Both COPs and POPs were identified by GC-MS by comparing their mass spectra against those of standard COPs, synthesised authentic samples of POPs and published data (Dutta, 2002). The TMS derivatives of SOPs were analysed according to the procedure described above for total sterols analysis by GC-MS.

## 4.4 Statistical Analysis

The data was analysed using Design Expert 8 (Stat-Ease Inc., Minneapolis, USA) to obtain linear, 2-factor interaction and quadratic mathematical models, as shown below (respectively):

$$y = a_0 + a_1x_1 + a_2x_2 \qquad \textit{(Equation 6)}$$

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_1x_2 \qquad \textit{(Equation 7)}$$

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_1x_2 + a_4x_1^2 + a_5x_2^2 \qquad \textit{(Equation 8)}$$

where:

y = dependent variable (chemical properties) (actual values)

$a_0$, $a_1$, $a_2$, $a_3$, $a_4$, $a_5$, = coefficients

$x_1$ (storage temperature), $x_2$ (storage time) = independent variables (coded values [Table 3]).

The derived equations were used to predict the properties at the different conditions. The relationships between the independent variables were plotted against the dependent variables determined on the vacuum fried beef and extracted oil after storage using SigmaPlot version 12 (Systat Software Inc., San Jose, CA, USA) to show the changes occurring in vacuum fried meat during storage over a defined temperature range.

**Table 3: Coded values for the independent variables of storage temperature (°C) and storage time (weeks)**

| | | Codes | | | | |
|---|---|---|---|---|---|---|
| | **Variable** | **-1.41** | **-1** | **0** | **1** | **1.41** |
| $x_1$ | Storage temperature (°C) | 11 | 15 | 25 | 35 | 39 |
| $x_2$ | Storage time (weeks) | 3 | 5 | 10 | 15 | 17 |

The percent difference between the predicted values and the actual experimental values, plus the mean relative percentage error (MRPE), was calculated for each dependent variable using the following equations:

$$\% \text{ difference} = \frac{\left( \left| Y_{\text{Predicted}} - Y_{\text{Experimental}} \right| \right)}{Y_{\text{Experimental}}} \times 100 \qquad \textit{(Equation 9)}$$

$$\text{MRPE (\%)} = \frac{\Sigma \text{ (\% difference)}}{\text{number of data}} \qquad \textit{(Equation 10)}$$

The % difference and MRPE were calculated to evaluate the reliability of the predicted values. According to Diamante, Durand, Savage and Vanhanen (2010), an MRPE of 13% or lower is satisfactory for most food engineering purposes.

The models generated by RSM analysis were also used to extrapolate the response of the dependent variables over extended storage periods and at temperatures not necessarily used in this experiment, but that would be reasonably expected to be encountered during the shelf life of vacuum fried beef strips in a retail context.

# 5.0 Results

Table 4 summarises the statistical analysis of the different properties of vacuum fried beef packaged in PET and aluminium foil laminate. Polyunsaturated, C18:2 and C18:3 fatty acids, *p*-AV, cholesterol, sitosterol and total SOPs of the samples stored in PET and aluminium laminate packaging were found to be not significantly different. Table 5 presents the mathematical model equations obtained for each property, the statistical significance of the different coefficients and the MRPE.

Using the different equations, the responses of the significant dependent variables were able to be predicted over a given set of storage conditions, namely 15, 20 and 25°C for 16, 24 and 32 weeks. The coded values were calculated based on the equations given in Appendix 2 (a) and (b) and are given in Table 6. It was assumed during the extended storage prediction that the nature of the response of the dependent variables remained the same as for the experimental storage conditions. The error associated with this assumption was not able to be measured.

The results of the predictions can be viewed in Appendix 3. Not all of the data was able to be satisfactorily predicted due to ill-fitting models, as shown by the high MRPE values in Table 5.

**Table 4: Summary of RSM analysis of vacuum fried beef strips stored at different temperatures for different lengths of time in two packaging materials**

| Dependent variable | Packaging type | |
|---|---|---|
| | PET | Aluminium foil laminate |
| Moisture content | * | NS |
| Water activity | *** | NS |
| PUFA | NS | NS |
| C18:2 | NS | NS |
| C18:3 | NS | NS |
| Free fatty acids | * | * |
| α-tocopherol | * | * |
| γ-tocopherol | * | *** |
| δ-tocopherol | *** | NS |
| Peroxide value | * | ** |
| p-Anisidine value | NS | NS |
| Totox | ** | NS |
| Cholesterol | NS | NS |
| Sitosterol | NS | NS |
| Total SOPs | NS | NS |

PUFA = Polyunsaturated fatty acids; SOPs = sterol oxidation products; PET = Polyethylene terephthalate packaging; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = Not significant.

## 5.1 Moisture Content

The response of moisture content of vacuum fried beef strips during storage can be viewed in Figure 2. A significant response ($p<0.05$) was only experienced for the samples packaged in PET (Table 4), and while the moisture content increased from the initial level of 4.17 ± 0.17% (db) (data not shown) it actually appeared to decrease with increasing storage temperature, reaching the lowest levels of around 4.50% (db) at the highest temperature

and longest storage time. Further modelling of moisture content during storage in PET packaging for up to eight months can be seen in Figure 2. There was no significant response to storage temperature or time for the samples packaged in aluminium foil laminate packaging and the mean moisture content was 5.95 ± 0.55% (db) (data not presented).

## 5.2 Water Activity

Significant changes were observed for the PET packaged samples only in response to the linear, quadratic and 2-factor interaction coefficients of storage temperature and time (Table 5). As for moisture content, $a_w$ of vacuum fried beef strips stored in PET packaging significantly decreased throughout the experiment but was higher than the initial $a_w$ of 0.19 ± 0.01 (data not shown). There was no significant effect for the aluminium foil laminate packaged samples, which had consistently lower $a_w$ than the corresponding PET packaged samples (data not shown). The mean $a_w$ of the samples stored in aluminium foil laminate packaging type was 0.21 ± 0.01 (data not shown) and was not affected by storage temperature or time.

**Table 5: Mathematical model equations generated by RSM analysis in terms of coded factors and corresponding mean relative percentage error (MRPE).**

| Dependent variable | | Equation | MRPE |
|---|---|---|---|
| Moisture content | PET | $7.00 – 1.52\ x_1{*} – 0.25x_2$ | 11.4 |
| | Al | - | - |
| Water activity | PET | $0.26 – 0.058\ x_1{**} + 9.398E\text{-}003\ x_2{**} – 0.015\ x_1x_2{**} – 0.019\ x_1{}^2{**} + 6.250E\text{-}003\ x_2{}^2{*}$ | 2.3 |
| | Al | - | - |
| Free fatty acids | PET | $1.78 + 0.54\ x_1{*} + 0.29x_2$ | 16.3 |
| | Al | $1.32 + 0.82\ x_1{**} + 0.16x_2 – 0.025x_1x_2 + 0.55\ x_1{}^2{*} + 0.32x_2{}^2$ | 19.4 |
| α-tocopherol | PET | $81.00 + 27.45\ x_1{**} – 3.36x_2$ | 20.1 |
| | Al | $1.53 + 36.08\ x_1{**} + 9.57x_2 + 18.21x_1x_2 + 34.35\ x_1{}^2{**} – 2.94x_2{}^2$ | 110.2 |
| γ-tocopherol | PET | $62.19 + 49.18\ x_1{*} – 42.73x_2 – 65.52x_1x_2$ | 118.7 |
| | Al | $106.34 + 243.29\ x_1{***} + 80.65\ x_2{*} + 134.35\ x_1x_2{**} + 147.53\ x_1{}^2{**} + 9.62x_2{}^2$ | 94.4 |
| δ-tocopherol | PET | $0.00 + 7.63\ x_1{**} – 15.35\ x_2{***} – 6.85\ x_1x_2{*} + 3.96x_1{}^2 + 12.56\ x_2{}^2{***}$ | 12.9 |
| | Al | - | - |

| | | | |
|---|---|---|---|
| **Peroxide value** | **PET** | $4.12 + 0.43x_1 + 1.14\ x_2{**} - 0.93\ x_1x_2{**} - 1.937E\text{-}003x_1^2 - 0.91\ x_2^2\ *$ | 32.6 |
| | **Al** | $4.12 + 0.077x_1 + 0.75\ x_2{**} - 1.03x_1x_2 - 0.23x_1^2 - 1.26\ x_2^2\ ***$ | 64.3 |
| **Totox value** | **PET** | $18.84 + 1.23x_1 + 2.00\ x_2{**} - 1.44\ x_1x_2{*} + 1.25x_1^2 - 0.076\ x_2^2{*}$ | 3.5 |
| | **Al** | - | - |

PET = Polyethylene terephthalate packaging; Al = Aluminium foil laminate packaging; MRPE = Mean relative percentage error; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Prediction of the response of $a_w$ to extended storage, as can be seen in Figure 1 (d), shows that it continues to increase, reaching nearly 0.55 by week 32 at 15°C. Even at this level of $a_w$, microbial proliferation is inhibited. Although the safety of stored vacuum fried beef strips was not of concern in the present study, it should form part of subsequent shelf life studies.

**Table 6: Extended storage response prediction coded values of the independent variables of storage temperature (°C) and extended storage time (weeks)**

| | **Variable** | | | | | |
|---|---|---|---|---|---|---|
| | **$x_1$** | | | **$x_2$** | | |
| | **Storage temperature (°C)** | | | **Storage time (weeks)** | | |
| | 15 | 20 | 25 | 16 | 24 | 32 |
| **Codes** | -1.00 | -0.50 | 0.00 | 1.20 | 2.40 | 4.40 |

## 5.3 Fatty Acid Profile

There was no significant effect on the total PUFA, C18:2 or C18:3 FAs in the vacuum fried beef oil after storage for either packaging type. The mean values as a percentage of total FAs and standard errors are presented in Table 7.

**Table 7: Mean values of PUFAs, C18:2 and C18:3 FAs (% of total FAs) in oil extracted from vacuum fried beef strips after storage**

| | **Packaging type** | |
|---|---|---|
| | **PET** | **Aluminium foil laminate** |
| **Total PUFA (%)** | 19.9 ± 0.7 | 20.1 ± 0.5 |
| **C18:2 (%)** | 14.2 ± 0.5 | 14.3 ± 0.3 |
| **C18:3 (%)** | 5.7 ± 0.2 | 5.8 ± 0.2 |

## 5.4 Free Fatty Acid Content

Storage temperature caused a significant increase in the FFA content of the vacuum fried beef oil for both packaging types (Table 4). Figures 2 (a) and (b) show the responses. The amount of FFAs as % equivalent oleic acid increased in both packaging types as the storage temperature was increased and reached almost 4% after 17 weeks storage at 39°C in aluminium foil laminate packaging. Storage time had no significant effect. The MRPE was 16.3% for PET packaging and 19.4% for the aluminium foil laminate packaging so extended storage prediction for FFAs was not carried out.

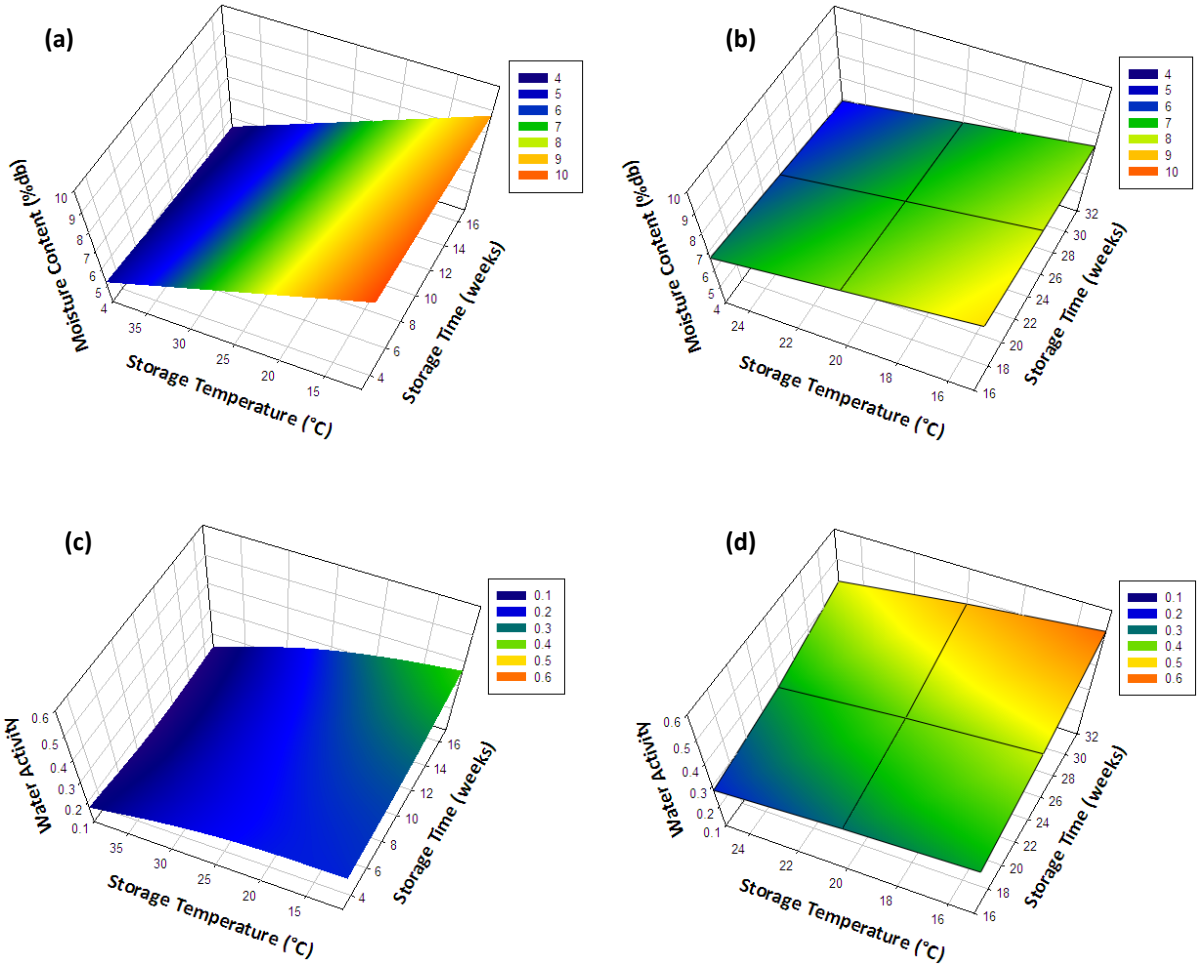


**Figure 1: (a) and (c) Responses of moisture content and $a_w$ of vacuum fried beef strips during storage in PET packaging; (b) and (d) Predicted responses up to 32 weeks storage at 15-25°C.**

## 5.5 Tocopherols

The tocopherols detected in the vacuum fried beef oil were contributed by the cooking oil, as the raw meat contained no tocopherols (data not shown). The levels of α-, γ- and δ-tocopherol in the vacuum fried meat samples were decreased from fresh (unused) canola

oil, presumably by the vacuum frying process (data not shown). The storage temperature appeared to have the greatest effect on tocopherol content. For the PET packaging type, α- and γ-tocopherol were affected by the linear coefficients of storage temperature while for the aluminium foil laminate packaging type α- and γ-tocopherol were affected by the linear and quadratic coefficients of storage temperature. The concentration of δ-tocopherol in the PET packaged samples was affected by the linear and quadratic coefficients of storage time and their 2-factor interaction coefficient (Table 5).

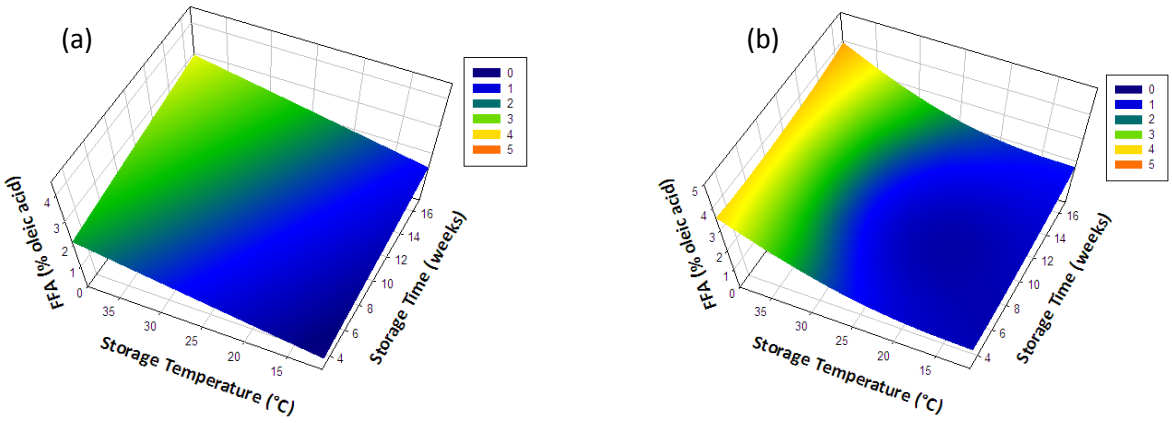


**Figure 2: Response of FFAs in oil extracted from vacuum fried beef strips after storage in (a) PET packaging and (b) aluminium foil laminate packaging.**

From the surface plots in Figure 3, the levels of α-tocopherol in the vacuum fried beef strips stored in aluminium foil laminate packaging looked to be overall lower than their PET counterparts, except at high storage temperature-long storage time combinations. Higher concentrations of γ-tocopherol were achieved in the aluminium foil laminate packaging type, especially again at high temperature-long storage time combinations.

The MRPE values calculated for α- and γ-tocopherol of both packaging types were well above 13% (Table 5) and therefore not further analysed. The quadratic model used for δ-tocopherol (PET packaging) was not able to model the predicted response to extended storage for 32 weeks satisfactorily.

## 5.6 Peroxide Value, *p*-Anisidine Value and Totox Value

The PV of the extracted oil from the vacuum fried beef samples significantly increased during storage for both packaging types, but the Totox value was only significantly affected in the

samples stored in PET packaging. The mean *p*-AV of vacuum fried beef strips was 12.6 ± 2.2 for PET packaging and 13.7 ± 2.7 for aluminium foil laminate packaging (data not shown), and there were no significant effects on *p*-AV of the oil for any of the stored beef samples. PV increased as the storage time was increased and the two-factor interaction coefficient of storage temperature and time ($p<0.04$) was significant for PV for the PET packaging type, indicating an interaction effect between storage temperature and time on peroxide formation. Totox value also increased with storage time (Table 5).

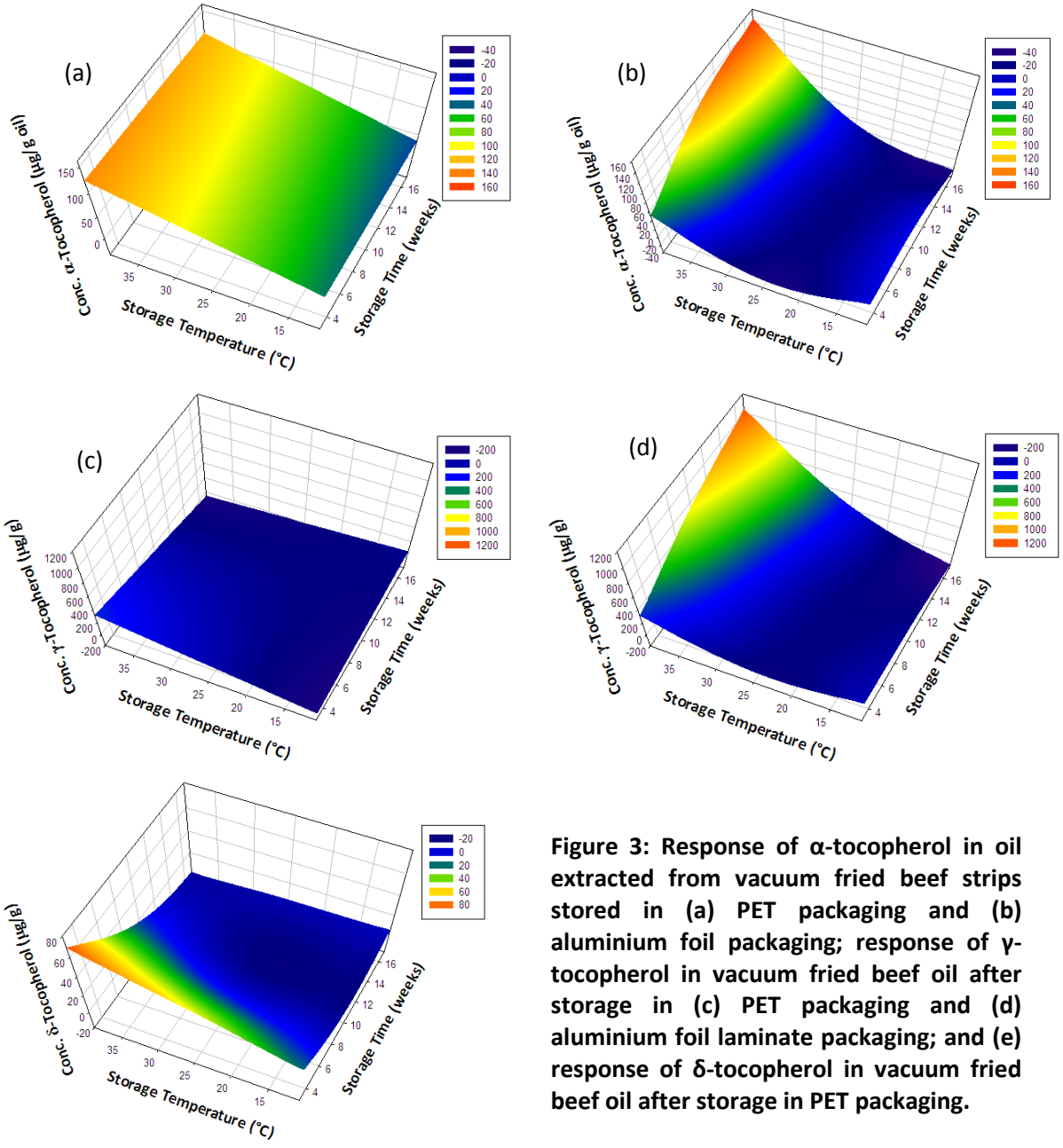


**Figure 3: Response of α-tocopherol in oil extracted from vacuum fried beef strips stored in (a) PET packaging and (b) aluminium foil packaging; response of γ-tocopherol in vacuum fried beef oil after storage in (c) PET packaging and (d) aluminium foil laminate packaging; and (e) response of δ-tocopherol in vacuum fried beef oil after storage in PET packaging.**

The MRPE values of PV for PET and aluminium foil laminate packaging were 32.6% and 64.3% respectively, and so PV was not further analysed. Extended storage prediction of Totox value for vacuum fried beef stored in PET packaging is given in Figure 4 (d) and shows the Totox value increasing as the storage time is increased. The experimental predicted Totox values did not exceed 25, and this was predicted to increase to approximately 32 after 32 weeks storage at 15°C.

## 5.7 Sterol Analysis

There were no significant changes in sterols in the oil extracted from vacuum fried beef strips after storage. The results were rather variable and neither packaging type appeared to give consistently higher or lower levels. There was no clear decreasing trend as would be expected, and at times the levels actually appeared to increase with storage which suggests the results were unreliable.

Cholesterol was the predominant sterol with a mean concentration of 4.4 ± 0.4 mg/g when stored in PET packaging and 4.1 ± 0.9 mg/g in aluminium foil laminate. This was followed by sitosterol, the predominant sterol in canola oil (Azadmard-Damirchi & Dutta, 2008), with mean concentrations of 1.1 ± 0.7 mg/g and 1.1 ± 1.0 mg/g in PET and aluminium foil laminate packaging, respectively. Campesterol, brassicasterol, squalene, stigmasterol and an unsaponifiable fraction were also identified, though at much lower concentrations.

## 5.8 Sterol Oxidation Product Analysis

The SOPs data gave very variable results and there were few significant changes when analysed by RSM, none of which were reported due to a lack of integrity in the data. 7α-hydroxy cholesterol, 7β-hydroxy cholesterol and 7-keto cholesterol were present in the highest concentrations with 8.4 ± 3.9 μg/g, 8.4 ± 4.5 μg/g and 10.3 ± 5.1 μg/g respectively when stored in PET packaging, but only 3.8 ± 3.2 μg/g, 3.6 ± 3.3 μg/g and 5.1 ± 4.3 μg/g (respectively) in aluminium foil laminate. The POP 7-keto sitosterol was consistently present at a mean level of 2.4 ± 1.3 μg/g in PET packaged samples and 1.5 ± 1.1 μg/g in aluminium foil laminate packaged samples. Total SOPs were on average 180.0 ± 49.0 μg/g in PET and 160.2 ± 39.8 μg/g in aluminium foil laminate packaged samples.

# 6.0 Discussion

## 6.1 Moisture Content

There was a significant decrease in the moisture content of the vacuum fried beef strips when stored in PET packaging, affected by storage temperature. Appendix 1 gives the relative humidity (RH) of the incubators that were used during the storage experiment and shows RH decreasing as the temperature increased. This undoubtedly was responsible for the observable decrease in the moisture content of the vacuum fried beef strips as the

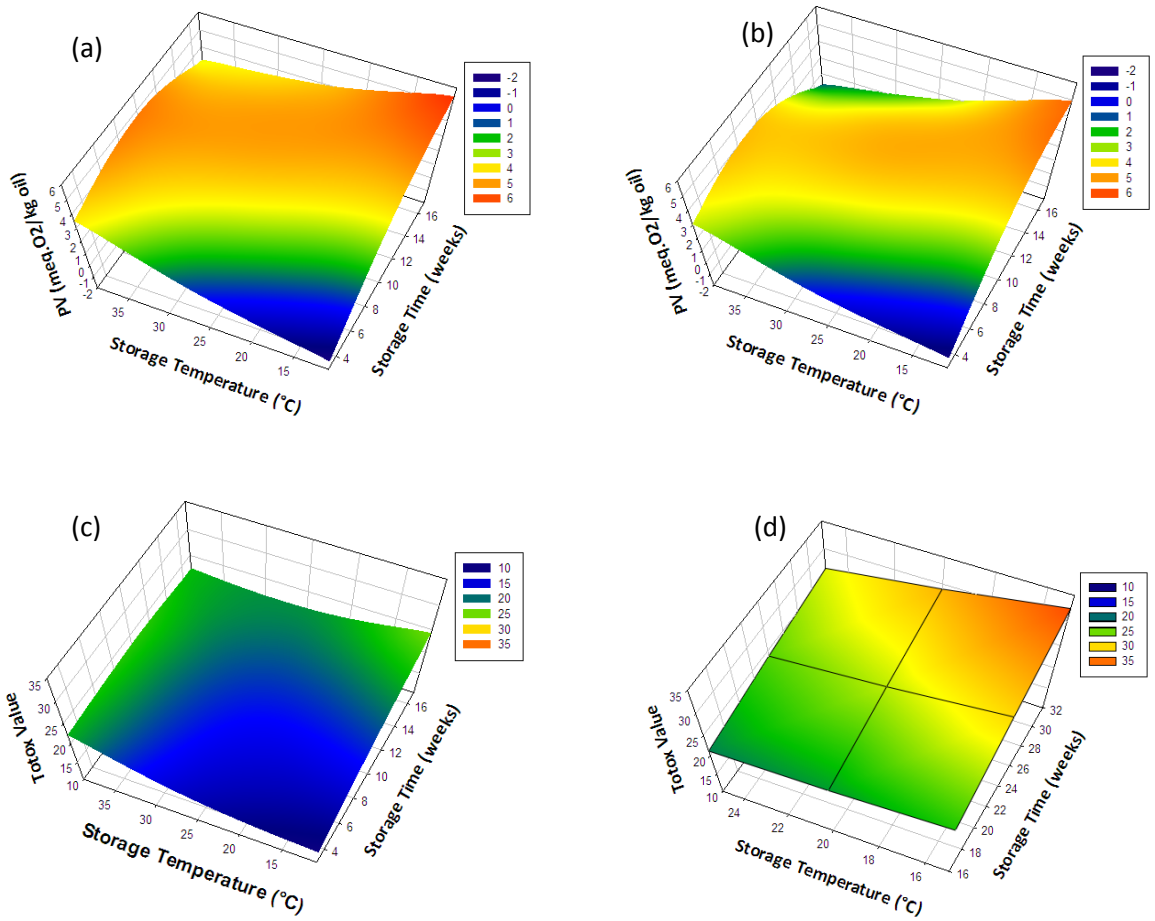


**Figure 4: Response of PV of oil extracted from vacuum fried beef strips stored in (a) PET packaging and and (b) aluminium foil laminate packaging; (c) response of Totox value of vacuum fried beef oil after storage in PET packaging; (d) Predicted response of Totox value after extended storage up to 32 weeks at 15-25°C.**

storage temperature was increased. However, the mean moisture content of the vacuum fried beef strips of both packaging types were above the initial moisture content of 4.1% (db) and thus it was concluded that storage overall did in fact cause the moisture content to increase. Based on the results of the present study, it appears that the moisture content of vacuum fried beef strips is relatively steady during storage of up to eight months.

The significant change that was only observed for the PET packaging type was attributed to the slightly lower WVTR of the aluminium foil laminate which allowed less atmospheric vapour to enter the package during storage. It was surprising that such a large difference in moisture content was observed for this small difference in WVTR.

## 6.2 Water Activity

The $a_w$ of vacuum fried beef strips increased during the storage experiment, which is in agreement with other dried meat product storage experiments (Modi *et al.,* 2007). Storage temperature and time both significantly affected the $a_w$ of the vacuum fried beef strips stored in PET packaging, but there was no significant increase in $a_w$ for the aluminium foil laminate packaged samples. This, and the fact that the aluminium foil laminate packaged samples had consistently lower $a_w$ than the PET packaged samples, is presumably again due to the lower WVTR of the aluminium foil laminate packaging type allowing less atmospheric water vapour to enter.

## 6.3 Fatty Acid Profile

Masson *et al.* (2002) and Kristensen *et al.* (2006) both observed a decrease in unsaturated fatty acids during storage of potato chips and butter blends, respectively, and determined that unsaturated fatty acids were the most unstable during storage. There were no significant changes in the levels of C18:2, C18:3 and total PUFAs in the present study however, which implies that the extent of lipid oxidation in the vacuum fried beef oil was minimal. Vacuum frying is known to reduce oxidative changes in foods due to decreased oxygen exposure and lower temperatures during processing (Fan, Mujumdar & Zhang, 2005; Shyu, Hau, & Hwang, 2005) and could be in part responsible. $N_2$ flushing of the storage packages before sealing would also have offered a protective effect, as would the tocopherols naturally present in the canola cooking oil, though these did overall significantly decrease during the storage trial.

While no significant changes occurred, the levels of C18:2, C18:3 and total PUFAs were consistently higher in the samples stored in aluminium foil laminate compared to the PET packaging type, particularly towards the end of the storage trial. This indicates that lipid oxidation was not occurring at the same rate for the different packaging materials. The OTR of the PET packaging was much higher than for the aluminium foil laminate (14.7 cc/($m^2$.day)

compared to only 3.0 cc/($m^2$.day)) and therefore it is likely that the PET packaged samples underwent PUFA oxidation to a greater extent than the aluminium foil laminate packaged samples.

## 6.4 Free Fatty Acid Content

FFA content increased in response to storage time for both packaging types. An increase in FFA levels in meat products during storage has also been observed in other studies (Modi, Mahendrakar, Rao & Sachindra, 2004a; Modi, Mahendrakar, Sachindra & Rao, 2004b; Modi *et al.,* 2007; Rao *et al.,* 1996). Concentrations in these studies ranged from very low (0.163%) to 19.4% and the levels in this study were in the lower region of this range. FFA formation is by hydrolysis reactions within lipids (Perkins, 2006) and their accumulation can be used as an indicator of lipid breakdown. Individual free fatty acids are also known to contribute flavours and aromas, not all of which are considered desirable in foods (Ledahudec & Pokorny, 1991), although the use of FFA as a food quality indicator is not recommended (Matthäus, 2006). Additionally, FFAs are more susceptible to oxidation than their esterified counterparts (Kinsella, Shimp & Mai, 1978). Based on these results it can be concluded that storage for extended periods of time and at moderate temperatures is detrimental to the quality of the oil in vacuum fried beef strips.

The results obtained for the different packaging types were somewhat contradictory to those hypothesised. As FFAs are produced by hydrolysis, it was expected that the vacuum fried beef strips with the highest moisture levels would have higher levels of FFAs; in this case, the samples stored in PET packaging. A satisfactory explanation for this effect should form part of the focus of subsequent studies on the shelf life of vacuum fried beef.

## 6.5 Tocopherols

Tocopherols exert an antioxidant effect in lipid-containing foods, due to their ability to donate a hydrogen atom to peroxyl radicals and form less reactive tocopheroxyl radicals (Lampi, Kataja, Kamal-Eldin & Vieno, 1999). As lipid oxidation increased with increasing storage time, illustrated by increases in PV and Totox value during storage, it is not surprising that tocopherol levels overall decreased. Indeed, Kristensen *et al.* (2006) also observed a correlation between increasing primary and secondary lipid oxidation products and decreasing α-tocopherol levels in butter blends, and similar relationships with antioxidants

have been observed by others (Rao *et al.,* 1996; Kim *et al.,* 2011; Modi *et al.,* 2007; Pelser *et al.,* 2007; Rhee, Cho & Pradahn, 1998). Lai, Gray, Booren, Crackel and Gill (1995) found the use of antioxidants significantly reduced the production of lipid oxidation products in chicken nuggets during frozen storage. Therefore the inclusion of antioxidants or antioxidant-containing ingredients such as herbs and spices in the marinades used for vacuum fried beef strips warrants consideration if storage stability is to be maximised.

It appeared that α-tocopherol levels were somewhat lower in the samples stored in aluminium foil laminate packaging and the reasons for this are not known. The levels of γ-tocopherol were higher for the aluminium foil laminate packaging type however, which can be explained by decreased oxidation due to a greater exclusion of atmospheric oxygen by this packaging type compared to the PET packaging material. As no significant changes in δ-tocopherol levels were observed for the samples stored in aluminium foil laminate packaging, it is assumed that the losses were minimal, and indeed the levels were higher than in the samples stored in PET packaging which were barely detected after five weeks of storage. This again can be attributed to the lower OTR of the aluminium foil laminate packaging.

The tocopherol data was unable to be modelled satisfactorily by RSM analysis. There was significant interaction between storage temperature and time for γ-tocopherol (PET packaging) and δ-tocopherol (aluminium foil laminate packaging), and it is possible that the tocopherols were also interacting with one or more of the other dependent variables, which cannot be assessed using this type of experimental design. Further research based on the results of the present study should include the use of tocopherols, given their potential to preserve the quality of vacuum fried beef strips during storage.

## 6.6 Peroxide Value, *p*-Anisidine Value and Totox Value

The length of storage was the main factor determining the PV of the extracted oil samples. Other researchers have also observed an increase in PV during storage of lipid-containing food products (Kristensen *et al.,* 2006; Pelser *et al.,* 2007; Rhee, Cho & Pradahn, 1998; Vanhanen & Savage, 2006). The PV data could not be accurately modelled by RSM analysis. As peroxide formation is a result of lipid oxidation, it is likely that interactions with the tocopherols naturally present in the cooking oil were influencing the PV obtained, as mentioned above. It was apparent however that the PV of the vacuum fried beef strips

stored in aluminium foil laminate packaging were lower than those stored in PET packaging, and this was attributed to the lower OTR of aluminium foil laminate. The PV for both packaging types did not exceed 6.0, which is considered low; Mariod, Matthäus, Eichner and Hussein (2006) stated that a PV less than 10 meq.$O_2$/kg oil was acceptable for frying oils, although the acceptable level for foods is unknown and PV is not considered to be useful for assessment of oils (Mariod *et al*., 2006).

As the peroxides in an oxidising fat or oil are transitory, *p*-AV was measured in addition to PV in order to gain a measure of the peroxide breakdown products present in the oil. Indeed, the rate of increase in the PV of the extracted oil samples decreased as the storage experiment progressed, which reflects decomposition of peroxides to secondary oxidation products (data not shown). However, the increase in *p*-AV was not significant in the present study over the given storage conditions. Che Man and Wan Hussin (1998) cited that the *p*-AV of a good quality oil should be less than 10; although recommended levels in foods are unknown, it is encouraging that the *p*-AVs encountered in the present study were not much greater than 10 and were relatively stable throughout the storage trial, indicating effective inhibition of oil oxidation and shelf stability.

The PV and *p*-AV were combined in the Totox value to give a more representative picture of the oxidised state of the oil in the vacuum fried beef. As the Totox value increased overall throughout the storage experiment, it is evident that lipid oxidation increased during storage in this experiment, although significant increases were only observed for the PET packaged samples; again, this was attributed to the higher OTR of PET. Prediction of the response over an extended storage period showed the Totox value increasing to approximately 32. Oils with a Totox value over 32 should be rejected for human consumption (Mariod *et al.,* 2006) and it is remarkable that this level was predicted to be reached only after eight months of storage in PET packaging at temperatures up to 25°C. While the acceptability threshold for Totox value in foods is unknown, it is clear that vacuum fried beef strips stored in both PET and aluminium foil laminate packaging were quite resistant to oil oxidation. A sensory analysis to determine the acceptability of stored vacuum fried beef strips would be most useful to determine the shelf life.

### 6.7 Sterols and Sterol Oxidation Products Analysis

The sterols found in vacuum fried beef strips are a combination of animal and plant sterols from beef and canola oil that the beef strips were cooked in. Cholesterol remains the dominant sterol and the main contributors to total SOPs were COPs.

Compared to sterols, oxidised sterols are present in very low levels and it would not be expected that total sterol levels would noticeably decrease with storage due to SOP formation. However it is less feasible that the levels would increase during storage, and Rao *et al.* (1996) found cholesterol to decrease slightly during storage of buffalo meat. The inherent heterogeneity of biological samples may in part account for the confusing results that were obtained. For example, while care was taken to exclude visible non-muscle fractions from the beef strips during preparation before frying, some intra-muscular gristle was inevitably present which contains different proportions of cholesterol and may have confounded the results. In addition, extensive storage of the extracted oil at -20°C until analysis (more than 8 months in some cases) was not recommended for accurate results (P. Dutta, Dec 2, 2011, pers. comm.). Future research on SOPs in vacuum fried beef strips is needed.

## 7.0 Conclusions

This research was successful in outlining the main lipid changes occurring during the storage of vacuum fried beef strips. Significant responses to temperature during storage were observed for moisture content, FFAs and tocopherols, while PV and Totox value were mainly only affected by the length of storage. The water activity was affected by both temperature and time and the two-factor interaction coefficient. There were few or no changes in FAs, *p*-AV, sterols and SOPs.

The main differences between the packaging types were related to moisture content and were due to the differences in OTR and WVTR. Aluminium foil laminate packaging appeared to be superior in most instances except FFAs. Lipid and sterol oxidation was minimal for both packaging types, indicating that vacuum fried beef strips were shelf-stable when packaged in either PET or aluminium foil laminate.

Further research regarding the shelf life of vacuum fried beef strips should include sensory analysis to determine consumer acceptability thresholds. This experiment shows that

vacuum fried beef strips may be able to be stored for up to 32 weeks without large changes in quality.

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# Appendices

## Appendix 1: Storage conditions for vacuum fried beef

**Table 8: Storage conditions for vacuum fried beef**

| | Temperature (°C) | | | | Relative humidity (%) | | |
|---|---|---|---|---|---|---|---|
| **Temperature code** | **Minimum** | **Maximum** | **Average** | | **Minimum** | **Maximum** | **Average** |

| | | | | | | |
|---|---|---|---|---|---|---|
| -1.41 | 9.4 | 10.7 | 9.7 | 65.5 | 99.2 | 87.9 |
| -1.00 | 15.0 | 15.7 | 15.0 | 49.3 | 53.3 | 52.7 |
| 0.00 | 25.0 | 25.1 | 25.1 | 26.3 | 49.8 | 35.0 |
| 1.00 | 34.4 | 36.0 | 35.1 | 13.0 | 26.3 | 15.3 |
| 1.41 | 26.0 | 40.2 | 39.8 | 13.6 | 31.5 | 15.7 |

## Appendix 2: Formulas for conversion between coded and actual values

### (a) Storage temperature (°C)

$$\text{Coded value} = \frac{\text{Actual temperature (°C)} - 25\ °C}{10°C} \qquad \textit{(Equation 11)}$$

### (b) Storage time (weeks)

$$\text{Coded value} = \frac{\text{Actual storage time (weeks)} - 10\ \text{weeks}}{5\ \text{weeks}} \qquad \textit{(Equation 12)}$$

## Appendix 3: Predicted responses during extended storage up to eight months

**Table 9: Predicted response of dependent variables after extended storage up to 32 weeks at 15-25°C, based on final equations generated by RSM analysis**

| Storage temperature (°C) | Storage time (weeks) | Response | | | | |
|---|---|---|---|---|---|---|
| | | MC (% db) | $a_w$ | FFA (% oleic acid) | | Totox value |
| | | PET | PET | PET | Al | PET |
| **15** | **16** | 8.2 | 0.34 | 1.6 | 1.7 | 22.9 |
| | **24** | 7.5 | 0.31 | 2.1 | 4.1 | 21.7 |
| | **32** | 6.7 | 0.28 | 2.5 | 8.1 | 21.1 |
| **20** | **16** | 7.8 | 0.42 | 1.9 | 1.7 | 27.9 |
| | **24** | 7.1 | 0.38 | 2.3 | 4.0 | 25.6 |
| | **32** | 6.3 | 0.34 | 2.8 | 8.0 | 23.8 |
| **25** | **16** | 7.4 | 0.53 | 2.1 | 2.0 | 32.5 |
| | **24** | 6.7 | 0.48 | 2.6 | 4.3 | 29.0 |
| | **32** | 5.9 | 0.42 | 3.1 | 8.2 | 26.2 |

MC = Moisture content; $a_w$ = Water activity; FFA = Free fatty acids; PET = Polyethylene terephthalate packaging; Al = Aluminium foil laminate packaging