THE SENSORY, LIPID AND HAEMOSTATIC PROFILE EVALUATION OF A POTENTIAL FUNCTIONAL FOOD USING RED PALM OLEIN

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AFRIKAANSE TITEL

Die sintuiglike, lipied en hemostatiese profiel evaluering van 'n potensiële funksionele voedsel met die gebruik van rooi palmoleïen

OPSOMMING

Motivering:

Dislipidemia en 'n hiperkoaguleerbare toestand is bekende risikofaktore vir kardiovaskulêre siekte (KVS), terwyl dieet 'n belangrike rol in die risikovoorkoming daarvan speel. Dieetvette en vetsure is dieetfaktore wat daarvoor bekend is om plasma lipiede en lipoproteïene te moduleer. Gekontroleerde dieeteksperimente het aangetoon dat plasma aktiwiteite van koagulasie en fibrinolitiese veranderlikes ook deur die vetsuursamestelling van die dieet beïnvloed kan word, maar min studies waarin die spesifieke effekte van individuele vetsure op die hemostatiese sisteem ondersoek is, is nog uitgevoer. Palmolie (PO), wat wydverspreid in die voedselindustrie as gevolg van verskeie voordelige funksionele eienskappe gebruik word, is 'n ryk bron van versadigde vetsure (VVS), veral palmitiensuur, sowel as mono-onversadigde vetsure (MOVS) en tokotriënole. Rooi palmoleïen (RPO) is die ongeraffineerde vorm van PO, en bevat tesame met die hoë tokotriënolinhoud daarvan, ook hoë vlakke van karotenoïede. Alhoewel die effekte van PO op lipiede en hemostatiese veranderlikes al redelik goed bestudeer is, is resultate steeds teenstrydig. Die effekte van RPO op hierdie veranderlikes is egter nog net tot 'n baie beperkte mate nagevors.

Doelwitte:

Die hoofdoelwitte van hierdie studie was om, met behulp van sintuiglike evaluering, die uitvoerbaarheid van die bekendstelling van RPO in die dieet van 'n stedelike, blanke Suid-Afrikaner populasiegroep te ondersoek, sowel as om die effekte van geraffineerde, gebleikte en ontreukde palmoleïen (POL) en RPO in vergelyking met sonneblomolie (SBO) op lipiedvlakke en hemostatiese profiele in hiperfibrinogenemiese volwassenes in 'n gerandomiseerde, gekontroleerde, enkelblinde parallelle studie te ondersoek. Om hierdie hoofdoelwitte te bereik, is die volgende sub-doelwitte vir elk van die twee studies gestel:

- Eerstens, om met behulp van 'n sintuiglike verbruikerspaneel, die aanvaarbaarheid van, voorkeur vir en voorgenome verbruik van muffins en beskuit wat RPO of SBO bevat te evalueer, ten einde die moontlikheid van suksesvolle insluiting van bogenoemde produkte, as draers vir die eksperimentele olies, in 'n opeenvolgende dieetintervensie-studie, te bepaal.
- Tweedens, om die dieetveranderinge en inskiklikheid deur die bepaling van voedsel- en nutriëntinnames te monitor; om die effekte van die insluiting van POL en RPO in die dieet op *plasmavetsure* [miristiensuur (C14:0), palmitiensuur (C16:0), palmitoleïensuur (C16:1, n-7), oleïensuur (C18:1) en linoleïensuur (C18:2, n-6)]; *serumlipiede* [totale cholesterol (TC), triasielgliserol (TG), hoë-digtheidslipoproteincholesterol (HDLC) en lae-digtheidslipoproteincholesterol (LDLC)]; *plasmahemostatiese faktore* [fibrinogeen, D-dimeer, plasminogeenaktiveerderinhibeerder-1 aktiwiteit ($PAI\text{-}1_{akt}$), weefselplasminogeenaktiveerderantigeen (tPA_{ag}), trombien-antitrombien-kompleks (TAT) en plasmien-antiplasmienkompleks (PAP)]; sowel as *fibriennetwerk eienskappe (FNE)* [massa-lengte-verhouding (MLV), permeabiliteit (K_s) en kompaksie] te ondersoek.

Metodes:

- **Verbruikerstudie:** In hierdie studie is die sintuiglike aanvaarbaarheid van, voorkeur vir en voornemende verbruik van muffins, gebak met RPO of SBO (kontrole), eerstens deur 'n algemene verbruikersgroep van 144 deelnemers geëvalueer, gevolg deur die evaluering van muffins en beskuit gebak met RPO en SBO deur 'n tweede groep van 67 verbruikers, wat ook vir die opeenvolgende intervensie-studie gewerf is. 'n Vyf-punt hedoniese en voedsel-aksie skattingskaal is vir evaluering deur beide groepe gebruik. Aanvaarbaarheid van voorkoms, kleur, tekstuur, en smaak is individueel geëvalueer om algemene aanvaarbaarheid te bereken.
- **Dieetintervensie-studie:** Nege-en-vyftig vry-lewende hiperfibrinogenemiese vrywilligers het in hierdie gerandomiseerde, gekontroleerde, enkelblinde, parallelle studie deelgeneem. Na 'n inloopperiode van vier weke, waartydens proefpersone 25g/dag SBO in die vorm van gebakte produkte (muffins en beskuit) ingeneem het, is hulle afgepaar volgens geslag, ouderdom en

liggaamsmassa-indeks (LMI) en gerandomiseer in drie groepe wat onderskeidelik 25g/dag van óf RPO, POL óf SBO in die vorm van gebakte produkte vir 'n volgende vier weke ingeneem het. Dieetinnames, antropometriese metings, serumlipiede, plasmavetsure, hemostatiese profiele en FNE is voor die inloop, sowel as na 4 en 8 weke, onderskeidelik, geneem.

Resultate:

- **Verbuikerstudie:** In die *eerste verbruikersgroep*, was die SBO muffins statisties hoër vir kleur, tekstuur, en algemene aanvaarbaarheid geëvalueer, en verbruikers was van voorneme om dit meer dikwels as RPO muffins te eet. Die praktiese betekenisvolheid van hierdie verskille was egter klein. Die gemiddelde telling vir algemene aanvaarbaarheid van RPO muffins was egter steeds baie hoog (4.2 op 'n 5-punt skaal), en verbruikers was van voorneme om dit dikwels te eet (een muffin per dag). Aangesien verbruikers aangedui het dat hulle slegs een RPO muffin per dag sal eet moes alternatiewe metodes ondersoek word ten einde 25g RPO per dag tydens die dieetintervensie in te sluit. Daar is dus besluit om ook hoë-vesel beskuit, wat die eksperimentele olies bevat, in te sluit. In die *tweede verbruikersgroep* is hoë-vesel muffins gebak van RPO en SBO as ewe aanvaarbaar ten opsigte van al die sintuiglike eienskappe geëvalueer en geen betekenisvolle verskille is in voorkeur vir, of voorneme van verbruik van RPO of SBO muffins in hierdie groep gevind nie. Verbruikers in die tweede groep het in vergelyking met dié in die algemene verbuikersgroep ook RPO muffins betekenisvol meer aanvaarbaar ten opsigte van verskeie sintuiglike eienskappe geëvalueer. RPO beskuit is ook deur die tweede groep baie aanvaarbaar op grond van alle sintuiglike eienskappe gevind, terwyl verbruikers voorts ook van voorneme was om RPO beskuit net so dikwels as SBO beskuit te eet, naamlik een per dag.
- **Dieetintervensie-studie:** Inskiklikheid, soos bepaal deur die versameling van oorblywende muffins en beskuit, is bepaal as 98.8±3.2%. Energie-innames was onveranderd gedurende die studie. LMI het gedurende die studie in al drie groepe effens verhoog, maar die verhogings was nie kliniese betekenisvol nie (mediaan van kleiner of gelyk aan 0.2 LMI-punte). Die gemiddelde TC- en LDLC-vlakke het, in vergelyking met die inname van RPO en SBO,

betekenisvol met die inname van POL verhoog (7% en 13%, onderskeidelik). Dié verhoging kan gedeeltelik deur die betekenisvolle verhoging in plasma C16:1, n-7, 'n metaboliet van C16:0, sowel as die betekenisvolle verlaging in plasma C18:2, n-6, wat tydens die inname van POL, maar nie RPO of SBO plaasgevind het nie, verklaar word. Alhoewel HDLC in al drie groepe ewe veel verhoog het, was die verhoging slegs in die RPO-groep betekenisvol, naamlik 7%. Geen betekenisvolle veranderinge is deur die inname van POL of RPO in $PAI\text{-}1_{akt}$, TAT, PAP, D-dimeer of fibrinogeenvlakke veroorsaak nie. RPO het die voordelige verandering van verlaging in tPA_{ag} ten opsigte van POL en SFO tot gevolg gehad. RPO en POL het nie onafhanklike effekte op FNE gehad nie. Al drie olies het, tot verskillende mates, FNE voordelig beïnvloed. POL het tot 'n verhoging in MLR en kompaksie gelei, RPO het tot 'n verhoging in kompaksie en die neiging tot verhoogde permeabiliteit gelei, terwyl SBO kompaksie verhoog het en tot 'n neiging in verhoogde MLR aanleiding gegee het. MLR het 'n betekenisvolle negatiewe verband met plasma C16:0, en 'n positiewe verband met totale plasma onversadigde vetsure getoon.

Gevolgtrekkings:

- **Verbuikerstudie:** RPO produkte is nie bo SBO produkte verkies nie, maar verbruikers in beide groepe het die algemene aanvaarbaarheid van RPO produkte steeds as baie hoog (≥4.0 op 'n 5-punt skaal) geëvalueer, en het die voorneme om dit dikwels te eet (ten minste een keer per dag), uitgespreek. Aanvaarbaarheid van, en inskiklikheid ten opsigte van RPO produkte kon dus potensieel beskou word as optimaal vir verbruik in die daaropvolgende dieetintervensie-studie.
- **Dieetintervensie-studie:** In hierdie studie het die inname van 25g RPO per dag deur hiperfibrinogenemiese proefpersone nie tot die toename in TC en LDLC, soos in die geval van POL-inname, gelei nie. RPO het die moontlike voordelige effek van verhoging in HDLC-vlakke gehad. RPO mag voorts ook 'n voordelige effek op risiko merkers van KVS hê deur die verlaging wat dit in tPA_{ag} tot gevolg gehad het. Alhoewel POL en RPO nie ander hemostatiese veranderlikes beïnvloed het nie, het dit ten minste geen negatiewe effekte daarop getoon nie. Onversadigde vetsure mag voordelige effekte op FNE hê, maar hierdie effekte behoort in ander studies met geskikte studie-ontwerpe

ondersoek te word voordat finale gevolgtrekkings in dié verband gemaak kan word.

RPO, 'n goeie bron van vitamien A voorlopers en vitamien E, kan dus moontlik as uitstekende, veilige en gesonde keuse vir gebruik in die voedselindustrie, sowel as vir huishoudelike gebruik, beskou word. Verdere studies is egter noodsaaklik om resultate van die huidige studie te bevestig en/of te verifieer.

Sleutelterme: Palmoleïen, rooi palmoleïen, sintuiglike evaluering, verbruikersaanvaarbaarheid, lipiede, hemostatiese veranderlikes, funksionele voedsels.

SUMMARY

Motivation:

Dyslipidaemia and a hypercoagulable state are known risk factors for cardiovascular disease (CVD), while diet plays an important role in the risk prevention thereof. Dietary fats and fatty acids are known dietary factors to modulate plasma lipids and lipoproteins. Controlled dietary experiments have indicated that plasma activities of coagulation and fibrinolytic parameters may also be affected by the fatty acid composition of the diet, but few studies have been performed to establish the specific effects of individual fatty acids on the haemostatic system. Palm oil (PO), widely used in the food industry as a result of several beneficial functional characteristics, is a rich source of saturated fatty acids (SFA), specifically palmitic acid, as well as mono-unsaturated fatty acids (MUFA) and tocotrienols. Red palm olein (RPO) is the unrefined form of PO, and contains, in addition to its high content of tocotrienols, also high levels of carotenoids. Although the effects of PO on lipids and haemostatic variables have been rather well studied, inconsistent results were found. The effects of RPO on these variables, however, have only been studied to a very limited extent.

Objectives:

The main objectives of this study were thus to investigate, by means of sensory evaluation, the feasibility of the introduction of RPO into the diet of an urban, white South African population group and subsequently, to investigate the effects of refined, bleached and deodorized palolein (POL) and RPO on lipid levels and haemostatic profiles when compared to sunflower oil (SFO) in hyperfibrinogenaemic adults in a randomised, controlled, single blind parallel study. To attain these main objectives, the following objectives for each of the two studies were stated as:

- Firstly, to evaluate, by means of a sensory consumer panel, the acceptance of, preference for and intended consumption of muffins and rusks containing either RPO or SFO in order to determine the possibility of successful inclusion of the above mentioned products, as carriers for the experimental oils, in a successive dietary intervention study.
- Secondly, to monitor dietary changes and compliance by estimating food and nutrient intakes; to investigate the effects of the inclusion of POL and RPO in the diet on *plasma fatty acids* [myristic acid (C14:0), palmitic acid (C16:0),

palmitoleic acid (C16:1, n-7), oleic acid (C18:1) and linoleic acid (C18:2, n-6)]; *serum lipids* [total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDLC) and low-density lipoprotein cholesterol (LDLC)]; *plasma haemostatic factors* [fibrinogen, D-dimer, plasminogen activator inhibitor-1 activity ($PAI\text{-}1_{act}$), tissue plasminogen activator antigen (tPA_{ag}), thrombin-antithrombin complex (TAT) and plasmin-antiplasmin complex (PAP)]; as well as *fibrin network characteristics (FNC)* [mass-length-ratio (MLR), permeability (K_S) and compaction].

Methods:

- **Consumer study:** In this study, the sensory acceptability of, preference for and consumption intent of muffins, baked with either RPO or SFO (control), was evaluated firstly by a general consumer group of 144 participants, followed by the evaluation of muffins and rusks baked with RPO or SFO amongst a second group of 67 consumers, who were also recruited for the subsequent intervention study. A 5-point hedonic and food action rating scale was used for evaluation by both groups. Acceptability of appearance, colour, texture and flavour was separately evaluated to determine overall acceptability.
- **Dietary intervention study:** Fifty-nine free-living hyperfibrinogenaemic volunteers participated in this randomized, controlled, single blind, parallel study. After a run-in period of four weeks during which the subjects received 25g/day of SFO in baked products (muffins and rusks) they were paired according to gender, age and body mass index (BMI) and randomized into three groups receiving either 25g/day of RPO or POL or SFO in baked products for another four weeks. Dietary intakes, anthropometrical measurements, serum lipids, plasma fatty acids, haemostatic profiles and FNC were measured before the run-in and after respectively 4 and 8 weeks.

Results:

- **Consumer study:** In the *first consumer group*, SFO muffins scored statistically higher for colour, texture, and overall acceptability, and consumers intended to eat it more often compared to the RPO muffins. The practical significance of these differences was, however, small. The mean score for

overall acceptability of the RPO muffins was very high (4.2 on a 5-point scale), and consumers intended to eat it often (one muffin per day). Because consumers indicated that they would only eat one RPO muffin per day, alternative methods had to be investigated for inclusion of the 25g/day of RPO in the dietary intervention study. It was decided to also provide high-fibre rusks containing the oils. Within the *second consumer group* high-fibre muffins baked with RPO and SFO were rated equally acceptable on all the evaluated sensory attributes and no significant difference was found in preference for, or consumption intent of muffins baked with either RPO or SFO in this group. Consumers in the second group rated RPO muffins significantly higher on several of the sensory attributes compared to the general consumer group. RPO rusks were also found very acceptable on all sensory attributes by this group, while consumers furthermore intended to eat rusks baked with RPO as often as those baked with SFO, namely one per day.

- **Dietary intervention study:** Compliance, as determined by collecting left over muffins and rusks, was determined as 98.8±3.2%. Energy intakes were unchanged during the study. BMI increased slightly in all three groups during the study, but the increase was not of clinical significance (median of equal or smaller than 0.2 BMI points). Mean TC and LDLC levels increased significantly with POL intake (7% and 13%, respectively), compared to intake of RPO and SFO. The increase may in part be explained by the significant increase in plasma C16:1, n-7, a metabolite of C16:0, and the significant decrease in plasma C18:2, n-6 with intake of POL but not with intake of RPO or SFO. Although the same increase in HDLC was found in all three groups, it was only significant in the RPO group, namely 7%. No significant changes with intake of POL or RPO were observed on PAI-1_{act}, TAT, PAP, D-dimer or fibrinogen. RPO beneficially changed tPA_{ag} levels by decreasing it compared to POL and SFO intake. RPO and POL did not have independent effects on FNC. All three oils, to different degrees, beneficially affected FNC. POL increased MLR and compaction, RPO increased compaction and tended to increase K_s, and SFO increased compaction and tended to increase MLR. MLR was significantly negatively associated with plasma C16:0 and positively associated with total plasma unsaturated fatty acids.

Conclusions:

- **Consumer study:** RPO products were not preferred to SFO products, but consumers of both groups evaluated the overall acceptability of RPO products as very high (≥4.0 on 5-point scale), and intended to eat it often (at least once/day). Acceptance of, and compliance with RPO products were thus considered to be optimal in the subsequent dietary intervention trial.
- **Dietary intervention study:** In this study the intake of 25g RPO per day by hyperfibrinogenaemic patients did not increase TC and LDLC as seen with POL intake. A beneficial effect of increased HDLC could possibly be attributed to RPO. RPO may furthermore even have beneficial effects on risk markers of CVD by the decreasing effect it had on plasma tPA_{ag} levels. Even though POL and RPO did not influence the other haemostatic variables it, at least, did not have any negative effects. Unsaturated fatty acids may have beneficial effects on FNC, but this effect needs to be examined in other studies with the appropriate study design before more definite conclusions can be made.

  RPO, a good source of vitamin A precursors and vitamin E, may thus possibly be regarded as an excellent, safe and healthy choice for use by the food industry as well as for home cooking. Further studies are, however, needed to confirm and/or verify results of the current study.

Key words: Palm olein, red palm olein, sensory evaluation, consumer acceptability, lipids, haemostatic variables, functional foods.

CONTENTS

LIST OF ABBREVIATIONS

%En	Percentage of total energy intake
Δ	Change
↑	Increase
↓	Decrease
↔	No effect
ANOVA	Analysis of Variance
ATIII	Antithrombin III
BMI	Body mass index
Cat.no.	Catalogue number
CV	Coefficient of variance
CHD	Coronary heart disease
CVD	Cardiovascular disease
ELISA	Enzyme-linked immunosorbent assay
FH	Familial hypercholesterolaemia
FNC	Fibrin network characteristics
FVIIc	Factor VII coagulant
HDLC	High density lipoprotein cholesterol
HMG-CoA	3-Hydroxy-3-methylglutaryl-co-enzyme A
IHD	Ischaemic heart disease
K_s	Permeability
LDLC	Low density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
MLR	Mass-length-ratio
MUFA	Monounsaturated fatty acid
$PAI\text{-}1_{act}$	Plasminogen activator inhibitor-1 activity
PAP	Plasmin-antiplasmin complex
PF_4	Platelet factor 4
$PGF_{1\alpha}$	Prostaglandin $_{1\alpha}$
PGI_2	Prostacyclin
PO	Palm oil
POL	Palm olein
PUFA	Polyunsaturated fatty acid
PU for CHE	Potchefstroom University for Christian Higher Education
RPO	Red palm olein
SFA	Saturated fatty acid
TAT	Thrombin-antithrombin complex
TC	Total cholesterol
TG	Triacylglycerol
tPA_{ag}	Tissue plasminogen activator antigen
TXB_2	Thromboxane B_2
VLDL	Very low density lipoprotein cholesterol

CHAPTER 1

INTRODUCTION

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1. BACKGROUND AND MOTIVATION

The aim of this chapter is to motivate the timeliness and uniqueness of this research relevant to the existing knowledge. For several years, the Nutrition Research Group in the School for Physiology, Nutrition and Consumer Sciences, Potchefstroom University for Christian Higher Education (PU for CHE) has been specializing in research regarding the relationship between diet, haemostasis and the risk of chronic diseases. Since the foundation of the Institute of Nutrition in 2000 in this school, the opportunity for interdisciplinary research was further reinforced as a result of the close relationship that exists, amongst others, between the fields of Foods and Nutrition. These factors, as well as the access to a unique study population and well established networks with national and international colleagues, facilitated one of the new directions in our research, namely the sensory evaluation of several potential functional food products, followed by the clinical evaluation of its effects on lipid and haemostatic variables in hypercholesterolaemic and/or hyperfibrinogenaemic subjects. Few, if any researchers, however, study both the fields of Foods and Nutrition in depth and the originality and value of this thesis, therefore, lies in the sequential nature of the two studies reported in these fields, both presented in the form of manuscripts.

As will be pointed out in this chapter, cardiovascular disease (CVD) remains the single biggest killer in the industrialized world (Anon, 2002; Murray & Lopez, 1996), while dyslipidaemia (Adult Treatment Panel II, 1994; Castelli, 1996) and an increased coagulation state (Aznar *et al.*, 1988; Danesh *et al.*, 1998) seem to be important contributing factors to the development of CVD (summarized by Oosthuizen, 1999). A short discussion on CVD will set the background against which the benefits of the development of functional foods, as a method of addressing the above mentioned health problem, will be discussed. After a general motivation for the research in this thesis is given, the aims and objectives of each separate study (as found in Chapters 3 and 4) will be stated. Subsequently, the structure of the thesis will be explained, followed by the author's and co-authors' contributions to the manuscripts presented.

1.1 Cardiovascular disease (CVD) and its risk factors

Atherosclerotic cardiovascular diseases were uncommon causes of death at the end of the nineteenth century. Even for the first 15 years of the twentieth century, myocardial infarction (MI) was not recognized as a clinical syndrome, but by the middle of the century, in Western, industrialized countries, including developing countries like South Africa (Bradshaw *et al.,* 1995), CVD and MI began to reach epidemic proportions (Wielgosz & Nolan, 2000). Although after the mid-1960s a decline in CVD mortality was noted in many countries, the underlying reasons for this decline remain the subject of ongoing research (Wielgosz & Nolan, 2000). The health problem of CVD, however, still strongly exists as it is currently (Anon, 2002), as was previously (Murray & Lopez, 1996), considered the leading cause of mortality and morbidity world-wide. In contrast to the earlier mentioned decline, CVD, in particular ischaemic heart disease (IHD) and stroke, is currently increasing with urbanization and acculturation in populations of developing countries (Famodu *et al.,* 1999).

Accompanying the observational data on CVD outcomes of morbidity and mortality, there has been a growing body of data on factors considered causally related. CVD has been thought of as a disease of lipid deposition, and cholesterol testing has been used as a tool to identify those at high risk. Yet half of those who suffer a MI do not have significantly increased cholesterol concentrations. As a result of efforts to understand these findings, researchers currently view CVD as more than a disease of lipid deposition. Because only about two thirds of cardiovascular events are related to well-established genetic and environmental risk factors, there is an ongoing search for new markers of cardiovascular risk (Pahor *et al.*, 1999). A combination of haemostatic factors (such as fibrinogen) with lipids have been reported as a better predictor of CVD than hyperlipidemia alone (Nair *et al.,* 1996). However, less is known about the role of haemostatic function in this regard. Data on haemostatic risk markers or factors of ischaemic heart disease and stroke specifically in African populations are scarce (Famodu *et al.,* 1999). Although it has been shown that dietary fats can affect certain factors involved in blood coagulation and fibrinolysis (Hornstra, 2001), the general relationship of total diet, as well as specific foods and nutrients, with the different haemostatic variables, are far from clear (Jerling, 2001; Vorster *et al.,* 1997).

The challenge of reducing cardiovascular risk appears to be increasing. Lifestyle choices, including smoking, dietary intake, and level of physical activity, are known modifiable risk factors for heart disease. Of these risk factors, diet is the most controversial and often the most confusing (Stewart-Fahs & Faucher, 2002). It has become clear that the control and prevention of CVD depend on a multidisciplinary approach that recognizes the importance and intricacies of lifestyle behaviours.

Other non-modifiable, as well as physiological and metabolic risk factors were concisely summarized by Oosthuizen (1999). Some of these, namely lipids and haemostatic risk factors as well as fats and oils (diet), will be discussed in more detail in Chapter 2 of this thesis. In spite of better treatments and some better outcomes, CVD remains a major health burden, but it seems that the cornerstone of the fight against heart disease has been and continues to be prevention.

1.2 Functional food - the relationship between food and health

As mentioned above, diet plays an important role in the primary and secondary prevention of CVD (Vorster *et al.,* 1997). There is, however, no silver bullet within food products that will completely prevent heart disease. A heart-healthy diet has many components. There is evidence to support the hypothesis that, by modulating specific target functions in the body, diet can have beneficial physiological and psychological effects beyond the widely accepted nutritional effects, namely reducing the risk for disease (Diplock *et al.,* 1999). This concept of functional foods has been defined by the International Life Sciences Institute of North America (ILSI) as "foods that, by virtue of physiologically active food components, provide health benefits beyond basic nutrition" (Clydesdale, 1999). It is hardly surprising to find that products for "heart health" are one of the most dynamic areas of activity both in functional foods and dietary supplements (Anon, 2002), while claims such as "reduce the risk of heart disease" are of the most important and often used ones. Interestingly, this trend was already predicted in 1998 by marketing and research and development executives (Sloan, 1998). Much research was, and still is needed to clarify whether a difference in clinical outcome exists as a result of consumption of whole foods versus isolated nutrients such as phytochemicals before specific recommendations could be provided to consumers (ADA, 1995).

Dietary fats and fatty acids are known dietary factors to modulate plasma lipids and lipoproteins (Sundram, 1997). Controlled dietary experiments have indicated that

plasma activities of coagulation and fibrinolytic parameters may also be affected by the fatty acid composition of the diet (De Bosch *et al.*, 1996; Marckmann, 1995), but few studies have been performed to establish the specific effects of individual fatty acids on the haemostatic system (Hunter *et al.*, 1999). Palm oil (PO), widely used in the food industry, is a rich source of saturated fatty acids (SFA), specifically palmitic acid (C16:0; 44.3%), as well as mono-unsaturated fatty acids (MUFA) (C18:1; 39.0%) (Ong & Goh, 2002). In South Africa, PO is the second most commonly used oil (27%) after sunflower oil (63%), with major applications especially in the food industry (Van Twisk, 2002; personal communication). It further contains large amounts of the antioxidant tocotrienol, a Vitamin E compound. Red palm olein (RPO) is the unrefined, unbleached, orange-red coloured oil extracted from oil palm fruit. Except for its high tocotrienol content, it is also considered the richest edible source of carotenoids (Cottrell, 1991). Although controversial, these antioxidant components, specifically tocotrienols, have shown beneficial effects on lipids and haemostatic profiles in several controlled intervention studies (Qureshi *et al.*, 1991; Qureshi *et al.*, 1995; Tan *et al.,* 1991). The effects of PO on lipids and lipoproteins have been well studied, but as indicated by the reviews of Ng (1994) and Sundram (1997), results are highly contradictory, ranging from hypocholesterolaemic or neutral, to hypercholesterolaemic. Contradictory, but not predominantly negative results, have been found in studies on the effect of PO on haemostatic variables, as compared with other SFA. According to Cottrell (1991), the earlier studies on the effect of PO were weakened by the fact that they were not designed to address the question of thrombosis in addition to its effect on lipids. In contrast, research on the effects of RPO *per se* is rather limited. Some human studies, as reviewed by Kritchevsky (2000), however, showed that it is indeed an useful addition to the present array of dietary fats and may be considered an excellent oil for human consumption. To a certain extent the food industry is moving away from using fats and oils only for their sensory characteristics, as emulsifiers, or as flavour and vitamin carriers. Instead, their roles in, amongst other, health and disease prevention are being explored in the development of new nutraceuticals or functional foods (Ong & Goh, 2002). Since the ultimate target of the food industry is consumer satisfaction, it is essential to consider not only the objective consumer needs (e.g. nutrition, safety, affordability), but also subjective aspects of consumer satisfaction (e.g. sensory properties and consumer attitudes). No matter how

successful a product is from the objective point of view of a scientist focusing, for instance, on nutrition and apparent functionality, the product is not successful if it does not please the consumer sufficiently to make him or her buy and consume it (Karel, 2000). For functional foods to be successful in the future, industry must thus accept the consumer's perception of food and health, and in doing so it is not more clinical studies that are needed, but also more consumer studies (Wennström, 2002).

It is clear that integrated research programmes with interdisciplinary activity among academical fields and universities, government and industrial laboratories are needed to solve key scientific and technological challenges and to exploit the scientific concepts in functional food science, as well as other important health issues like CVD.

2. AIMS AND OBJECTIVES

The aims and objectives of this thesis were:

2.1 **Main aim:** To investigate, by means of sensory evaluation, the feasibility of the introduction of red palm olein (RPO) into the diet of an urban, white South African population group.

Objectives: To compare, by means of consumer panels the

- the acceptance of,
- preference for, and
- intended consumption of high-fibre muffins and rusks baked with RPO as vehicle for inclusion of this test oil in a dietary intervention study, to control products, baked with sunflower oil (SFO).

2.2 **Main aim:** To investigate the effects of refined, bleached and deodorized palm olein (POL) and RPO on lipid levels and haemostatic profiles when compared to SFO in hyperfibrinogenaemic adults in a randomised, placebo-controlled, single blind parallel study.

Objectives:

1) To monitor dietary changes and compliance by estimating food and nutrient intakes;

2) to investigate the effects of the inclusion of POL and RPO in the diet on:

- **Plasma fatty acids:** Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1, n-7), oleic acid (C18:1) and linoleic acid (C18:2, n-6);
- **Serum lipids:** Total serum cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLC) and low-density lipoprotein cholesterol (LDLC);
- **Plasma haemostatic factors:** Plasma fibrinogen, D-dimer, plasminogen activator inhibitor-1 activity ($PAI\text{-}1_{act}$), tissue plasminogen activator antigen (tPA_{ag}), thrombin-antithrombin complex (TAT) and plasmin-antiplasmin complex (PAP);
- **Fibrin network characteristics (FNC):** Mass-length-ratio (MLR), permeability (K_S) and compaction (as discussed in detail in the Ph.D. Thesis of a co-worker [Pieters, 2002]).

3. STRUCTURE OF THIS THESIS

This thesis is presented in article format. The experimental work consisted of two studies, of which the first was in the field of food science, and the second in the field of clinical nutrition. Following this introductory chapter which motivates the necessity of such interdisciplinary research efforts, Chapter 2 gives an overview of the literature considered important for the interpretation of data from the manuscripts in this thesis. This includes the concepts of dyslipidaemia and imbalanced haemostasis as risk factors for CVD, followed by the two main topics of this thesis, namely the effect of POL and RPO and its constituents on lipids and haemostatic variables, as well as research and development regarding new functional food and the importance of sensory evaluation with consumers in this whole process. Chapter 3 consists of a submitted manuscript on the consumer acceptance of the products used as vehicles for the experimental oils in the subsequent intervention trial, namely high-fibre muffins and rusks baked with either RPO of SFO (submitted for publication in International Journal of Food Science and Technology). The questionnaire used in this study is presented as Addendum A at the end of the thesis. In Chapter 4, the effects of POL and RPO on lipids, haemostatic factors and FNC are investigated in hyperfibrinogenaemic subjects (submitted for publication in American Journal of

Clinical Nutrition). The 24h-dietary recall form and calendar reminding subjects to collect their experimental food used in this study are presented as Addenda B and C, respectively. In Chapter 5, a general discussion and summary of all the results are provided, conclusions are drawn and recommendations are made. The relevant references of Chapters 3 and 4 are provided at the end of each chapter according to the authors' instructions of the specific journal to which the manuscripts were submitted. The references used in the unpublished Chapters 1, 2 and 5 are provided according to the mandatory style stipulated by the PU for CHE.

4. AUTHORS' CONTRIBUTIONS

The two studies reported in this thesis were planned and executed by a team of researchers. The contribution of each of the researchers is given in Tables 1.1 and 1.2. Also included in this section is a statement from the co-authors confirming their individual role in each study and giving their permission that the two articles may form part of this thesis.

Table 1.1 Consumer acceptance of high-fibre muffins and rusks baked with red palm olein as potential functional foods

Name	***Role in the study***
Miss SC Scholtz M.Sc (Food Scientist, Nutritionist)	Co-responsible for design, planning and execution of total study, adaptation of questionnaires, statistical analyses and compilation of the data, as well as literature searches and preparation of manuscript. Part of Ph.D. study.
Prof. MJC Bosman Ph.D (Food Scientist)	Promoter. Co-responsible for design, planning, approval of final protocol and execution of study. Supervised the writing of this manuscript.

I declare that I have approved the above-mentioned article, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that it may be published as part of the Ph.D. thesis of Miss SC Scholtz.

MJC Bosman

Prof MJC Bosman

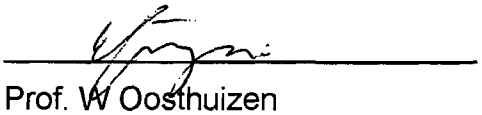
Table 1.2 The effect of red palm olein and refined palm olein on lipids and haemostatic factors in hyperfibrinogenaemic subjects

Name	***Role in the study***
Miss. SC Scholtz M.Sc (Food Scientists and Nutritionist)	Preparation and dissemination of muffins and rusks. Responsible, together with W Oosthuizen, JC Jerling, MJC Bosman and M Pieters for the execution of the total study. Responsible for literature searches, statistical analyses, processing of data and the writing of the manuscript.
Prof. MJC Bosman Ph.D (Food Scientist)	Promoter. Preparation and dissemination of muffins and rusks. Involved with execution of the total study. Supervised the writing of this manuscript.
Prof. W Oosthuizen Ph.D (Nutritionist)	Co-promoter. As clinical study co-ordinator, responsible for the execution of the total study, laboratory analyses, statistical analyses and compilation of the data. Supervised the writing of this manuscript.
Miss M. Pieters M.Sc (Dietitian, Nutritionist)	Together with SC Scholtz, W Oosthuizen, JC Jerling and MJC Bosman, responsible for the execution of the total study. Involved with and partly responsible for laboratory analyses, statistical analyses and compilation of the data. Part of Ph.D study.
Prof. JC Jerling Ph.D (Nutritionist)	Design, planning and approval of final protocol. Involved with execution of the total study.
Prof. HH Vorster D.Sc (Physiologist, Nutritionist)	Design, planning, approval of final protocol.

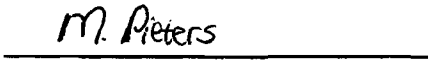
The following is a statement from the co-authors confirming their individual role in each study and giving their permission that the articles may form part of this thesis.

I declare that I have approved the above-mentioned articles, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that it may be published as part of the Ph.D. thesis of Miss SC Scholtz.

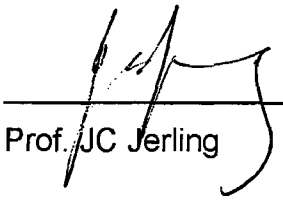
Prof. MJC Bosman



Prof. W Oosthuizen



Miss. M Pieters



Prof. JC Jerling

Prof. HH Vorster

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CHAPTER 2

LITERATURE REVIEW

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LITERATURE REVIEW

1. INTRODUCTION

As several of the important concepts that are to be discussed in this chapter have been reviewed by other authors, only the most important aspects and relevant contributions have been synthesized and will be addressed. The aim is to put all the literature in context, to give the reader the necessary factual background for the understanding and interpretation of the two manuscripts presented (Chapters 3 and 4), as well as to provide insight into the study as a whole.

A short introduction to cardiovascular disease (CVD), as well as some general risk factors thereof, will set the background for the rest of the discussion. The author will subsequently explore in more detail saturated dietary fats and oils as CVD risk factor, especially focusing on a controversial but extremely relevant issue, namely the effects of specifically palm oil on lipids and haemostasis. Palm oil (PO), widely consumed around the world today, has a distinct place and function in the food industry. Red palm olein (RPO), the partially refined counter part of palm oil, has also shown great potential for use in the food industry (O'Holohan, 1997). It was thus considered very important to review and study the health effects of these oils, as well as their main constituents, for human consumption.

The above is followed by a general discussion on food, nutrition and health, which leads to the concept of functional foods. This concept is expanded by a description of research and development concerning functional foods, including a valuable contribution by the author of this thesis on the importance of consumer testing and the sensory evaluation of new functional foods prior to nutrition intervention trials, as well as commercialization of these functional food products. Furthermore, the importance of interdisciplinary research regarding research and development of new functional foods is highlighted. This chapter concludes with the impact of functional food on consumers in the 21st century.

2. CARDIOVASCULAR DISEASE (CVD) AND ITS RISK FACTORS

CVD is still the number one killer around the globe, accounting for more than 30% of deaths worldwide and 45% of deaths in industrial or developed nations. According to the World Heart Federation, up to 40% of all deaths will be related to CVD by 2020.

While death rates are falling in many industrialized countries, heart diseases are still responsible for 50% of all deaths (Anon, 2002b). In South Africa, mortality rates due to ischaemic heart disease (IHD) are the highest amongst Asians and whites (Bradshaw *et al.,* 1995). CVD is a multifactorial disease precipitated by a host of interrelated risk factors in genetically susceptible individuals. The typical Western diet, high in fat and low in fibre with inadequate micronutrient intakes, followed by many South Africans (Vorster *et al.,* 1997b) could probably explain the high CVD rates among the above mentioned population groups. Furthermore, South Africa is unique in the sense that the prevalence of familial hypercholesterolaemia (FH) amongst white South Africans is estimated at 1 out of 70 (Seftel *et al.*, 1995), whereas the world-wide estimation is 1 out of 500 (Goldstein & Brown, 1983). FH is, in fact, probably one of the most common genetic diseases found among white Afrikaans-speaking South Africans, especially among certain surnames such as Kruger and Van der Walt (Torrington & Brink, 1990).

CVD has a multifactorial aetiology, as illustrated by the existence of numerous risk indicators. Only after a cause-and-effect relationship has been established between the disease and a given risk indicator (called a risk factor in that case), modifying this factor can be expected to affect disease morbidity and mortality (Hornstra, 2001). Risk factors associated with an increase in the risk of CVD's occurrence do thus not essentially indicate causative roles (Chetty *et al.,* 1997). Not being a cause does, however, not diminish the value of the risk factor as a way to predict the probability of the disease (Vorster *et al.,* 2000).

2.1 CVD Risk factors

Prospective epidemiological studies were mainly responsible for the identification of several CVD risk factors. These risk factors, as concisely summarized by Oosthuizen (1999), may be divided into lifestyle or behavioral, physiologic and metabolic, and other non-modifiable risk factors. Diet seems to be one of the major controllable risk factors involved in this degenerative disease. For the purpose of this study, the author will only focus on those risk factors most closely related to the research theme. As the intricacies of lipoprotein metabolism have been unraveled over the last three decades, a wide body of data indicates that, while high levels of total cholesterol (TC), low density lipoprotein cholesterol (LDLC) and triacylglycerol (TG) are positively associated with CVD, high density lipoprotein cholesterol (HDLC),

as risk indicator, shows a negative association (Adult Treatment Panel II, 1994; Castelli, 1996; Khosla & Hayes, 1994). Furthermore, the fatty acid composition of serum phospholipids or cholesterol esters are also an independent risk factor for coronary heart disease (CHD) (Salomaa *et al.,* 1996). Recent literature revealed the growing perception that the haemostatic system is not only physiologically and pathologically involved in the process of thrombus formation, but that haemostatic imbalance in the form of hypercoagulability also contributes to the process of atherosclerosis or atherothrombosis (Vorster *et al.,* 2000) and thus CHD.

To bring these last mentioned facts in perspective with the rest of this study and the variables that were evaluated in Chapter 4, the author will consequently briefly refer to specific dietary fats and oils as CVD risk factors.

2.2 Dietary fats and oils

Several strategies to prevent the development of CVD have been researched and it seems that dietary intervention, together with other lifestyle changes, form an essential part of the management of some of the risk factors for CVD (Oosthuizen, 1999; Vorster *et al.,* 1997a). The efficacy of dietary manipulations depends on several factors, including the individual's genetic constitution. Although the relationship of the total diet, as well as specific foods and nutrients with lipoprotein metabolism has been more thoroughly researched (summarised by Oosthuizen, 1999), this relationship is far from clear with respect to the different haemostatic variables (Jerling, 2001; Vorster *et al.,* 2000). Numerous dietary factors have been implicated altogether, but one of the most important variables that has come under the most scrutiny is fats and fatty acids. Before the influence of dietary fats and fatty acids on lipids and haemostatic factors will shortly be discussed, the specific characteristics of palm oil and red palm oil first needs to be addressed for the purpose of this thesis.

2.2.1 Characteristics of palm oil and red palm oil

Palm oil (PO), derived from the mesocarp or flesh of the palm fruit (*Elaeis guineensis*), is the main oil produced from the oil palm which is grown in India, Malaysia and some African countries. Malaysia is by far the largest producer and exporter of palm oil in the world (O'Holohan, 1997). Two methods of refining (as summarised by O'Holohan, 1997 and Pieters, 2002) are in widespread use, namely

the physical process (more common) and the chemical or alkali method (more flexible) in order to produce refined, bleached and deodorized palm oil. Dry fractionation of PO yields palm olein (POL, liquid fraction: 70-80%) and palm stearin (solid fraction: 20-30%). POL contains less saturated fatty acids (SFAs), and more polyunsaturated fatty acids (PUFAs) than PO, thus having a P/S ratio of 0.3 versus the 0.2 ratio of PO (see Table 2.1). PO is a semi-solid fat at ordinary room temperature due to the presence of solid, fully saturated triglycerides and the high melting point mono-oleoglycerides (Choo, 1994). For this reason it is also considered a suitable *trans*-free main ingredient in margarines, shortenings, etc. in the food industry (Ong & Goh, 2002). As a result of the relatively high monounsaturated fatty acid (MUFA) (41.5%) and SFA (46.8%) content of POL, as well as the presence of natural antioxidants (Table 2.2, discussed in section 2.4), it has a high stability towards free radical oxidation. The stability of refined POL is further enhanced by the fact that approximately 3.5% free fatty acids are readily removed by physical refining. For this reason it is an excellent choice for home cooking, as well as deep frying in the food industry (Ong & Goh, 2002). The nut of the palm fruit contains the palm kernel, from which palm kernel oil, with its totally different chemical and physical properties, is obtained. This oil has a higher content of medium and short-chain SFAs (47.2-73.8%), with lower MUFA (15.6-37.0%) and PUFA (3.2-9.8%) levels compared to PO (Berger, 1986). This point is important because the two oils are often confused by nutritionists. Whereas PO is mainly used for food, palm kernel oil is mainly used for the oleochemical industry (Ong & Goh, 2002). The differences in fatty acid content between the above mentioned PO fractions, are briefly summarized in Table 2.1. Palmitic acid (C16:0) is the second most abundant fatty acid (after oleic acid) and the most abundant SFA in the USA and UK, accounting for approximately two-third to three-quarter of all SFAs consumed (8-10 %En) (Chandrasekharan & Basiron, 2001).

Table 2.1 Saturated, monounsaturated and polyunsaturated fatty acid content of several palm oil fractions (adapted from Ong & Goh, 2002).

Oil	SFA	MUFA	PUFA	P/S ratio
Palm oil	49.5	40.3	9.6	0.30
Palm olein	46.8	41.5	12.0	0.30
Palm kernel	84.0	14.0	2.0	0.02

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; P/S ratio = Polyunsaturated fatty acid/Saturated fatty acid ratio

Red palm olein (RPO) is the unrefined, unbleached, orange-red coloured oil extracted from the oil palm fruit with its carotenoid content, of which α- and β-carotene constitutes 80-90%, still intact. Crude PO differs in its total carotene content among the different varieties, species and hybrids, ranging from *E. guineensis* var. *pisifera* with 428ppm carotene to *E. oleifera* with 4 600ppm carotene (Choo, 1994). A modified refining process has, however, also been developed which involves mild reactions (compared to ordinary refining methods) to remove the free fatty acids and reduce peroxide values. RPO thus represents the richest food source of carotenoids (Cottrell, 1991) while it is also rich in vitamin E isomers (tocopherol and tocotrienol), which have the capacity to retard peroxidation and scavenge free radicals, while specifically tocotrienols also have hypocholesterolaemic potential (Qureshi *et al.,* 1995; Qureshi *et al.,* 1991b; Rukmini, 1994). Its fatty acid composition compares well with that of refined PO (summarised in Table 2.3). Several studies have also confirmed the bio-availability of RPO carotenoids and proved that RPO is a good substitute for synthetic vitamin A in supplementation programmes and preventive therapy (Manorama *et al.,* 1997; Van Stuijvenberg *et al.,* 2000). According to Manorama and Rukmini (1992), RPO is not suitable as deep frying medium or for deep fried products if the aim is to enhance the consumption of β-carotene, as deep frying may cause changes in the physical, chemical (e.g. destruction of ß-carotene) and sensory properties of the oil, mainly due to heat deterioration. It was found by these authors that 88% of the oil's ß-carotene content was, however, retained in cooked and baked products, in the latter case probably as a result of thorough mixing with the other ingredients and indirect heat exposure.

The oil fractions that were used in the present study (Chapter 4) were refined, bleached, deodorised POL and PRO. This RPO fraction is commercially available and marketed as Carotino®. The vitamin E isomer and carotene content of the test oils specifically used in this study, are presented in Table 2.2. The importance of differences in biological activity and abundance of these compounds are further described in section 2.3.

The combination of all the above mentioned characteristics of RPO makes it an excellent oil for human consumption and may even lead to extended use in the food industry. Considerable research and development input are, however, needed to utilize β-carotene in this oil optimally as a source of dietary β-carotene, as well as an edible oil. More studies should thus be done to support the growing contention that

RPO can be a useful and healthful addition to the human diet, and strategies to increase the use of this oil, especially in developing countries, should therefore be addressed without hesitation.

Table 2.2 Vitamin E isomer and carotenoid content of POL and RPO used in the current study.*

Nutrient	POL (µg/g)	RPO (µg/g)
Vitamin E isomers:		
α-tocopherol	130	140
α-tocotrienol	150	213
γ-tocotrienol	187	302
δ-tocotrienol	57	73
Total vitamin E	524	728
Carotenoids:		
α-carotene	ND	273
β-carotene	ND	282
cis-α-carotene	ND	61
lycopene	ND	7
other	ND	48
Total carotenoids	ND	671

* As analysed by the Palm Oil Research Institute of Malaysia; POL = palm olein; RPO = red palm olein; ND = none detected

A further discussion on reasons why PO and RPO can be used successfully in the food industry, as well as in chemical processes applied to these oil, can be found in section 3. Taking into consideration the above mentioned background information on the physical and chemical characteristics of PO and RPO, the effects thereof, as well as to a lesser extent that of other fats and fatty acids on lipids, lipoproteins and haemostatic factors, will consequently be discussed.

2.2.2 *Effects of fats and fatty acids on lipids and lipoproteins*

The science behind the effects of dietary fat on human health is so complex that there are no simple and straightforward answers to the questions in the field today (Dunford, 2001). Since our normal diets contain mixtures of different fats and fatty acids, the net effect on TC or individual lipoproteins will be the sum of, in all likelihood, numerous and possibly opposing effects. It is therefore of the utmost

importance to decipher the effect of each individual fatty acid, as opposed to classes of fatty acids (Khosla & Sundram, 1996). For the purpose of this study, there will be a focus on the fatty acids in the saturated class. Of all the SFAs, palmitic (C16:0) and stearic acid (C18:0) predominate in the diet, with C16:0 being the most abundant. It is well established that diets high in SFA raise plasma TC (Hegsted *et al.,* 1965) and in the Seven Countries Study (Kromhout *et al.,* 1995), the average intake of all major SFAs was significantly associated with 25-year mortality rates from CHD. Thus, several official nutritional recommendations include reduction of SFA intake to ≤10 % total energy (%En) to decrease the risk of CHD. Evidence indicates that all SFA do not affect lipoprotein profiles equally (Hegsted *et al.*, 1965; Zock *et al.,* 1994). Much inconsistence persists concerning the impact of specific dietary fatty acids on plasma cholesterol, and more importantly, concerning their underlying mechanism of action on LDLC and HDLC dynamics (Hayes *et al.,* 1995). C18:0 has a neutral effect, while lauric (C12:0) and myristic acid (C14:0) have potent cholesterol raising effects (Grundy & Denke, 1990). The potency of C14:0 as a cholesterol-raising FA has been calculated by some authors (Hayes & Khosla, 1992; Sundram, 1997) to be four times that of C16:0, while other authors' data show that it is only about 1.5 times as cholesterol-raising as C16:0 (Zock *et al.,*1994). Generally, the hypercholesterolaemic effect of fatty acids relative to each other is much debatable, while the effect of PO and C16:0, which is well studied, seems to be very inconsistent. Although earlier studies identified palmitic acid as hypercholesterolaemic, these findings have recently been questioned by a number of researchers. Opposed to this, the use of RPO *per se* in research has been rather limited (Kritchevsky, 2000) and few human studies (Manorama *et al.,* 1999; Wood *et al.,* 1993) on its effect on lipids and lipoproteins could be found in the literature search. To focus on the theme of this thesis, Table 2.3 represents the fatty acid distribution of specifically the palm olein and red palm olein used in and analyzed for the current study by gas chromatography, as well as a simplified summary of its effects on blood cholesterol. Although fatty acid compositions stated by other literature sources (Cottrell, 1991; Ong & Goh, 2002) differ somewhat from the values given in Table 2.3 as a result of difference in hybrid, variety or growth region, stated values from the current study were considered more applicable for reference by the author. This is followed by a summary (Table 2.4) of only those human studies in which the effect of palm oil and palm olein, red palm oil or palmitic

acid on lipids was compared to other oils or fatty acids, as compiled after a literature search on the Science Direct Database (also including MEDLINE publications).

Table 2.3 Fatty acid composition of POL and RPO used in our study* and their effects on blood cholesterol.

Fatty acid	Effect on blood cholesterol#	POL (%)	RPO (%)
Lauric acid – C12:0	↑	0.25	0.27
Myristic acid – C14:0	↑	0.96	0.90
Palmitic acid – C16:0	↑, ↓, Neutral	40.93	36.20
Stearic acid – C18:0	Neutral	4.28	3.70
Oleic acid – C18:1	↓	41.69	46.70
Linoleic acid – C18:2n-6	↓	11.32	12.80
Linolenic acid – C18:3n-3	↓	0.20	0.41

* As analysed by the Palm Oil Research Institute of Malaysia; # Adapted from Chandrasekharan & Basiron, 2001; POL = palm olein; RPO = red palm olein; ↑ = increase; ↓ = decrease

It should be emphasized that exact comparisons of the effects of fatty acids on lipids and lipoproteins in human studies are impossible as it is frequently complicated by many variables in the study design, such as the level of total fat (or fatty acid) in the diet, percentage of fat replacement for test fats (level of %En exchange), type of test fat (synthetic or natural), type of test diet (whole, solid food or liquid formula), total calorie level of the diet, total dietary cholesterol intake (Hunter, 2001; Sundram, 1997), as well as intake and specific threshold of C18:2 in the study population (Hayes *et al.*, 1995). The duration of the feeding period, whether all foods or only test foods were provided and thus if the study was free-living or under controlled conditions, age of subjects and whether subjects are hyper or normo-cholesterolaemic, also play an important role (Hunter, 2001).

According to Table 2.4 and the above discussion, it is clear that the first few studies (mostly before 1990), which are often cited as examples of the cholesterol raising properties of PO, are characterized by the use of liquid formula diets in which fats contributed about 40 %En, the use of relatively older subjects with moderate to severe hypercholesterolaemia and the feeding of atypical diets in which the test fat is usually provided in excess (Sundram, 1997). The study by Zock *et al.* (1994) is probably the only study that has shown a hypercholesterolaemic effect of C16:0 in young, normocholesterolaemic subjects consuming solid food diets with moderate

cholesterol intakes, but the use of fat blends containing atypical triglyceride moieties may have been partially responsible for these contradictory results (Hayes *et al.,* 1995; Khosla & Sundram, 1996). Khosla & Hayes (1994) conclude that, to derive valid information about the physiological impact of dietary fat, and specifically fatty acids, it should be fed at the levels that the body normally encounters. In some of the studies summarized above, an extreme (>15 %En) exchange between the test fats (Bonanome & Grundy, 1988; Denke & Grundy, 1992; Mattson & Grundy, 1985), which is not normally possible with natural diets, was used. In normal diets <10 %En can be exchanged when natural oils are used as sources of the fatty acids. As seen in the more recent studies (after 1990), normally, when solid-food diets are utilized and more realistic fatty acid exchanges and mildly hypercholesterolaemic to normal cholesterolaemic younger subjects are used, the hypercholesterolaemia attributed to C16:0 is either muted or disappears. Furthermore, in comparison to diets enriched by canola, rapeseed and olive oils, palm olein generally appears to be comparable in its ability to modulate the lipids and lipoproteins. The difference between desaturation of C16:0 and C18:0 (3.9% vs 9.2%) provides rationale to partially explain the observation (Bonanome & Grundy, 1988; Schwab *et al.,* 1996; Tholstrup *et al.,* 1994a) that C18:0 is less hypercholesterolaemic than C16:0 (Emken *et al.,* 1993). Following the hypothesis by Hayes and Khosla (1992) that C16:0 would be neutral in situations where LDL receptor activity is not compromised (e.g. by dietary cholesterol), and taking into account the above mentioned variabilities in study design, another hypothesis by Sundram *et al.* (1995) was formulated. According to it, C16:0 may be equivalent to C18:1 (and even C18:2) provided that: (1) test subjects are normocholesterolaemic (<200mg/dL); (2) dietary cholesterol intake is <300mg per day; (3) the PUFA content of a diet does not exceed 20 %En, where depression of HDLC might be a factor; and (4) the exchange between fatty acids occur above the critical "threshold" (±5 %En) for C18:2 intake. More research, however, seems necessary to confirm and/or verify this "threshold", as the upper limit of C18:2-intake was reported to be 3 %En by the National Institutes of Health (NIH) (Simopoulos *et al.,* 1999). The above hypothesis is further strengthened by analyses of accumulating data which show that 85% of the observed variation in serum cholesterol could be explained solely on the basis of C14:0 and C18:2 when dietary cholesterol intake is ≤300mg per day (Khosla & Hayes, 1994; Khosla & Sundram, 1996).

Animal studies have shown that the positional fatty acid distribution of some fatty acids influences its absorption in the small intestine (Ong & Goh, 2002; Renaud *et al.*, 1995). Fatty acids in the *sn*-2 position of dietary triglycerides are preferentially absorbed through the intestinal wall and those esterified to the *sn*-1 and *sn*-3 positions, especially long-chain SFA, are released in the intestinal tract and partly excreted in the feces. Mostly fatty acids in the *sn*-2 position are thus able to influence lipemia and platelet reactivity (Ong & Goh, 2002; Renaud *et al.*, 1995). In natural PO, 87% of the fatty acids found in position 2 are unsaturated, whereas the saturated fatty acids mainly occupy positions 1 and 3 (Ong & Goh, 2002). The absorption of C16:0 from PO is therefore not very high and this might contribute towards an explanation for the fact that natural PO did not have the same hypercholesterolaemic effects in several of the above studies as did other SFA's. This, however, will have to be further examined, as the importance of positional distribution of fatty acids on lipoprotein metabolism seems debatable by some researchers (Nestel *et al.,* 1995; Zock *et al.*, 1995). These studies favour the conclusion that interesterification *per se,* during which equal amounts of fatty acids are redistributed or randomized to each of the three positions of the glycerol molecule, does not affect cholesterol-raising or lowering properties of the fat.

From the above it can be concluded without doubt that all SFA are not equal in their effects on lipids and lipoproteins. In general, the reported behavior of PO which is generally different from that of other SFAs, may be attributed to the absence of shorter chain SFAs unlike in milk and coconut oil, its high oleic acid content, and even more importantly, to its high tocotrienol content (Cottrell, 1991; Qureshi *et al.,* 1991a). Most of the recent findings thus merit a reevaluation of the nutritional properties of palm oil and palm olein in a more positive light, to emphasize the important role it may play as major edible oil for human consumption worldwide.

Table 2.4 Summary of human studies investigating the effect of palm oil or palmitic acid relative to other fatty acids on lipids and lipoproteins.

Reference	Study characteristics	Main results
Ahrens *et al.*, 1957	40% En as PO, LF, metabolic ward conditions.	PO ↑ TC compared to corn oil. TC after PO was lower than baseline values.
Grande *et al.*, 1961	PO enriched diet vs stearic acid diet derived from cocoa butter.	PO ↑ TC relative to stearic acid diet. Confirms Key's findings that stearic acid is normocholesterolaemic.
Anderson *et al.*, 1976	12 subjects. Diets providing 35 %En SFA predominantly as PO vs safflower oil diet (PUFA).	PUFA resulted in lower TC than SFA diet.
Baudet *et al.*, 1984	Diets containing 30 %En fat contributed predominantly by either palm oil, sunflower oil (SFO), peanut oil or milk fat.	SFO ↓ TC and LDLC compared with other diets. TC and LDLC similar after PO and peanut oil, whereas milk fat resulted in higher TC and LDLC than all other diets.
Mattson & Grundy, 1985	Metabolic ward conditions. 20M, age 59y, LF providing 40 %En contributed by either PO, high C18:1 safflower oil or high 18:2 safflower oil.	After 4wks, both safflower oil diets produced lower TC and LDLC than PO diet. HDLC on high C18:2 was lower than similar HDLC's of high C18:1 and PO diets.
Bonanome & Grundy, 1988	Metabolic ward conditions. 11M, age 64y, LF providing 40 %En PO, high C18:1 safflower oil and an high stearate interesterified fat blend (43% C18:0 + 40% C18:1).	TC and LDLC was higher after PO than values on both other diets. Study concluded that stearic acid had neutral impact on cholesterol and lipoproteins in humans. Stearic diet ↓ HDLC compared to PO or C18:1 diet.
Grundy & Vega, 1988	11 PP, LF containing 40 %En PO, coconut oil or high C18:1 safflower oil (high fat), compared to 20 %En low fat diet.	PO and coconut oil ↑ TC. TC and LDLC were lower on C18:1 diet compared to all other diets. TC, LDLC and HDLC on PO diet were lower than on coconut and habitual diets.
Marzuki *et al.*, 1991	Normal, healthy volunteers. SF containing either PO or soybean oil.	TC and LDLC were not affected by PO or soybean oil diets, thus PO and soybean oil had comparable effects on TC, LDLC and HDLC.
Ng *et al.*, 1991	61M, 22F, NC subjects, aged 20-34y, SF diets prepared with PO, corn oil and coconut oil and providing ±75 %En of fat.	Switching from coconut to PO and corn oil ↓ TC (-19%, -36%), LDLC (-20%, -42%) and HDLC (-20%, -26%), respectively. LDLC/HDLC ratio ↓ 8% by PO and 25% by corn oil. Thus, PO is hypocholesterolaemic compared to coconut oil.
Denke & Grundy, 1992	Metabolic ward conditions, 14M, ages 44-71y, LF diets providing 40 %En fat and comparing lauric acid (C12:0) from a synthetic high-lauric oil, palmitic acid (C16:0) from palm oil, or oleic acid (C18:1) from oleic-rich sunflower seed oil.	C16:0 ↑ TC and LDLC more than did C12:0. (↑LDLC in C12:0 about two-thirds that of C16:0). Thus C16:0>C12:0>C18:1 in effect on TC and LDLC. No differences in TG or HDLC for all diets.

Reference	Study characteristics	Main results
Heber *et al.*, 1992	13M, NC subjects, ages 22-43y, SF diets providing 35 %En fat of which 50% would be test fat, namely PO, coconut oil or hydrogenated soybean oil.	PO diet did not change TC, LDLC, apo A or apo B, but ↑ HDLC. Coconut oil ↑ TC, LDLC and apo B.
Nestel *et al.*, 1992	Mildly HC M subjects. SF diets in which a *trans* elaidic rich fat was compared with a C16:0-rich blend, and with a C18:1-rich control diet.	Both test blends resulted in higher TC and LDLC than the C18:1 control diet. Essentially no difference between TC and LDLC in test diets. C16:0 ↑ HDLC. This led authors to conclude that there is little benefit from avoiding PO use by substituting *trans* fatty acids in food formulations.
Ng *et al.*, 1992	20M, 13F, NC subjects, ages 22-42y, SF diets providing 31 %En, <200mg/d cholesterol and 3 %En from C18:2. Test diets contained palm oil (C16:0) or olive oil (C18:1-rich).	7 %En exchange between C16:0 and C18:1 resulted in identical TC, LDLC, HDLC and TG values. Thus, in healthy, NC subjects, dietary C16:0 can be exchanged for C18:1 within the range of these fatty acids normally present in typical diets without adversely affecting serum lipids and lipoprotein levels.
Sundram *et al.*, 1992	38M, NC subjects, ages 19-45y, maximal replacement (70%) of usual sources of SFA in Dutch diet (animal fats and hydrogenated oils) by PO.	Replacement by PO had no effect on TC, LDLC or most other lipoprotein fractions. PO consumption resulted in 4% ↑ in apo A, 4% ↓ in apo B, 11% ↑ in HDL_2, 8% ↓ in LDL:HDL_2 + HDL_3 ratio and 9% ↓ in TG of LDL-fractions relative to control the diet. Thus, when PO replaces a major part of the normal fat content in a Dutch diet, it may have some cardiovascular benefits.
Wood *et al.*, 1993	29M, ages 30-60y, NC subjects, SF diets providing 38 %En fat of which 50% would be test fat, namely butter, crude PO, hard margarine, refined PO, 80% refined PO + 20% SFO, and SFO.	Diets containing either crude or refined PO did not ↑ TC relative to habitual diet, or LDLC and apolipoproteins levels relative to any other test diet. Crude PO ↓ LDLC. HDLC and apo A levels on the refined PO diet was the highest of all diets.
Nestel *et al.*, 1994	34M, HC subjects, age 49y, SF diets providing 37 %En fat and palmitoleic acid (C16:1), C16:0 or C18:1 as test fats.	TC and LDLC were similar with C16:1 and C16:0, but higher compared to C18:1. HDLC was lower with C16:1 than with C16:0. The study confirms that, at least in HC men, a 4 %En ↑ in C16:0 raises LDLC relative to C18:1 (+3% En), even when dietary cholesterol is low (< 165 mg/day). C16:1 (+4% En) behaves like a SFA and not a MUFA in its effect on LDLC.
Sundram *et al.*, 1994	17M, NC subjects, ages 19-22y, SF diets providing ±30 %En as fat and <200mg/d cholesterol, in which 5 %En was exchanged between C16:0 and C12:0 + C14:0.	Compared with the C12:0 + C14:0-rich diet, the C16:0 diet produced 9% lower TC, as reflected mainly by a lower (11%) LDLC and, to a lesser extent, HDLC. None of the diets induced changes in the other lipoprotein levels

Reference	Study characteristics	Main results
Tholstrup *et al.*, 1994a	15M, ages 22-30y, SF diets providing 40 %En from fat, of which 36% was derived from test fats, namely shea butter (C18:0), PO (C16:0), and palm-kernel oil with high-oleic SFO (C14:0 + C12:0).	C18:0 ↓ TC, LDLC, HDLC, Apo A and Apo B compared to C16:0. C16:0 ↓ TC, HDLC and apo A compared to C14:0 + C12:0.
Zock *et al.*, 1994	36F, age 29y, 23 M, age 28y, NC subjects, SF diets containing 39 %En as fat and ±10 %En by either C16:0, C18:1 or C14:0. Natural fat sources were not used.	Replacing 10 %En from C16:0 by C18:1 ↓ LDLC. C14:0 ↑ LDLC, HDLC and apo A relative to C16:0, and can be considered about 1.5 times as cholesterol-raising as C16:0. C4:0 and C16:0 both caused high LDLC and apo B levels and low HDLC/LDLC ratios.
Choudhury *et al.*, 1995	10M, 9F, age 27y, NC subjects, SF diets providing 31 %En as fat, <200mg/d cholesterol and 3-4 %En from C18:2, in which the effect of POL (C16:0) and olive oil (C18:1-rich) was compared.	Both C16:0 and C18:1 ↓ TC, TG and HDLC relative to subjects' usual diet. POL ↓ TC by 19%. 5 %En exchange between the test fats resulted in almost identical TC and LDLC values, so that when C16:0 replaced C18:1 the expected ↑ in LDLC was not seen and thus, C16:0, though saturated, is not always a TC raising FA.
Ghafoorunissa *et al.*, 1995	Study 1: Metabolic ward conditions, 12M, age 35y. Study 2: "in-home" study, 12M, 12F, ages 30-52. SF diets (27 %En fat) in which PO was substituted for groundnut oil (C18:2).	In both studies SFAs availability was effectively doubled and the content of C18:2 was decreased by half using PO. Despite these major shifts in FA composition, TC, LDLC, HDLC and VLDLC were unaltered by the type of test oil in both studies.
Nestel *et al.*, 1995	27M, mildly HC subjects, age 49y, SF diets providing 10-15 %En fat, supplemented by 20 %En from test fat (margarine), namely (1) high-linoleic acid, moderate *trans* FA, (2) high-PO blend or (3) an interesterified form of the high-PO margarine with <200mg/d cholesterol.	Both high-PO margarines led to similar LDLC, which were higher than LDLC on high-linoleic margarine. This study shows that interesterification of oils used to harden margarines does not raise plasma cholesterol more than does the margarine's constituent FAs.
Schwab *et al.*, 1995	15F, ages 19-34y, NC subjects, SF diets providing 36 %En as fat in which a substitution of 4 %En with C16:0 or C12:0 were made in the experimental diets, and 4 %En as monoenes in the baseline diet.	C16:0 caused no changes in TC, LDLC, HDLC, VLDLC or TG. There were no differences in serum total, lipoprotein lipids or apo A and apo B between the experimental diet periods.
Sundram *et al.*, 1995	23M, NC subjects, ages 19-24y, SF diets providing 31 %En as fat and <200mg/d cholesterol with at least 6 %En from C18:2. Exchange of dietary C16:0 was compared with either C18:2 (American Heart Association Step 1 fat blend, ±4 %En exchange) or C18:1 + C18:2 (Canola, ± 7 %En exchange).	TC, VLDLC, and LDLC were not affected by the diets. The Canola diet (low SFA, high MUFA) and C16:0 diet (high SFA, low PUFA) produced identical HDLC, while the AHA-diet (moderate SFA, moderate PUFA) ↑ HDLC with 17% compared to the other two diets. Neither apo A, apo B or Lp(a) were affected by the diets.

Reference	Study characteristics	Main results
Schwab *et al.*, 1996	12F, NC subjects, age 23.5y, SF diet providing 37 %En of fat, with a substitution of 5 %En of C16:0 or C18:0 in the experimental diets for 5 %En of MUFA's in the baseline diet.	TC, HDLC and apo A was higher on the C16:0 diet than on the C18:0 diet (8%, 9% and 11%, respectively), while LDLC and TG did not differ significantly. Within the C16:0 diet-group, TC, LDLC (12% and 15%, respectively) and LDLC/HDLC ratio were higher after intervention (compared to baseline).
Temme *et al.*, 1996	12M, 18F, NC subjects, ages 20-60y, SF fatty acid enriched diets providing 40 %En as fat, of which 28% was supplied by either C12:0, C16:0 or C18:1. Exchanges of 8 %En were made between the test fats.	C12:0 ↑ TC and LDLC compared to C16:0. C16:0 ↑ TC relative to C18:1, while HDLC did not differ between C16:0 and C18:1. No effects were seen in TG or Lp(a). It appears as though C12:0 *per se* was more cholesterol raising than C16:0.
Cater *et al.*, 1997	Metabolic ward conditions, 9M, mildly HC subjects, ages 55-75y, SF diets providing 53 %En of fat with 43 %En from either medium-chain triacylglycerols (MCTs), PO, or high oleic acid sunflower oil (HOSO).	TC was higher on PO than on HOSO. MCT produced TC and LDLC levels not different from those by PO, but higher than those by HOSO. There were no differences in HDLC. On the basis of %En this study suggests that MCT has one-half the potency that PO has at raising TC and LDLC.
Choudhury *et al.*, 1997	24M, 18F, NC subjects, 24 aged <40y, 18 aged >40y. Men consumed 3x50g of crisps fried in either C16:0 (POL) or C18:1 (HOSO), while women consumed 2x50g of crisps fried in either of the oils. These amounts provided ±50% of subjects' daily fat intake.	TC and LDLC were 7% lower (in older and younger subjects) on C18:1 than on C16:0. Fasting TG were not different between test oil periods.
Cook *et al.*, 1997	36M, NC subjects, ages 20-32y, 4 SF diets providing ±29 %En as fat were formulated, to provide combinations of a high (± 10-12 %En) or low (±3 %En) level of C16:0 in relation to a high or low level of PUFA (C18:2).	TC and LDLC were not affected by the high level of C16:0 when diets also contained high levels of C18:2. C16:0 had no effect on HDLC on either high or low levels of intake. Results indicate that C16:0 had no effect on serum lipoproteins in the presence of recommended intakes for PUFA.
Sundram *et al.*, 1997	18M, 9F, NC subjects, ages 19-39y, SF diets providing 31 %En and low cholesterol (<225mg/d) were followed. Dietary *trans* C18:1 (elaidic acid at 5.5 %En) was exchanged for *cis* C18:1, C16:0 or C12:0 + C14:0.	The *trans*-rich fat ↑ TC, LDLC and apo B, and ↓ apo A relative to C16:0-rich and C18:1-rich fats, while it ↓ HDLC relative to all other test fats. The C12:0 + C14:0-rich fat ↑ TC compared to C16:0-rich and C18:1-rich fats. Identical effects on lipoproteins were elicited by C16:0 and *cis* C18:1. TG values were unaffected by all fats.
Zhang *et al.*, 1997a	31M, 20F, mildly HC subjects, ages 32-68y, SF diets providing ±30 %En fat and <200mg/d cholesterol, in which the test fats PO and peanut oil (PE) accounted for 60-65% of total dietary fat.	Compared to entry levels, TC, LDLC and TC/LDLC ratio ↓ in PO group (-6.5%, -9.0%, -11.5%), while not appreciably altered in PE group after intervention. PO, used as cooking oil in the Chinese diet, will thus not lead to any adverse effect on blood lipids and will not increase CVD risks.

Reference	Study characteristics	Main results
Zhang *et al.*, 1997b	Experiment 1: 120 M, NC subjects, ages 18-25y, SF diets providing ±30 %En as fat, 75-80% of which was contributed by test oils, namely PO, soybean oil (SO), lard (LA) and peanut oil (PE). Experiment 2: 31M, 20F, mildly HC subjects, ages 32-68y, SF diets providing ±30 %En fat and <200mg/d cholesterol, in which the test fats PO and peanut oil (PE) accounted for 60-65% of total dietary fat.	Experiment 1: Compared with entry level, TC and LDLC ↓ with 6.7 and 13.1%, respectively, in PO group and ↑ with 22.8 and 30.7%, respectively, in the LA group. At end of intervention TC, LDLC and TC/HDLC ratio in PA group were lower than those of LA group. PE had no influence on serum lipids. Experiment 2: Compared to entry levels, TC, LDLC and TC/LDLC ratio ↓ in PO group (-6.5%, -9.0%, -11.5%), while not appreciably altered in PE group after intervention.
Cuesta *et al.*, 1998	14F, HC subjects, age 63y, SF diet providing ± 400mg/d cholesterol in which a dietary exchange between C16:0 and C18:1 (oleic acid rich SFO) was made, while C18:2 was kept at a relatively lower level (4 %En). Experimental oils provided ± 62% of total fat intake.	POL ↑ TC (17.7%) and apo B (18.0%), while LDLC was higher following the C16:0 than the C18:1 diet. No differences were observed in the TC/HDLC ratio between the dietary periods. Although C16:0 ↑ LDLC, it better protected LDL particles agains peroxidation, mainly in women with high TC, than did C18:1.
Müller *et al.*, 1998	27F, ages 19-42y, SF diets providing 30-31 %En. The effects of 3 test margarines, one based on PO (PALM-margarine), one based on partially hydrogenated soybean oil (*TRANS*-margarine) and on with a high PUFA-content (PUFA-margarine), were compared. Test margarines provided 26 %En in all diets.	No differences were found in TC, LDLC and apo B between *TRANS*- and PALM-diets. HDLC and apo A were higher on PALM- compared to *TRANS*-diet. TC, LDLC and apo B were lower on PUFA-diet compared to other 2 diets. TG and Lp(a) was not different among diets. Thus: C16:0 from PO may be a reasonable alternative to *trans* fatty acids in margarine if the aim is to avoid *trans* fatty acids, but it is still less favorable than a margarine based on a more PUFA vegetable oil.
Nestel *et al.*, 1998	12M, 8F, HC subjects, age 51y, SF diets in which the effects of C18:0 (as structured TG) and C16:0 (PO), which differed by ±5 %En, was compared in the form of 2 test margarines. Foods containing 40-55g of the test margarines were eaten daily after a run-in of 2wks on a low-fat diet.	Neither C16:0 nor C18:0 raised TC or TG back to habitual values after the run-in period. There were no differences in TC, LDLC, HDLC or TG between the 2 test-fat periods. Thus, a similar increase in intake of C16:0 and C18:0 (differing by 5%En) resulted in TC and LDLC that did not differ from those values measured during a period of low-fat intake.
Mutalib *et al.*, 1999	53M, NC subjects, n=10 in reference group on their habitual diet. N=43 divided in 3 groups consumed highly SFA diets containing either PO, hydrogenated soya fat (HSO – *trans* C18:1-rich) or hydrogenated rapeseed fat (HRSO - *trans* C18:1-rich).	PO diets (contributing 26 %En) ↑ TC, LDLC and HDLC at end of 8wk intervention. HRSO and HSO diets ↓ TC and HDLC. PO ↓ TG after 4wks, but not HSO or HRSO. PO could be a natural alternative to SFA derived from animals or hydrogenated vegetable fats if total SFA do not exceed 10 %En.

Reference	Study characteristics	Main results
Clandinin *et al.*, 2000	24M, NC and HC subjects, ages 20-32y, SF diets providing 28.9%±1.6% En as fat, from high (± 10-12 %En) or low (±3 %En) combinations of C16:0 in relation C18:2n-6. [low C16:0, low C18:2; low C16:0, high C18:2; high C16:0, low C18:2 and high C16:0, high C18:2].	High C16:0 ↑ TC and LDLC when the diet was low in C18:2. When the diet was high in C18:2, raising the level of C16:0 did not have an effect on TC or LDLC. At high levels of C18:2, high C16:0 led to ↓HDLC. Results indicate that C16:0 has no effect on serum lipid profiles in the presence of recommended intakes for C18:2n-6 (10 %En).

%En = % of total energy; M = male; F = female; LF = liquid formula; SF = solid / natural food; NC = normocholesterolaemic; HC = hypercholesterolaemic; ↑ = increased; ↓ = decreased; all indications of "higher", "lower", "similar" or "different" values indicate statistical significance of at least $p \leq 0.05$; PO = palm oil; POL = palm olein; SFO = sunflower oil; TC = total cholesterol; LDLC = low density lipoprotein cholesterol; TG = triacylglycerol; HDLC = high density lipoprotein cholesterol; apo A = apolipoprotein A-1; apo B = apolipoprotein B; Lp(a) = lipoprotein (a); SFA = saturated fatty acid; PUFA = polyunsaturated fatty acid; MUFA = monounsaturated fatty acid; C12:0 = lauric acid; C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 = oleic acid; C18:2 = linoleic acid.

Before the effects of fats and fatty acids on haemostasis will subsequently be discussed, the author considered it necessary to give the reader a synthesized overview of the functioning of the haemostatic system.

2.2.3 The haemostatic system

The haemostatic system consists of four closely related and complementary systems, namely the vascular endothelium, blood platelets, coagulation factors and inhibitors, and the fibrinolytic promoters and inhibitors. These systems normally function together in complicated, co-ordinated and tightly controlled processes to prevent thrombus formation and thus ensure the fluidity of blood. The level, activity, function or breakdown products of a specific haemostatic variable should thus be interpreted in context of balance in the total system (Vorster *et al.,* 2000). The roles of the different haemostatic variables that were measured in this study, are illustrated in a simplified manner in Figure 1.

The coagulation cascade is initiated by endothelial cell injury. Upon damage to the endothelium, tissue factor is secreted and activates coagulation. Coagulation is an auto-catalytic, self-limiting process in which thrombin, playing a central role, is generated through a series of cascade-reactions. Thrombin converts fibrinogen into fibrin. Fibrinogen is positively related to other risk factors of CVD and these relationships, together with its causative role in both atherosclerosis and thrombosis, are supported by plausible biological mechanisms and evidence from a variety of studies. Fibrin combined with aggregated platelets forms a clot to arrest bleeding (Lottenberg & Kitchens, 1987; Mackie & Bull, 1989; Vorster *et al.,* 2000).

Thrombin can, however, be inactivated by antithrombin III, and this complex is known as thrombin-antithrombin complex (TAT). In addition to the physiological balance between coagulation factor activities and coagulation inhibitor activities (Lowe & Rumley, 1999), there is also a physiological balance between fibrin network formation (coagulation) and dissolution (fibrinolysis) (Vorster *et al.,* 2000). The fibrinolytic system is thus consequently activated (Emeis *et al.,* 1985) and inactive plasminogen is converted to plasmin by tissue plasminogen activator (tPA). Plasmin breaks down the fibrin clot into fibrin degradation products, of which D-dimer is one type. The presence of D-dimer is therefore an indication that both thrombin and plasmin generation have occurred. It has been shown in several prospective studies that D-dimer at baseline is significantly related to and thus a predictor of future CVD

events. In the circulation, tPA binds to active plasminogen activator inhibitor-1 (PAI-1), forming an inactive complex. Because two-thirds of the total tPA in the plasma is bound to PAI-1, it can be expected that tPA antigen should have a positive relationship with CVD, and free, active tPA, a negative one (Vorster *et al.*, 2000). These relationships have been confirmed in several cross-sectional and case-control studies (Fukao *et al.*, 1992; Hellsten *et al.*, 1992; Smith *et al.*, 1995; Yamada *et al.*, 1996; Yamauchi *et al.*, 1992). Another inactivator of fibrinolysis is α_2-antiplasmin which inactivates plasmin.

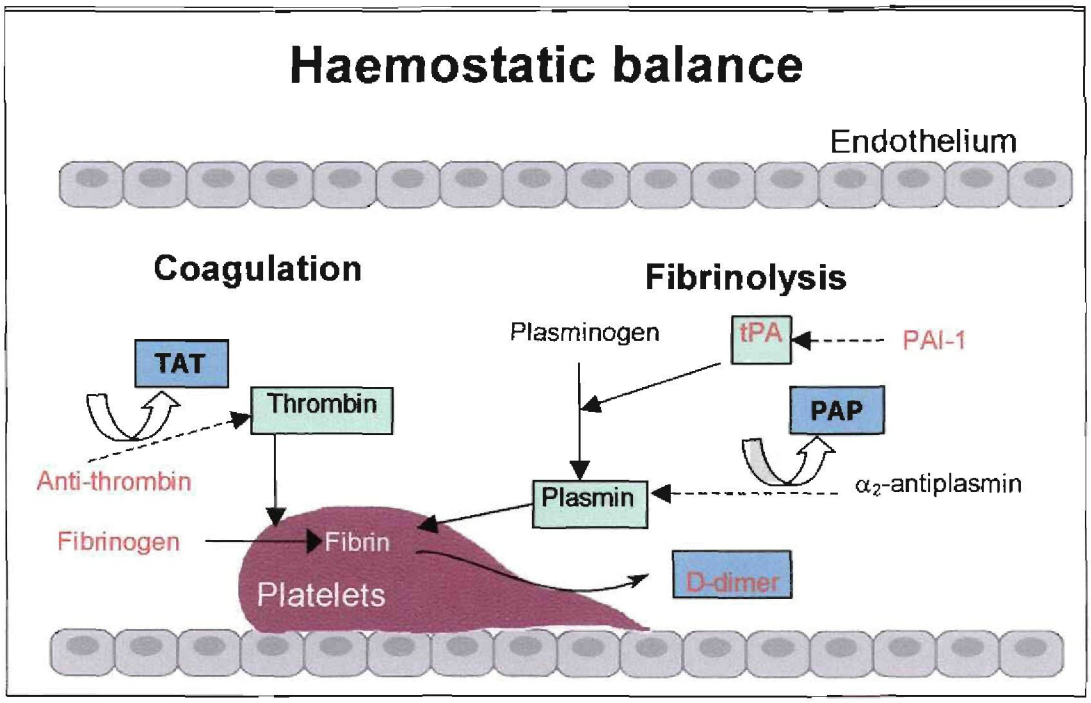

Figure 2.1 The haemostatic system

tPA: Tissue plasminogen activator; PAI-1: Plasminogen activator inhibitor-1; TAT: Thrombin-antithrombin complex; PAP: Plasmin-antiplasmin complex; Red font = CVD risk factor
= active form; = Marker of activity of the haemostatic system; ----▶ Inhibitor

Under normal physiological circumstances there is a "balance" between coagulation and fibrinolysis. In many circumstances an increase in coagulability is accompanied by a compensatory increase in fibrinolytic activity and *vice versa*, resulting in a new steady state or balance (Jerling, 1998). Over exposure to endothelial damage and subsequent initiation of the coagulation system may result in a state where the anti-coagulation and/or fibrinolytic activities fail to compensate for these increases. The result, as indicated previously, may be a hypercoagulable state and increased risk for CVD (Vorster *et al.*, 2000). The haemostatic balance can, however, be measured by

comparing thrombin and plasmin generation. The thrombin-antithrombin complex (TAT) is often used as a marker of thrombin generation, while the plasmin-α_2-antiplasmin complex (PAP) can serve as a marker of plasmin generation (Mair *et al.*, 1997; Takahashi *et al.*, 1990).

2.2.4 Effects of fats and fatty acids on haemostasis

It has consistently been reported that dietary lipids can modify haemostatic function both in the long term, after dietary intervention and acutely after a meal (Hunter *et al.*, 1999). Earlier human experiments on the influence of diet on thrombogenesis have primarily focused on the effect of total dietary fat content or the ratio of PUFAs to SFAs in diets on fasting fibrinolytic variables (Tholstrup *et al.*, 1994). Given that humans are in the postprandial state over much of a 24h-period and that the processes of atherogenesis and thrombosis may be accelerated as a result of changes in the haemostatic system which occur during nutrient absorption (Miller, 1997), researchers are increasingly focusing on postprandial metabolism and how specific dietary components can modify haemostatic properties during this period (Hunter *et al.*, 1999). In the Seven Countries Study (Kromhout *et al.*, 1995), it was found that palmitic and stearic acids were more strongly related to CHD mortality rates than to serum cholesterol levels. This suggests that these SFA's may also exert their effect on CHD through other metabolic pathways, such as arterial thrombosis. Few studies have been performed to establish the specific effects of fatty acids on the haemostatic system (Hunter *et al.*, 1999) and even these produced inconsistent results (Hornstra, 2001). Although it is known that dietary fat type can affect endothelial integrity, results in this regard are also inconsistent and often difficult to interpret in terms of arterial thrombosis tendency (Hornstra, 2001). There are, however, several studies that have investigated the effect of PO and its constituting fatty acids and antioxidants on the coagulation system and different haemostatic factors. To the knowledge of the author, the only study on the effect of crude palm oil (RPO) on some coagulation factors is again that of Wood *et al.* (1993). In order to aid the reader of this thesis to interpret the second manuscript (Chapter 4), the author will subsequently only focus on the above mentioned studies concerning PO and RPO. With the addition of a few studies, Table 2.5 represent an adapted summary of animal and human studies from a MEDLINE search (PubMed) by Pieters (2002), giving an indication of what types of studies have been done and the different variables that have been investigated.

For the specific aim of this chapter, other studies on the effect of the above fatty acids of which the source was not palm oil, was not included in this literature summary. It is difficult to draw final conclusions from the results of these studies, as they differ in experimental design. However, it seems that in most cases, PO (although it contains a high content of SFA) and palmitic acid, had no effect or did not differ in its effect on coagulation factors relevant to other oils or fatty acids. It even tended to inhibit thrombus formation in some cases. It also seems not to affect platelet reactivity unfavourably. Evidence has been presented to suggest that the poor effect of PO in promoting blood clotting compared to other oils might be due to an alteration in the production of the eicosanoids that influence platelet aggregation (Rand *et al.,* 1988). In some of the studies summarized above, PO even seems to contribute towards a better haemostatic profile compared to the other dietary fats. Further studies are, however, needed to confirm these results, to examine the effects of PO, POL and RPO on other important haemostatic variables and to explore the mechanisms involved.

Table 2.5 Summary of animal and human studies investigating the effect of palm oil on haemostatic variables (adapted from Pieters, 2002).

Study	Interventions	Subjects and study design	Main results
Rand *et al.*, 1988.	PO, sunflowerseed oil and a control of low fat diet.	61 male Wistar rats, aged 5 wks, were fed for 8 weeks in a parallel study design.	Arterial thrombosis tendency was ↓ by sunflower-seed oil while PO-group showed an insignificant ↓ (compared to control). PO had no effect on collagen-induced platelet aggregation while it was ↑ in the sunflowerseed oil group (compared to control). PO ↓ TXA_2 production but had no effect on PGI_2 production. PO had no effect on platelet membrane fluidity but it was ↑ by sunflowerseed oil.
Heemskerk *et al.*, 1989.	PO, sunflowerseed oil, hydro-genated coconut oil and fish oil were compared.	24 male Wistar rats, aged 5 wks, were fed for 8 weeks in a parallel study design.	Coconut oil resulted in ↓ platelet activation (by thrombin and collagen) compared to the other diets.
Qureshi *et al.*, 1991a.	Standard diet supplemented with tocotrienol-rich fraction of PO compared to a standard diet.	8 normocholesterolaemic and 8 hypercholesterolaemic pigs received meals for 8 weeks in a parallel study design.	Tocotrienol diet ↓ TXB_2 and PF_4 only in the hypercholesterolaemic pigs.
Pereira *et al.*, 1991.	Palm oil compared to butter fat.	12 male Wistar rats, pathogen free, weighing 70g, were fed for 10 weeks in a parallel study design.	PO ↓ fibrinogen, ↑ AT III (resulting in ↑ clotting time), ↓ platelet aggregation (associated with ↑ bleeding time) compared to butter fat. These findings suggest that PO may be antithrombotic.
Schick *et al.*, 1993.	Hydrogenated PO, olive oil, n-3 fatty acid ethyl esters and standard chow.	18 male fort Detrick Dunkin-Hartley guinea pigs, 300 - 350g, received diets for 36 days in a parallel study design.	PO had no effect on platelet aggregation and TXB_2 production. N-3 fatty acids ↓ TXB_2 production and olive oil ↑ platelet sensitivity.

Study	Interventions	Subjects and study design	Main results
Wood *et al.*, 1993	Diets providing 38 %En fat of which 50% would be test fat, namely butter, crude PO, hard margarine, refined PO, 80% refined PO + 20% SFO, and SFO.	29 normocholesterolaemic men, aged 30-60y, received experimental diets for 6 weeks, followed by 6 weeks of habitual diet.	Participants were each tested for TXB_2 or $PGF_{1\alpha}$ levels on a maximum of three of the dietary test fats. No differences were found for TXB_2 on any diet, while butter, hard margarine and refined PO diets ↓ $PGF_{1\alpha}$. TXB_2 and PGI_2 levels are apparently more sensitive to other influences than dietary fat.
Tholstrup *et al.*, 1994a.	PA compared to shea butter (stearic acid) and palm kernel and sunflower oil mix (myristic and lauric acid).	15 young healthy men received the 3 diets consecutively with 1-month washout in between.	Stearic acid ↓ $FVIIc_{act}$ compared to PA. There was no difference between the treatments and habitual diet for t-PA_{act}, t-PA_{Ag}, PAI-1_{act}, PAI-1_{Ag} or euglobin fibrinolytic activity.
Tholstrup *et al.*, 1994b.	PA compared to synthetic fat high in myristic acid.	12 healthy young men received 3-week treatments before cross-over.	There were no differences between the PA and myristic acid groups for clotting time, PAI-1_{Ag} or fibrinogen but both fatty acids ↑ $FVIIc_{act}$.
Ghafoorunissa *et al.*, 1995.	POL and groundnut oil. Two studies.	Study 1: 12 healthy Indian men received 8-week treatments before cross-over. Study 2: 24 healthy Indian (12) men and (12) women received 16-week treatments before cross-over.	Platelet aggregation and erythrocyte membrane fluidity were essentially similar for POL and groundnut oil, in both studies.
Tomeo *et al.*, 1995.	Palm Vitee (γ-tocotrienol and α-tocopherol rich fraction of PO) compared to palm super olein without vitamin E.	50 patients with carotid artery atherosclerosis (23 men, 27 women) received diets for 18 months in parallel study design.	Palm Vitee resulted in carotid atherosclerotic regression and ↓ platelet peroxidation compared to the control.
De Bosch *et al.*, 1996.	PO compared to SFO. 3 Studies.	Study 1: 40 healthy rural subjects received diets for 90 days. Study 2: 137 healthy rural subjects received diets for 150 days. Study 3: 60 healthy urban subjects received diets for 150 days.	Platelet aggregation remained unchanged in all 3 studies. Plasma fibrinogen increased in both PO and SFO groups in studies 1 and 2 (rural subjects). PAI-1_{act} and $FVII_{act}$ increased in both urban groups (PO and SFO, study 3) and their initial values were higher than the ones of the rural subjects.

Study	Interventions	Subjects and study design	Main results
Mata *et al.*, 1997.	PO compared to olive oil, SFO and fish oil.	24 healthy individuals (12 men, 12 women) received the 4 treatments consecutively for 5 weeks each.	Olive oil ↓ smooth muscle cell proliferation through ↓ in smooth muscle cell DNA-synthesis, compared to other treatments.
Salomaa *et al.*, 1997.	Phospolipid palmitic, linoleic and dihomogammalinoleic acid were compared.	338 men and 363 women aged 45 – 64 took part in this cross-sectional, epidemiological study.	Palmitic acid was positively associated with plasminogen. Linoleic acid was negatively associated with plasminogen and fibrinogen. Dihomogammalinoleic acid was positively associated with FVIIc, fibrinogen and plasminogen amongst women.
Zhang *et al.*, 1997a	Palm oil (PO) compared to peanut oil (PE), each accounting for 60-65% of total dietary fat of ±30 %En.	31 men and 20 mildly hypercholesterolaemic women, aged 32-68y, parallel study, 2 groups. Following a run-in of 3wks on PE, subjects consumed either PO or continued on PE for another 6 wks.	The plasma $TXB_2/PGF_{1\alpha}$ ratio was significantly reduced. No changes were observed on the whole blood platelet aggregation in both groups.
Temme *et al.*, 1998.	Study 1: Palmitic acid compared to lauric and oleic acid. Study 2: Medium chain fatty acid (MCFA) diet compared to myristic or oleic acid-rich diet.	Study 1: 18 women, 14 men, healthy, aged 20-60y underwent 3 diet periods of 6 weeks each in this cross-over study design. Study 2: 37 women, 23 men, healthy, aged 20-60y. After 3 wk oleic acid run-in, 3 groups of subjects followed a parallel design, each consuming one of the test fats.	There was no difference in aggregation velocity between the SFA diets and the MUFA diet. TXB_2 measured in collagen activated samples, which correlated with aggregation velocity, did not differ between the lauric or palmitic compared with the oleic acid diet.
Mensink *et al.*, 1999.	Palm olein, containing α-tocopherol and tocotrienols. PO without tocotrienols was used as control.	40 hypercholesterolaemic men, without history of CVD received diets for 6 weeks in parallel study design.	No difference was observed in either group in: collagen-induced platelet velocity, maximum aggregation, TXB_2 production (whole blood), urinary TXB_2, 11-keto-thromboxane B_2, coagulation and fibrinolytic markers.

Study	Interventions	Subjects and study design	Main results
Mutalib *et al.*, 1999.	PO, hydrogenated soy fat and rapeseed oil, with habitual diet as control.	53 healthy Scottish males received diets for 8 weeks in parallel study design.	PO had no effect on whole-blood aggregation but it was ↑ in the soy fat group.
Temme *et al.*, 1999	Palmitic acid compared to lauric and oleic acid.	18 women, 14 men, healthy, aged 20-60y underwent 3 diet periods of 6 weeks each in this cross-over study design.	PAI-1 act was higher on the palmitic acid compared with the oleic acid and the lauric acid diet. No differences between diets were observed for AT III act, fibrinogen, fragment 1+2, plasminogen or α2-antiplasmin activity.
Müller *et al.*, 2001	Three margarines containing either palm oil (PO), partially hydrogen-nated soybean oil (PHSO, *TRANS*) or high in PUFA were compared.	9 healthy female students, consuming in random order diets containing either of the 3 margarines for 17 days each.	PO had the most favourable effect on tPA_{act}. *TRANS*-diet ↓ diurnal postprandial state level of tPA_{act} compared to PO-diet. No differences in either fasting levels or circadian variation of tPA Ag, $PAI\text{-}1_{act}$, $PAI\text{-}1_{Ag}$, $FVII_{act}$, or fibrinogen between the 3 diets.

↓ = decreased; ↑ = increased; PO = palm oil; POL = palm olein; PA = palmitic acid; SFO = sunflower oil; PGI_2 = prostacyclin; TXA_2 = thromboxane A_2; TXB_2 = thromboxane B_2; $PGF_{1\alpha}$ = prostaglandin$_{1\alpha}$; PF_4 = platelet factor 4; AT III = antithrombin III; $PAI\text{-}1_{Ag}$ = plasminogen activator inhibitor-1 antigen; FVIIc = factor VII coagulant; tPA_{act} = tissue plasminogen activator activity; tPA_{Ag} = tissue plasminogen activator antigen; $PAI\text{-}1_{act}$ = plasminogen activator inhibitor-1 activity; FVII = factor VII. All indications of "higher", "lower", "similar" or "different" values indicate statistical significance of at least $p \leq 0.05$.

From the above discussions in 2.2.2 and 2.2.4 it is clear that dietary fats and fatty acids play a major and important role in lipid metabolism, as well as in the haemostatic system. To extend the discussion on dietary intervention and risk for CVD, the effect of certain antioxidants will subsequently be discussed.

2.3 The effects of Vitamin E and carotenoids on CVD risk factors

Tocopherols are present in most vegetable oils and are more common in the diet than tocotrienols, which are found at relatively high concentrations in PO, one of its richest sources (Cottrell, 1991; Ong & Goh, 2002). Tocotrienols are the unsaturated, structural analogs of the saturated tocopherols, differing only in having a farnesyl rather than a phytyl side chain (Dunford, 2001; Kang *et al.,* 1998). The concentration of γ-tocotrienol in PO is more than double that of α-tocotrienol (Choudhury *et al.,* 1997). α-Tocopherol is the most biologically active of all the tocopeherol homologues, followed by β-, γ- and δ-tocopherol, while it seems that of the tocotrienols, only α-tocotrienol has significant biological activity (Bramley *et al.*, 2000). Compared to one another, these different forms of vitamin E are, however, exhibiting different degrees of antioxidant protection, as it seems that α-tocotrienol has a much higher antioxidant activity than α-tocopherol (Serbinova & Packer, 1994). The edible grade of unrefined RPO is considered the richest food source of carotenoids, especially β-carotene (Cottrell, 1991), as well as a good source of tocopherol and tocotrienol (Kritchevsky, 2000; Rukmini, 1994). Palmvitee®, the trade name for the vitamin-rich distillate of palm oil enriched with tocopherol and tocotrienol, is available in capsule form. The above mentioned tocopherol and tocotrienol-rich fraction of PO is often referred to as TRF. Furthermore, SFO is one of the best sources of the most biologically active homologue α-tocopherol, while it contains no tocotrienols (Cottrell, 1991). The carotenoid, tocotrienol and tocopherol content of the oils used in the current study, are subsequently summarized in Table 2.6. These values are represented in mg/25g of oil, which was the amount that was recommended to be consumed daily by subjects in the nutrition intervention study.

Table 2.6 Carotenoid, tocotrienol and tocopherol content of the oils used in the current study.*

Variables (mg/25g oil)	POL	RPO	SFO
Total Carotenoids	-	16.8	-
Beta-carotene	-	7.1	-
Alpha-carotene	-	6.8	-
Alpha-tocopherol	3.3	3.5	14.1
Total tocotrienol	9.9	14.7	0.1

*As analyzed by the Palm Oil Research Institute of Malaysia; POL = palm olein; RPO = red palm olein; SFO = sunflower oil

Animal studies with Palmvitee® and the tocotrienol rich fraction (TRF) of PO showed cholesterol-lowering effects by down-regulation of hydroxymethyl-glutaryl-CoA (HMG-CoA) reductase activity in the cholesterol biosynthetic pathway (Qureshi *et al.*, 1991a). The results on cholesterol-lowering in human trials are, however, inconsistent as TC and LDLC were decreased in some studies (Qureshi *et al.*, 1991b; Qureshi *et al.*, 1995; Tan *et al.*, 1991), while neutral effects (Mensink *et al.*, 1999; Wahlqvist *et al.*, 1992) have also been observed. These discrepancies seem partly to be ascribed to dose-dependent effects as Khor and Chieng (1997) concludes that lower dosages of Palmvitee® tends to have positive hypocholesterolaemic effects, whereas higher dosages tend to be neutral in effect. More studies on the effects of PO and RPO and its constituents are needed to verify these inconsistent results.

Regarding the haemostatic system, the effect of tocotrienols seems inconsistent in humans as some authors found no effects on platelet aggregation to collagen or ADP (Wahlqvist *et al.*, 1992), or other coagulation and fibrinolytic variables (Mensink *et al.*, 1999), while others observed that tocotrienols significantly reduced platelet factor 4 and thromboxane, as well as platelet aggregation in whole blood or platelet rich plasma (Qureshi *et al.*, 1991b). In the Swedish MONICA Study, a cross-sectional population study, tPA_{act} correlated directly and $PAI\text{-}1_{act}$ correlated inversely with plasma β-carotene. However, when adjusted for confounders these correlations were not significant any more (Eliasson *et al.*, 1995). In the only intervention study that examined the effect of β-carotene on haemostasis, Van Poppel *et al.*, (1995) found that β-carotene had no influence on fibrinogen, tPA_{ag}, fibrin and its degradation

products in smokers and non-smokers. Thus, CVD protection from β-carotene via a beneficial effect on haemostasis seems improbable.

Probably the most important and well researched cardiovascular protective effect of Vitamin E and carotenoids is through its antioxidant properties. Basic research provides a plausible mechanism by which antioxidant vitamins might reduce the risk of atherosclerosis, while a large number of descriptive, case-controlled, and cohort studies provide data that sustain the antioxidant hypothesis, suggesting that the consumption of antioxidant vitamins might reduce the risk for CVD (reviewed by Gaziano, 1999; Marchioli *et al.,* 2001). The antioxidant hypothesis furthermore postulates that sub-optimal levels of principal antioxidant micronutrients (vitamin C, vitamin E [tocopherol and tocotrienol] and β-carotene) are to a certain extent underrated risk factors for cardiovascular diseases (Gey, 1995). In human observational research the association of antioxidants with reduced risk of CVD appears to be stronger for vitamin E than for β-carotene (Gaziano, 1996). In contrast to the above, results of antioxidant supplementation showed no protective effect on CVD morbidity and mortality in several large-scale randomized clinical trials such as the Finnish Alpha Tocopherol Beta-carotene Lung Cancer Prevention Study (ATBC), the Carotene and Retinol Cancer Prevention Trial (CARET), the Cambrigde Heart Antioxidant Study (CHAOS), the Physicians' Health Study (PHS) and the MRC/ BHF Heart Protection Study (Heart Protection Study Collaborative Group, 2002; reviewed by Kritharides & Stocker, 2002; reviewed by Marchioli *et al.,* 2001), while some studies (ATBC and CHAOS) even indicated an elevated risk of fatal heart disease and an increased risk for lung cancer in smokers or people exposed to asbestos (ATBC and CARET), with supplementation of especially β-carotene (Kritharides & Stocker, 2002; Lee, 1999; Rapola, 1997). Overall evidence from published clinical trials does thus not confirm or indicate a protective role of β-carotene on CHD (Tavani & La Vecchia, 1999; Van den Berg *et al.*, 2000).

While ecological studies either suggest the absence of, or a weak relation between β-carotene plasma levels and incidence of CHD mortality, evidence from cohort studies is to some extent compatible with a protective action of β-carotene. Case-control or observational studies support an inverse relation between β-carotene and CVD, which is possibly stronger in current smokers (Christen *et al.*, 2000; Tavani &

Vecchia, 1999). The role of β-carotene in decreasing CVD risk thus seems inconsistent.

At this point, in general antioxidants thus represent a possible but, as yet, unproven means to reduce the risk of CVD (Gaziano, 1999). Most researchers, however, agree that consumption of fruits and vegetables is an important part of a healthy diet (Gaziano, 1996). The author speculates that antioxidants in its natural state as part of whole foods may be more effective in contributing to cardiovascular protection.

Although sometimes inconsistent, it is clear from the above that PO and RPO, as a result of its antioxidant content, indeed could potentially have some health benefits for human consumption. For this reason, the author found it necessary briefly to discuss its role in the food industry, as well as certain applied chemical processes for enhancing its functional characteristics.

3. USES OF, AND APPLIED PROCESSES ON PALM OIL AND RED PALM OIL IN THE FOOD INDUSTRY

To a certain extent the food industry is moving away from using fats and oils only for their sensory characteristics, as emulsifiers, or as flavor and vitamin carriers. Instead, their roles in, amongst other, health and disease prevention are being explored in the development of new nutraceuticals or functional foods (Ong & Goh, 2002). Nutritionally beneficial compounds naturally present in edible fats and oils (e.g. β-carotene and sterols) can be enriched through new refining techniques or modifications of conventional techniques, leading to potentially greater use as nutraceutical ingredients (Dunford, 2001). For this reason, background information on the health and sensory properties and changes associated with the processes applied to oils in the food industry, is necessitated. The author will also refer to some intrinsic physical and functional characteristics of PO and RPO that distinguish it from other oils and thus favour its use in the industry.

A minimum degree of solid triglyceride is needed for the processing of foods such as pastries, baked goods and biscuits. Hydrogenation of vegetable oils is often used to accomplish a more solid textured fat and thus particular functional effects in foods. Hydrogenation is the chemical process where the degree of saturation in a fat or oil is increased by addition of hydrogen atoms to the fatty acid double bonds in the presence of a metal catalyst, usually nickel. The result is the conversion of

unsaturated to saturated bonds (Stauffer, 1996). During this process, *trans* isomers are produced, but their quantity can be manipulated by altering the hydrogenation conditions. This process may also enhance the stability of certain oils to oxidation. As hydrogenation progresses, the melting point of the fat or oil increases gradually (Love, 1992). Partially hydrogenated vegetable oils, containing geometrical (*cis-trans*) and positional isomers of mono and di-unsaturated fatty acids, thus constitute a major ingredient in margarine production. *Trans* fatty acids have, however, reemerged as a big nutritional concern as studies reported that LDLC and TG concentrations are raised and HDLC is lowered with the intake thereof (Mensink & Katan, 1990; Sundram *et al.,* 1997; Wood *et al.,* 1993). Besides increasing the ratio of LDLC to HDLC, *trans* fatty acids have also shown to increase Lp(a) lipoprotein levels when they are substituted for saturated fatty acids (Ascherio *et al.,* 1999). As a result of its semisolid texture at room temperature, PO can be used without hydrogenation and in combination with small percentages of other hydrogenated vegetable oils, to achieve a certain hardness of margarine products (Müller *et al.,* 1998). Although not necessarily caused by a component present in PO, replacing habitual dietary fats by PO may have beneficial effects on lipids. As shown by Hornstra *et al.* (1991), a PO enriched diet can result in the reduced consumption of *trans* fatty acids. This replacement even had the effect of reducing serum Lp(a) levels in normocholesterolaemic volunteers.

An example of a suitable formulation for cake margarine is 50% palm stearin, 15% hardened soya oil, 15% coconut or palm kernel oil and 20% palm oil (Berger & Teah, 1988). Several studies (Müller *et al.,* 1998; Sundram *et al.,* 1997) have thus concluded that nutritionally, some SFAs (e.g. palmitic acid from PO) may be a reasonable alternative to *trans* fatty acids, as it does not seem to have the same adverse effect on plasma lipids. This is specifically applicable in the case of partially hydrogenated vegetable oils in margarine if the aim is to avoid trans *fatty* acids (Müller *et al.,* 1998). Another approach or alternative to harden liquid oils without leading to *trans* fatty acid formation, is through interesterification. The structure of naturally occurring triglycerides is genetically determined. The process of interesterification (randomization) does not change the fatty acid composition, but it permits the exchange of fatty acids within the triacylglycerol so that all three positions of the triglyceride molecule contain an equal portion of the acids (Hunter, 2001; Renaud *et al.,* 1995). It can be accomplished by enzymatic (highly controlled) or

chemical (relatively uncontrolled) methods. This rearrangement of fatty acids changes the physical characteristics of the oil or fat. In the specific case of PO, interesterification causes a substantial transfer of palmitic acid from its usual *sn*-1,3 positions in the triacylglycerol molecule into the middle *sn*-2 position (Renaud *et al.*, 1995). Although the possibility exists that interesterification may increase the likelihood of the fat raising LDLC more than the native oil (Kritchevsky, 1995), a study by Nestel *et al.* (1995) showed that interesterification of palm oil to harden margarine did not raise plasma cholesterol more than does the margarine's constituent fatty acids. More research, however, is needed to ensure that TC is not increased by interesterification of specifically PO.

Furthermore, as a result of its relative high SFA and low PUFA content, as well as the presence of several natural antioxidants like vitamin E isomers (tocopherols and tocotrienols) and carotenes (in the case of RPO), PO and RPO are very resistant to oxidation and therefore have a longer shelf life. In the process of oxidative rancidity, oxygen reacts with unsaturated fatty acids either spontaneously on exposure to air or in the presence of oxygen, light and a sensitizer. In the case of autoxidation, the hydrogen that is most weakly bound will be abstracted from an unsaturated fatty acid. Oxygen reacts at these positions and produces mixtures of hydroperoxide isomers. The major consequence of these reactions is the production of volatiles that impart rancid odors and flavours to foods (Love, 1992). Quite often, oil suppliers are thus faced with the problem of their oil being rejected based on flavour criteria (off-flavour). The peroxide value is a common measurement of lipid oxidation, but it might perhaps not serve as a true indicator of the actual state of oxidative rancidity in an oil or fat. Sensory evaluation (as described in section 4.2) is thus important because it is the ultimate test of oil quality (Idris *et al.*, 1992). Whereas other oils may therefore need to be converted to more stable forms through processing or the use of additives, PO and RPO have an advantage regarding oxidative stability. Because of this oxidative stability, the fact that it does not contain linolenic acid (found in soybean oil), which gives rise to "roomy" odors during frying (Edionwe & Kies, 1993), and that PO is more resistant to heat deterioration, PO is considered one of the best choices for home and industrial frying purposes (see section 2.2.1).

As discussed in section 2.2.2, the positional fatty acid distribution of some fatty acids influences its absorption in the small intestine (Ong & Goh, 2002; Renaud *et al.*, 1995) and may thus influence its effect on lipoprotein metabolism. In synthetic fats,

the fatty acids are randomly distributed over the glycerol backbone, whereas in natural fats like PO, the distribution depends on the fat source and is not similar among the three positions. As a result of the unique fatty acid distribution of PO (as referred to in section 2.2.1), the absorption of C16:0 from PO is not very high and the influence on lipemia restricted (Ong & Goh, 2002; Renaud *et al.*, 1995). Contradicting the above, some studies of the effects of fats with different positional distributions of palmitic and stearic acids do, however, suggest comparable effects on serum lipids and lipoproteins (Nestel *et al.,* 1995; Zock *et al.,* 1995).

Other important characteristics of PO in determining its incorporation into food products include the presence of high melting point triglycerides together with its relatively low solid content at 10°C, which is beneficial in the formulation of products with a wide plastic range, thus making it suitable for hot climates and certain industrial applications. (Palm olein generally has a melting point of about 21°C and a cloud point of 8°C.) It also has a tendency to crystallize in the small beta-prime crystals, a property desirable for some applications in, for example, margarine and cakes (Cottrell, 1991). Shortenings containing high proportions of PO products have even been said to have a cake-baking performance comparable with the best commercial product. PO and POL is used all over the world for industrial frying of potato crisps, french fries, instant noodles, doughnuts and other snack foods (Berger, 1986). Newer applications include its use in emulsion-based powdered and consumer foods such as mayonnaise, soup-mixes, imitation cheese and microencapsulated PO. PO is furthermore often most price competitive to other oils (Cottrell, 1991).

RPO (as described in section 2.2.1) has a deep orange-yellow colour due to its high content of β-carotene. Keeping in mind that the colour of food is a significant factor in determining its acceptance, Manorama and Rukmini (1992) found that RPO can successfully be used in those foods or recipes where the yellow-orange colour it imparts, is acceptable. Most Indian foods, especially savoury curries, but even fried items and cake, having a pleasant light brown/orange colour, were found acceptable by consumers. Blending of RPO with other commonly consumed oils to decrease the intensity of its colour, could also be regarded as a valuable alternative. Exciting new products such as healthy cooking and salad oils (Carotino®) have also been introduced in the market.

The wide range of food applications described above justify the claim that PO, and soon perhaps also RPO, could be the most versatile of natural fats. Furthermore, the development of innovative lipid-based functional foods will improve consumer perception, economic value and market share of products. The question, however, remains if, despite its versatility and health benefits, the majority of especially South African consumers could afford the house-hold use of PO or RPO (even more expensive) instead of SFO.

In the subsequent sections, the relationship between food, nutrition and health will be discussed in more general terms, followed by the importance of interdisciplinary research and the impact of functional foods on consumers nowadays.

4. FOOD, NUTRITION AND HEALTH

Nutritional epidemiology has generated hypotheses linking foods to disease incidence (Wrick, 1993). Today we stand at the threshold of a new frontier in nutrition sciences. Overwhelming evidence indicates that our Western diet plays a major role in atherogenesis and thus also CHD. To the contrary, the philosophy that certain foods can be health promoting beyond its nutritional value is gaining acceptance within the public arena and among the scientific community as mounting research links diet or food components to disease prevention and treatment (ADA, 1995). According to a survey in the United States, nearly two-thirds of grocery shoppers report that their purchasing decisions are driven by their desire to either reduce the risk of, or manage a specific health condition. These shoppers are also more likely to purchase foods that are naturally nutritious as tools, rather than supplements (Sloan, 2000). A new concept in nutrition, namely "indulgent nutrition" has thus seen the light. According to this concept, accepted foods and new foods that delight the palate are used to deliver significant health benefits. Furthermore, it acknowledges the fact that most people are unwilling to compromise taste for long-term health benefits. If consumers are, however, convinced that they will not have to sacrifice taste in order to eat healthy, they would also be more likely to use nutrition as tiebreaker in deciding which foods to buy. Therefore, taste and not only nutrition, has been the marketing focus for a new generation of functional foods (Johnson, 2001).

4.1 Functional food

There is currently no universally accepted definition of functional foods. Whereas the International Food Information Council (IFIC) defines it as "foods that provide health benefits beyond basic nutrition", the International Life Sciences Institute of North America (ILSI) defines it quite similar as "foods that, by virtue of physiologically active food components, provide health benefits beyond basic nutrition" (ADA, 1999). A food can thus be regarded as "functional" if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, and in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. They must, however, remain foods and demonstrate their effects in amounts that can normally be expected to be consumed in the diet (Diplock *et al.,* 1999). Functional foods need not always be newly developed foods – even familiar foods for which recent research findings have highlighted new health benefits or dispelled old dogma about potential adverse health effects, may be included in this category.

The term "functional foods" actually originated in Japan. As a result of an escalating bill for its national healthcare program and an aging population, the government launched a program in the mid-1980s to promote the development of foods with healthful – even medicinal – properties. The term used for these foods was "functional", and this program led to a frenzy of new product development activity (Hollingsworth, 1995). From 1991, this category of foods was called Foods for Special Health Use (FOSHU) in Japan (Wrick, 1993). Today, the increased interest in functional foods in general is likely occurring for three reasons, namely increased health care costs and life expectancy, recent legislation (e.g. on health claims), and scientific discoveries (Milner, 2000). European manufacturers ranked CVD number one in terms of conditions having a very great influence on the functional food market, followed by cancer, obesity, osteoporosis, gut health and immunity (Leatherhead, 1999). Food technology thus has a role to play in combating these chronic diseases, especially CVD (as mentioned in previous sections).

In addition to the nutrients that are involved in normal metabolic activity, functional foods contain specific components that may provide additional health benefits. These non-nutrient , bioactive food constituents, referred to as phytochemicals, are derived from naturally occurring ingredients (ADA, 1995). Other terms such as

"bioengineering" and "designer foods" relate to the technology available to develop phytochemical-rich foods. Currently, there is an increasing interest in determining which phytochemicals have the most beneficial properties. To evaluate the biological effects of phytochemicals, carefully designed human feeding studies have to be performed (Kurzer, 1993). One example that demonstrates the need for enhancing the phytochemical content of foods is apparent in the research related to β-carotene. Current dietary intake levels of β-carotene are estimated at 1.5mg daily. The best estimate of appropriate intake level, based on intakes reflected in populations with the lowest rates of CVD and cancer, is approximately 6mg/day. The advantage of improved dietary intake and/or food fortification/modification over supplementation is that the consumer will continue to consume food to meet β-carotene requirements while at the same time consuming other naturally occurring health-promoting factors, such as fibre. Scientific research has also uncovered more reasons why cereal products are healthy foods. In addition to providing essential nutrients and fibre, they may also contain disease-preventing phytochemicals (Wrick, 1993). This line of argumentation forms part of the motivation behind the current study (see Chapters 3 and 4) in which RPO, a rich source of β-carotene, was incorporated into high-fibre baked food products, to investigate the effect of this oil on lipids and haemostasis.

4.1.1 Research and development of functional food

The research, design and development of functional foods is a scientific challenge that, by means of interdisciplinary cooperation, should rely on a stepwise process, as presented in Figure 2.2. The process begins with basic scientific knowledge of body functions and the identification of a specific interaction between one or a few components present in a food. This step is regarded as fundamental research and should lead to one or more proposals for hypothetical mechanisms of the identified interactions as well as to the development and validation of relevant biomarkers (Roberfroid, 2000). If these markers represent an event *directly* (i.e. causally) involved in the process they should be considered as *factors,* whereas if they represent correlated events, they should be considered as *indicators.* Furthermore, markers relevant to functional foods can also be classified according to whether they relate to exposure to the food component under study, relate to the target function or biological response, or relate to an appropriate intermediate endpoint of an improved state of health and/or reduction of disease risk (Diplock *et al.,* 1999). The next step

in the process is generation of new hypothesis-driven human intervention studies that will include the use of these validated, relevant markers (Roberfroid, 2000). To be able to make health claims about the functional ingredients or functional foods, unequivocal proof of its functional effects should also be established during these correctly planned scientific studies. Subsequently, during the development of functional food, sensory evaluation of the products should be performed to ensure that it will be acceptable to intended consumers. New and advanced techniques will, however, be required as the focus will increasingly be on trace compounds rather than on macronutrients as in the past (Karel, 2000). The objective of technology-based development of food products and processes is to assure safety, health-promoting qualities, and a high level of acceptability combined with affordability. One of the great challenges to food technology, as indicated above, is to put the development of "health-promoting-foods" on a rational basis. This step could be followed by actual consumer and marketing research, which requires successful teamwork (Carter & Riskey, 1990). Consumer testing is an essential element in the overall decision making process with regard to the likelihood for product success (Stone, 1988), from the development stage, through to product launch and market consolidation (Land, 1988; Van Kleef *et al.,* 2002). If the product proves to be acceptable, it would probably be commercialized successfully. If not, it should be reformulated or adapted with the aid of sensory evaluation, and the previous steps should be repeated. After product development and evaluation for acceptability, the functional food could also be tested and re-tested, as a whole food and not merely as functional ingredients, in human nutrition intervention trials. Results, which must be interpreted with caution, could give substantiation to prove and verify the food product's functionality. Nutritionists, food scientists and food industries should communicate research results on phytochemicals and functional food to consumers, taking into account that marketplace success of foods containing these ingredients depends largely on consumer satisfaction with the products themselves, as well as their comfort with the entire concept of foods specially designed to prevent or decrease the risk for specific diseases, as in the case of designer foods (Wrick *et al.,* 1993).

The development of functional foods thus provides an unique opportunity to contribute to improvement of the quality of the food offered to consumers who want to benefit their health and well-being. Integrated research programmes with

interdisciplinary activity are needed to solve the key scientific and technological challenges and to exploit the scientific concepts in functional food science. Functional foods can only reach their maximum potential if the food industry, government and health professionals work together to improve communication between themselves and consumers and also to educate consumers, thereby allowing them to make informed decisions about their dietary choices. Thus, the importance of interdisciplinary research in this whole process will consequently shortly be discussed in more detail.

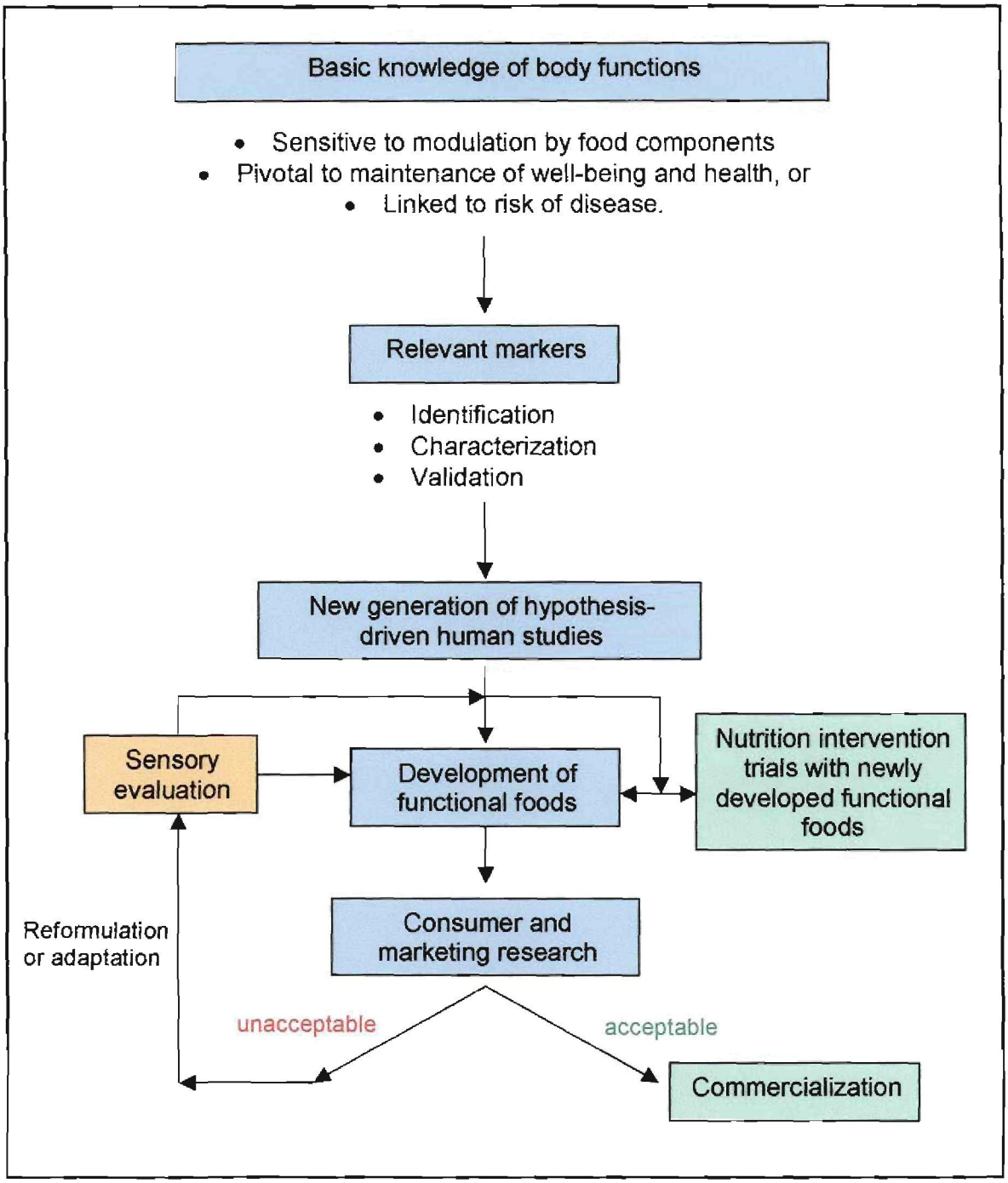

Figure 2.2 Stepwise process of the design and development of functional foods (adapted from Roberfroid, 2000; further developed by the author of this thesis).

4.1.2 The importance of interdisciplinary research

Developing successful food products is a high risk venture, as product failure is becoming all the more expensive. As compared to the situation a few years ago (Sloan, 1994), the success rate for new product market introductions generally remains poor. Although in general new product development has dropped in the last few years, functional food markets, on the contrary, are steadily growing (Sloan, 2000). Today's competitive marketplace dictates that a comprehensive approach be used to develop quality products that are well liked and that satisfy specific consumer needs. A comprehensive approach to product development must make use of technology to yield a product that has unique sensory characteristics (Stone, 1988). Interdisciplinary research and close collaboration between academic institutions and the food industry will be necessary to advance the objectives of food technology. Food research includes a complexity of food materials, several properties controlling the behavior of raw food materials, the variety of methods used in food processing, and the complex nature of food quality as it affects the consumer. It clearly necessitates a combination of expertise of various institutions in academia and to collaborate closely with industry when major research projects of national, regional or global impact are undertaken (Karel, 2000). These collaborative actions are furthermore needed to identify the most potent food ingredients (phytochemicals), to determine optimal intakes and the mechanisms of action, and to assure their safety (Kurzer, 1993). It is believed that successful teamwork between, amongst others, food scientists, nutritionists, sensory evaluation and marketing research begins with the broader comparison of marketing and sales versus technology (Carter & Riskey, 1990).

Dietetic professionals and nutritionists have the opportunity, given their depth of nutrition knowledge, to work collaboratively with food scientists and researchers, educators, the food industry and government to promote accurate and appropriate research, dissemination of information, product development, regulation and consumer education in this area. Only a rigorous scientific approach producing highly significant results will guarantee the success of this new discipline of food science and nutrition and before it can be considered an economic challenge at all, functional food must thus remain a scientific challenge (Roberfroid, 2000). Sound scientific evidence should, however, be put into practical dietary applications for the consumer (ADA, 1995), as information is a powerful marketing tool when it comes to

the sale of healthy foods (Sloan, 2000). It is therefore of the utmost importance that marketers should also be educated in the sensory and health aspects of foods, especially to have faith in the new functional food developments and to successfully communicate these to the consumer. Consumers are much more interested in positive marketing aspects of food than in disease messages, as disease-risk reduction does not seem to be a strong motivator for all but a very few consumers, and then primarily ones who are at real risk of the disease. In the chaotic universe of health claims and promotional campaigns, a major task for nutrition, toxicology and food professionals is to provide a scientific basis for assessing, evaluation and assuring the efficacy of food components (Karel, 2000). Consumers and those who make recommendations to consumers have the right to require guarantees about the reliability of claims regarding functional foods and the scientific data supporting them. Health authorities, in collaboration with the food industry and academia, will thus have the responsibility to elaborate procedures for authorizing claims that meet these legitimate requirements (Roberfroid, 2000).

From the above, it is evident that the success of any new functional food is highly reliable on collaboration and interdisciplinary research. As it is also one of the major focus points of this thesis, the specific importance of one of these disciplines, namely sensory science and evaluation, in product development and consumer acceptability of food products will consequently be discussed.

4.2 Sensory evaluation and consumer acceptability of food products

Competition within the food industry continues to increase, while the consumer is without doubt still the key to success of newly developed products (McIlveen & Armstrong, 1998). Since the ultimate target of the food industry is thus the satisfaction of the consumer, it is essential to consider not only the objective consumer needs (e.g. nutrition, safety, affordability), but also subjective aspects of consumer satisfaction (e.g. sensory properties and consumer attitudes). No matter how successful a product is from the objective point of view of a scientist focusing for instance on nutrition and apparent functionality, the product is not successful if it does not please the consumer sufficiently to make him or her buy and consume it (Karel, 2000). For this reason, sensory evaluation of food products, especially by consumer panels, have re-emerged as a "new" science of inestimable importance in collaboration with nutrition research and functional food development. Consumer

behaviour is becoming increasingly less predictable, more fragmented and less consistent (Imram, 1999) and this complicates the food industry's task. Great taste remains an imperative and this is probably the key factor why nutrition, in combination with great taste, may thus be considered the most important component to survival in tomorrow's dynamic marketplace. There are not many signs that people are strongly influenced to buy anything based on functionality or health. People are, on the contrary, very much buying on taste, or perceived taste (Vierhile, 2002). It is furthermore also a widely acknowledged fact that taste remains the most important barrier to improving nutrition (Johnson, 2001). Less-than-optimum taste, high prices and complicated dosage regimens were all contributing factors in the disappointing sales of earlier mass-market functional products (Sloan, 2000). However, even nutritionally unmotivated consumers would be open to consuming nutritionally beneficial components added to foods they already like, which may be seen as an engraved invitation for new indulgent nutritious foods, as described in section 4 (Johnson, 2001).

The essential cornerstone for embracing the functional foods revolution thus seems to be what the Centre for Food & Health Studies in the United States call the A+ approach to food and health: Functional food should be *available* on shelves where people normally go shopping, *affordable* within the means of most people and *accessible*, a combination of the above two factors. Finally, it also should be *acceptable*. If foods are not accepted in the minds of consumers and if they do not feel positive about these products or like the taste, these functional foods will certainly fail (Anon, 2002a).

Physicochemical characteristics of food arise from ingredients, processing and storage variables and results in specific sensory characteristics of the food product, as detected by the human senses. The study of the interaction between these physicochemical properties with the human senses is known as psychophysics. In food science, applied forms of psychophysics are known as sensory evaluation (Imram, 1999). Since 1950 it became evitable that the field of sensory evaluation has become a field of sensory science, with methods becoming models, and classical statistical tools becoming tools to understand behaviour (Ennis, 1998). Sensory science is furthermore increasingly seen as providing the tools to aid companies to understand those product characteristics (e.g. taste) which are important in determining consumer likes an dislikes (McEwan, 1998), and thus overall

perception and acceptance of a food product (Imram, 1999). A combination of sensory analysis with trained panels and market research with consumers is recognized by many companies as a valuable step in product optimization, to ensure that product development is targeted and focused (McEwan, 1998). Sensory evaluation techniques are thus employed from the initial planning stages right up to the formulation-reformulation stages leading to full scale production. Understanding the human response to sensory properties, however, presents problems because of the difficulty of correlating sensory properties with chemical composition and physical structure, and of quantifying customer response to several demographical, environmental, social and economical factors (Karel, 2000). From the above it is clear that the study of consumer perception of food quality and acceptability is complex and interdisciplinary, encompassing scientific disciplines including food science and technology, nutrition, psychology, physiology, marketing and hospitality (Imram, 1999).

Flavour and texture have been shown to have a profound effect on perception and acceptability. Visual sensations also help to contribute to this perception since the first encounter with food products is often visual and will affect subsequent willingness to accept a product. Colour and appearance may even have a halo effect which modifies subsequent flavour perception and food acceptability (Imram, 1999). Those responses made in reaction to physical stimuli for sight, taste, smell, touch and sound are collectively referred to as sensory factors, while hedonic factors are better defined as the evaluative or like-dislike dimensions of foods. Hedonic acceptability measures are considered appropriate for laboratory or field testing of individual items, whereas consumption is a better measure of acceptance of groups of foods. The acceptability of a product is defined by Land (1988) as the level of continued purchase or consumption by a specified population. Food acceptance can also be measured by an indication of reported frequency of consumption behaviour (Schutz, 1988). Generally, it is considered that consumption measures might even provide a better index of acceptance than hedonic measurements (Meiselman *et al.*, 1988). Acceptance of a food as represented by preference is also an important factor in decisions made with regard to utilization of food products. The concept of food acceptance is a complex one in which preference alone is only one factor in considering the actual acceptance of a food (Schutz, 1988). It is, however, only by understanding the preferences of individuals that the role of sensory factors in food

choice may be understood. Other factors such as price and nutritional benefits will likewise operate through the person's beliefs and affects (Shepherd, 1988). Factors specifically influencing acceptability can roughly be divided into *people factors* that cannot easily be controlled, such as expectations, physiological state, personality, occasion, finance and mood, as opposed to *product factors* that can be better controlled, such as preparation, processing, packaging, breed or cultivar and availability. The presence of certain expectations or internal reference standards of an individual is an essential feature of acceptability. The absence of such standards may, to some degree, explain neophobia. Standards thus give stability and also confidence to try out new products (Land, 1988). Thus, the greater the degree to which a consumer's experience with a product matches his/her pre-established expectancies of it, the greater is his/her liking of the product (Cardello *et al.,* 1985). Variations in response to products as a result of differences in sensory sensitivity among individuals, are one of the contributory elements which produce market segments of products for particular groups of consumers (Land, 1988). Nevertheless, consumer feedback is necessary to guide product developers to fulfil consumer needs and to set food manufacturer's quality assurance parameters (Setser, 1993; Van Kleef *et al.,* 2002). As stated by Ennis (1998), sensory research has played a key role in the development of our modern food supply and will continue to do so as long as people consume food and beverages.

The different types of methods and tests that may be used for measuring the above mentioned sensory attributes are listed in Table 2.7. For the purpose of this thesis, only affective tests involving untrained consumer participation, will be classified in detail, whereas a few examples of analytical tests in each category will be given. In the present study (Chapter 3), one test in each of the three quantitative affective categories has been used for consumer evaluation of the test products (see Addendum A and B). The acceptance test used in this study consisted of a 5-point hedonic scale, with each point accompanied by a descriptor. This was followed by a simple preference test where subjects could indicate which of the evaluated samples they preferred, if any. According to Warwick (1990), the three main factors influencing the acquisition of taste preferences in humans are innate predispositions, early feeding experiences and peer or family modeling. These may, however, vary in the degree to which they may override physiological signals of nutritive needs and satiety. Finally, a 5-point food-action rating scale was included, on which consumers

could indicate their intention to consume the evaluated food samples, ranging from 1 (I will never eat it) to 5 (I will eat it often / thrice per day). This test was included as researchers recognized that a product that a person likes very much may not necessarily be a product that the person will consume very often or in great quantities. Rather, consumers may like certain foods very much but may consume them infrequently, perhaps because those foods are too satiating physiologically, or because they may rapidly tire of those products. Measuring frequency in the simplest format thus requires a question about the number of times during a fixed interval that a consumer would consume a food (Moskowitz, 1991).

Table 2.7 Classification of methods and tests used in sensory evaluation (adapted from Setser, 1993).

Analytical		**Affective (Consumer panels)**	
Discriminative	**Descriptive**	**Qualitative**	**Quantitative**
Sensitivity/Threshold tests: Frequency methods *Forced-choice:* Paired comparison Duo-trio Triangle	*Profiling tests:* Flavour Texture Free-choice *Quantitative:* Quantitative descriptive analysis Scaling: Interval, ranking, ratio	Focus groups Focus panels One-on-one interviews	*Acceptance scales:* Hedonic scale (degree of like/dislike) Just-right scale *Preference tests:* Paired preference Rank preference Multiple paired preference *Food-action scales:* Willingness to purchase or consume

Data obtained from the affective test are usually "subjective" because they are influenced by each evaluator's food habits, cultural background, personality, interpretation of the scale, recent eating history, health, age and factors interacting to influence a person's individual likes and dislikes. In order to be truly representative of consumers' attitudes, preferences and/or acceptance of products, relatively large numbers (>40) of respondents are required (Setser, 1993).

From the above discussion it is clear that sensory evaluation and consumers have a major role to play in the success of functional foods. Although the importance of consumer input in the development and marketing of new products has been extensively reviewed and acknowledged, according to the literature studied for this

chapter, not enough attention was and/or is given to evaluation of acceptability of functional food products prior to its incorporation in human clinical trials. As also discussed in section 4.1.1, the author strongly feels that consumer feedback should be obtained not only in order to successfully commercialize new food products, but also preceding human nutrition intervention trials, so as to be able to optimize consumer compliance with test products. By ensuring better compliance of subjects with the test products, researchers could also expect better consumption and thus more reliable results on the functional effects of the active food ingredients tested. To a large extent, the above factors were the main driving force behind, or motivation for conducting the sensory evaluation of the potential functional food products (Chapter 3) prior to the main intervention study (Chapter 4) in this thesis.

To conclude this chapter, the author will briefly refer to the current growth of the functional food market, as well as the impact that functional food has on consumers nowadays.

4.3 The impact of functional food on consumers in the 21st century

While the precise size of the functional food market is difficult to determine, there is general agreement that it has large potential for growth (Sloan, 2000). The increasing number of consumers believing that foods may directly contribute towards their health, combined with advances in various scientific domains, provide companies with unique opportunities to develop an almost infinite array of new functional food concepts (Van Kleef *et al.,* 2002). However, there is an ongoing debate about the extent to which functional foods or nutraceuticals will make people healthier in the long term and how they might impact on population or public health, and not just individual health. Despite this, the current impact of functional food in providing new healthy choices can be no better illustrated than by studying the shelves of supermarkets in countries like the United States of America, the Netherlands, Sweden or Finland, that have been transformed by functional food (Anon, 2002a).

The functional foods segment is currently driven by the health-directed, financially well-off, aging Baby Boomers, who are expected to dominate (at least in the United States of America) the market for the next 30 years. As the population ages, stress levels and time pressures increase, and sedentary lifestyles become a way of life, functional food developers will face an ever evolving series of markets based on

performance (e.g. energy or sleeplessness) and quality of life (e.g. joint pain or immunity). Recognizing the long-term strength of the functional food market, industry activity is skyrocketing (Sloan, 2000). A few important trends in the functional food market have been identified by Sloan (2000). Traditional manufacturers can now participate in a truly new generation of "healthy foods" by taking advantage of existing health claims that provide access to major markets related to diseases such as CVD, by simply incorporating familiar and accepted foods in their production lines. Another trend with the most long-term potential for functional food is customization. Male and female directed products (focusing on prostate cancer and hearth health, as well as calcium and iron fortification or relief of menopausal symptoms, respectively) are leading the mass market for personalized functional foods. Another strategy to customization is "ingredient bundling" – targeting products toward preventing/ treating a specific condition, for example heart health. Furthermore, snacking or individualized consumption, like snack-size packages, is another important underlying trend. People are influenced more and more by eating on-the-go, in their cars or in front of the keyboard and thus pre-portioned, healthy foods are definitely a trend that cannot be ignored (Vierhile, 2002).

Two things, however, are clear: most consumers don't want to give up their favourite foods in their quest for health (Sloan, 2000), and taste still seem to be the number one reason for buying food (Hollingsworth, 2000). Today's consumer has much access to information, especially through the media. However, the saturation of news, information, opinion and superfluous messages sometimes serve to overload the senses. Especially the impact of reports on single studies or conflicting messages create burnout and jaded consumers who are less receptive to health messages (Hollingsworth, 2000). Consumers should be encouraged to make use of reliable sources of health information. In this regard, dietitians, nutritionists and food scientists have an unique opportunity to translate specifically more complicated health messages into practical, useful information to consumers.

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CHAPTER 3

CONSUMER ACCEPTANCE OF HIGH-FIBRE MUFFINS AND RUSKS BAKED WITH RED PALM OLEIN AS POTENTIAL FUNCTIONAL FOODS

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Summary

The acceptance, preference for and consumption intent of high-fibre muffins and/or rusks baked with either red palm olein (RPO) or sunflower oil (SFO, as control) were evaluated by two consumer groups, in order to determine the possibility of its successful inclusion as carriers for the oils in a subsequent nutrition intervention trial. A 5-point hedonic and food action rating scale was used for sensory evaluation. Although the SFO muffins and rusks scored higher than the RPO products in acceptance attributes, all differences were not significant. RPO products were not preferred to SFO products, but consumers of both groups evaluated the overall acceptability of RPO products as very high (≥4.0 on 5-point scale), and intended to eat it often (at least once/day). Acceptance of, and compliance with RPO products in the nutrition intervention trial would thus probably be optimal. These results could also be of great value to the food industry.

Running title Consumers accept red palm oil products

Keywords Sensory evaluation, consumption intent, preference, nutrition intervention, dietary oils

Introduction

Cardiovascular disease (CVD) is a leading cause of mortality and morbidity in most Western industrialised countries. In South Africa, with its developed / developing population mix and where there is a population shift from rural communities to urban areas, CVD is on the increase (Bradshaw *et al.,* 1995; Vorster *et al.,* 1999). The health hazards of excessive intake of dietary fats, especially those rich in saturated (Ng, 1994) and *trans* fatty acids (Sundram *et al.,* 1997), have often been highlighted in the scientific media. Since red palm olein (crude or mildly refined palm olein, RPO) became available commercially, its role as an excellent natural source of tocopherols and tocotrienols, as well as β-carotene (a pro-vitamin A carotenoid), became all the more evident (Rukmini, 1994). Carotenoids along with tocopherols and tocotrienols are powerful anti-oxidants, and although controversial, it has been associated in several studies with decreased risk for chronic diseases (E-Siong, 1992; Mayne, 1996) and in the case of the vitamin E isomers, decreased platelet aggregation and prostaglandin production (Steiner & Anastasia, 1975; Qureshi *et al.,* 1991). In some studies the tocotrienol-enriched fraction of palm oil (palmvitee) has showed to be hypocholesterolaemic by decreasing serum total cholesterol (TC) and low density lipoprotein cholesterol (LDLC) (Qureshi *et al.*, 1995; Qureshi *et al.*, 1991; Tan *et al.,* 1991) partly through inhibiting hydroxylmethyl-glutaryl-CoA (HMG-CoA) reductase. It can thus be hypothesised that the intake of RPO may positively influence lipid profiles and haemostatic factors (Kritchevsky, 2000; Rukmini, 1994) and a nutrition intervention study was subsequently designed by the Potchefstroom Institute of Nutrition (PU for CHE) to test this hypothesis.

Although research on the effects of RPO *per se* is rather limited, some human studies, as reviewed by Kritchevsky (2000), showed that it is indeed an useful addition to the present array of dietary fats and may be considered an excellent oil for human consumption. In contrast, the effects of refined, bleached palm oil (PO), a rich source of saturated fatty acids (SFA), specifically palmitic acid (44.3 %), and the mono-unsaturate fatty acid, oleic acid (39.0%; Ong & Goh, 2002), on cholesterol levels have been well studied. Results are controversial as several studies showed that PO has neutral effects on cholesterol levels, especially in normocholesterolaemic subjects, while PO may, however, increase cholesterol levels in hypercholesterolaemic subjects or subjects with

abnormal cholesterol metabolism (Sundram, 1997). To a certain extent the food industry is moving away from using fats and oils only for their sensory characteristics, as emulsifiers, or as flavour and vitamin carriers. Instead, their roles in, amongst other, health and disease prevention are being explored in the development of new nutraceuticals or functional foods (Ong & Goh, 2002). Products containing red palm oil may be considered functional foods if they prove to fit the definition thereof and thus provide "health benefits beyond basic nutrition" (Clydesdale, 1997), by either improving the state of health and well-being and/or by reducing risk of disease (Blades, 2000). According to Manorama and Rukmini (1992), RPO is not suitable as medium for deep frying or for deep fried products, as it may cause changes in the physical, chemical (e.g. destruction of ß-carotene) and sensory properties of the oil mainly due to heat deterioration. It was found by these authors that 88% of the oil's ß-carotene content was, however, retained in a baked product, probably as a result of thorough mixing with the other ingredient, indirect heat exposure and lower temperatures (<200°C).

In the light of the above and after several other product options were considered, it was decided to use well known South African baked products (Bosman, 1998), namely high-fibre muffins and rusks, as vehicles for RPO in the intervention study. Rusk is a typical South African, bread-like product which is dried at a low temperature for several hours after being baked, thus having a crisp / brittle texture. Sunflower oil (SFO), rich in linoleic acid (18:2) and the most commonly consumed edible oil in South Africa, was used as control. Although these products are normally dark brown, the ß-carotene content of the RPO caused it to be slightly orange to reddish-brown in colour and it was thus important to assess if these products were still acceptable to subjects of the intervention study and consumers, as marketplace success and thus continuous consumption of functional foods, depends largely on consumer satisfaction (Wrick *et al.*, 1993). Furthermore, according to Johnson (2001), taste and not necessarily nutrition, has been the marketing focus for the new generation of functional foods. If consumers are convinced that they will not have to sacrifice taste, they are more likely to use nutrition as the tiebreaker in deciding which foods to consume. The success of subjects' compliance during the dietary intervention, therefore, will largely depend on their acceptance of, and willingness to consume these products containing RPO.

The aim of this study was thus to assess the acceptance of, preference for and intended consumption of muffins and rusks containing either RPO or SFO by means of consumers, to evaluate the possibility of successful inclusion of the above mentioned products in a successive dietary intervention trial. The acceptability evaluation of these new, potential functional foods containing RPO and thus being good sources of β-carotene, may also render valuable information to the food industry.

Materials and methods

Subjects

The target population of the first consumer study was defined as actual or potential consumers of high-fibre muffins. Academic, administrative and technical personnel of three employment institutions in the urban area of Potchefstroom were recruited by telephone calls and via electronic mail on a basis of availability and willingness to participate. A total of 144 white South African consumers (53 men and 91 women), aged 21-55 years, volunteered to take part in this study. Subsequently, the second consumer group consisted of 67 subjects (38 men and 29 women, aged 21-55 years) already recruited from the Lipid Clinic (PU for CHE) to take part in an intervention study with RPO. The consumer tests were conducted in single fold at the taste panel room (PU for CHE) and other central locations. Subjects were not offered incentives, but each received a small token of appreciation for their participation after the evaluation session.

Experimental design

In general, the same experimental design was followed in both studies. Results from the first consumer group (among general consumers) necessitated the evaluation of an additional vehicle for the oils in the second consumer group (among subjects from the intervention trial). In the first study, subjects were presented with questionnaires as well as two coded, high-fibre muffin samples of 30g each. One of the samples was baked with RPO while the other contained SFO. In the second study, subjects evaluated two 30g, coded samples each of high-fibre muffins, as well as rusks, baked with either RPO or SFO. After completing their demographic information, sensory evaluation followed

and subjects had to assess the acceptability of, preference for and intended consumption of each sample.

Questionnaires

A pre-screened and pre-tested sensory evaluation questionnaire, consisting of two sections, namely for demographic information and a score sheet for assessing acceptability of, preference for and consumption intent were used in evaluation of the different samples. This questionnaire was designed and published by Bosman (1998), and adapted by the authors for this study.

Sample preparation and presentation

All procedures were standardised and pre-tested on preparation, handling and serving. Two batches of high-fibre muffins were freshly mixed and baked before sensory evaluation, as described by Bosman *et al.* (1996). The recipe, however, was adapted by increasing the oil content to from 100ml to 125ml per recipe in order to ensure that each subject consume the optimum amount of test oil per day, as required in the successive nutrition intervention study. In the second consumer study, additionally to muffins, high-fibre rusks were also freshly baked according to a standardised recipe (unpublished data, Reitsma & Bosman, 1993), cut and dried. The fatty acid composition of the oils used in the study was analysed by the Palm Oil Institute of Malaysia, using gas chromatography (presented in Table 1).

Although all batters contained the same amount of different oils, namely RPO in the form of the cooking oil, Carotino®, provided by the Palm Oil Research Institute of Malaysia (PORIM), or SFO, bought from a local supermarket, the fat and carotenoid analyses of the baked products differed slightly (Table 2).

Table 1 Fatty acid composition of the oils extracted from high-fibre muffins and rusks used in this study.#

Fatty acid (%)*	SFO muffins	SFO rusks	RPO muffins	RPO rusks
Lauric acid – C12:0	nd	nd	0.3	0.3
Myristic acid – C14:0	0.2	nd	1.1	0.9
Palmitic acid – C16:0	6.3	6.4	32.6	32.1
Stearic acid – C18:0	4.9	4.8	3.4	3.2
Oleic acid – C18:1 n-9	25.7	26.1	47.5	48.1
Linoleic acid – C18:2 n-6	62.1	61.6	14.1	14.5
Linolenic acid – C18:3n-3	0.1	0.2	0.5	0.5
Arachidic acid – C20:0	0.2	0.2	0.2	0.2

* per 100g of oil; # Analysed by the Palm Oil Research Institute of Malaysia, 2001
nd = not detected in sample
SFO = sunflower oil; RPO = red palm olein

The motivation behind the decision to include dried rusks in the intervention study and thus in the second consumer panel evaluation, was largely based on the fact that it is a much more concentrated source of fat and carotenoids compared to muffins, and subjects would not have to consume such large amounts of the products per day.

Table 2 Analysis of fat and carotenoid composition of muffins and rusks.#

Sample	% Fat content	Carotenoids µg/g fat extracted	Carotenoids mg/100g of product	β-Carotene mg/100g of product
Dried SFO rusks	28.8	9.0	0.26	0.08
SFO muffins	12.6	9.5	0.12	0.04
Dried RPO rusks	29.9	503.0	15.02	7.51
RPO muffins	16.1	500.0	8.03	4.02

# Analysed by the Palm Oil Research Institute of Malaysia, 2001
SFO = sunflower oil; RPO = red palm olein

After being baked and/or dried, the different samples were covered with translucent plastic and presented in identical containers. All samples were assigned a three-digit

code and were presented to subjects in balanced order to minimise order effects. The RPO muffins were coded plk and the SFO muffins as svg, while the RPO rusks were coded vlp and the SFO rusks as dgs. Evaluation sessions of 10-15 minutes were conducted between 09h30 and 11h30 under controlled conditions in either the taste panel room (PU for CHE) or at the other institutions. On arrival subjects were welcomed, seated and informed about the purpose of the consumer test. Subjects were also supplied with water (22°C) for palate cleansing before and between samples. Each sample was evaluated once by each subject.

Consumer sensory tests

Three separate tests were conducted in order to evaluate the muffin and/or rusk samples. Firstly a 5-point hedonic scale (1 = extremely unacceptable; 5 = extremely acceptable) was used for the independent hedonic rating of appearance, colour, texture and taste of each sample. Overall acceptability was calculated statistically from these attribute acceptance scores. Secondly, a preference test followed and subjects had to indicate whether they preferred one of the samples to the other, and if so, which one. Thirdly, by using a 5-point food action or attitude scale, a food action rating test was executed to assess intended consumption of each sample. Response categories ranged from "eating it very often" (3/per day; score 5) to "never eating it" (score 1). Subjects rinsed their palates with water before and between tasting and re-tasting of samples.

External validity

According to Schutz (1988), factors which influence the external validity of evaluations of food stimuli relative to food acceptance can be identified as firstly, the type of respondent, secondly, the type of stimuli and thirdly, the measurement procedure. These dimensions are related and consist of several sub-categories that increase or decrease the external validity. In the present study, consumers were recruited to be representative of a particular target population, food items (not merely substances or food names) were evaluated and simple affective judgements were followed by measures of use intention, thus ensuring good external validity.

Statistical analyses

Demographic data of subjects were analysed using descriptive statistics. T-tests ($p \leq 0.05$) were applied to the hedonic attribute scores for muffin and rusk samples, using Statistica® ('99 Edition, StatSoft Inc, Tulsa, USA). Analysis of variance (ANOVA) and Tukey's multiple comparison test ($p \leq 0.05$) were applied to determine the effect of age, gender and order of presentation on the different attributes. Non-parametric tests were used for the variable 'consumption intent' as evaluated on a food action rating scale, which did not have equal distances between the different categories on the scale. Significant differences in medians within groups were determined by using the Wilcoxon matched pairs test, and significant differences between groups by using the Mann-Whitney U test (Baker, 1994). The Chi-square test was performed to determine which of the samples, if any, in each formula category was preferred. For means that differed significantly, practical significance (significance based on effect sizes) was calculated. In practice, the assumption usually made, based on a common standard deviation for two populations, is not realistic. When dealing with two samples, the effect size helps the researcher to decide whether a statistically significant difference between the means is in fact an important difference in practice. Taking μ_1 and μ_2 to be the means of the two populations and σ as their (common) standard deviation, a sensible measure of practical significance would be the standardised difference (d) $\Delta = (\mu_1 - \mu_2) / \sigma$, also called an effect size. This value of practical significance is thus independent of units or sample size (Steyn, 2000).

Results

Within consumer group one (Table 3), SFO muffins scored statistically higher for colour (4.36 ± 0.72; $p=0.000$), texture (4.26 ± 0.69; $p=0.0441$), and overall acceptability (4.35 ± 0.52; $p=0.0021$) than RPO muffins. However, in all the above cases, the practical significance of these statistical differences was small ($d \leq 0.3$) and RPO muffins still received high scores (≥ 4 on the 5-point scale) on all the evaluated sensory attributes. Consumers intended to eat muffins baked with RPO and SFO often ($4.0 \pm [3.0\text{-}4.0]$ vs ($4.0 \pm [3.0\text{-}5.0]$). However, more consumers intended to eat three SFO muffins per day (score 5 on the 5-point scale) compared to RPO muffins, thus resulting in a statistical

difference (p=0.0069) with small practical significance (d=0.02) in consumption intent. Within the second consumer group, there were no statistical differences between any of the evaluated acceptance attributes or consumption intent of muffins baked with either RPO or SFO, while for both formulas scores were very high (≥4,0 on the 5-point scale).

When comparing the two consumer groups, muffins baked with RPO scored significantly higher for appearance (4.51 ± 0.56; p=0.033), colour (4.45 ± 0.63; p=0.0001), texture (4.45 ± 0.63; p=0.0001), overall acceptability (4.46 ± 0.51; p=0.0002) and consumption intent (4.0 ± (4.0-5.0); p=0.0000) among the subjects of the intervention study than among the general consumers in group one.

Furthermore, in the case of texture (4.51 ± 0.61; p=0.012), overall acceptability (4.52 ± 0.46; p=0.024) and consumption intent (5.0 ± (4.0-5.0); p=0.0000), consumers in the second group also rated the SFO muffins significantly higher than their counterparts in consumer group one. The practical significance of these differences in acceptability ranged from small to medium in effect size, while the practical significance for consumption intent had a big effect size.

Table 3 Comparison within and between consumer groups of acceptance and intended consumption of muffins containing either red palm olein (RPO) or sunflower oil (SFO).

Acceptance*	Consumer group 1 n=144 Mean ± SD	d-value	Consumer group 2 n=67 Mean ± SD	p-value between groups	d-value between groups
Appearance-RPO	4.31 ± 0.65		4.51 ± 0.56	0.033	0.3
Appearance-SFO	4.43 ± 0.67	-	4.55 ± 0.50	ns	-
Colour-RPO	4.01[a] ± 0.81		4.45 ± 0.63	0.0001	0.5
Colour-SFO	4.36[b] ± 0.72	0.3	4.51 ± 0.59	ns	-
Texture-RPO	4.10[a] ± 0.70		4.45 ± 0.63	0.0006	0.5
Texture-SFO	4.26[b] ± 0.69	0.2	4.51 ± 0.61	0.012	0.4
Taste-RPO	4.25 ± 0.80		4.45 ± 0.66	ns	-
Taste-SFO	4.33 ± 0.78	-	4.51 ± 0.68	ns	-
Overall acceptability RPO	4.17[a] ± 0.52		4.46 ± 0.51	0.0002	0.5
Overall acceptability-SFO	4.35[b] ± 0.52	0.3	4.52 ± 0.46	0.024	0.3
Consumption intent#	**Median (25-75 percentiles)**		**Median (25-75 percentiles)**		
RPO	4.0[a] ± (3.0-4.0)		4.0 ± (4.0-5.0)	0.0000	0.8
SFO	4.0[b] ± (3.0-5.0)	0.2	5.0 ± (4.0-5.0)	0.0000	0.7

Consumer group 1 = general public; Consumer group 2 = subjects in nutrition study
RPO: red palm olein; SFO: sunflower oil
[ab] Means with different superscripts differ significantly within groups ($p<0.05$)
d = effect / practical significance: d=0.2 : small effect; d=0.5 : medium effect; d=0.8 : big effect
(no practical significance indicated when statistical significance lack)
* 0-1 = extremely unacceptable; 4-5 = Extremely acceptable
\# 0-1 = Will never eat 1-2 = Will eat only when no other food is available
2-3 = Will eat once a week 3-4 = Will eat often (1/day)
4-5 = Will eat very often (3/day)

With only one exception (namely taste), in neither of the consumer groups did gender, age or order of presentation of samples have any effect on the acceptance attributes evaluated. Taste was the only attribute on which gender had any significant effect among consumers in the first group. According to men (Table 4), the taste of SFO muffins was statistically more acceptable than those containing RPO (4.40 ± 0.69 vs 4.09 ± 0.93; p=0.04), but practical significance was once again small (d=0.3). Women in this group, however, rated taste of the different muffin formulas almost identical. Furthermore, gender, age and order of presentation had no effect on consumption intent of any sample in either consumer group (results not shown).

Table 4 The effect of gender in consumer group 1 on acceptance* of taste.

	Muffin formulas		
Gender	**RPO**	**SFO**	**Practical**
	Mean ± SD	**Mean ± SD**	**significance (d)**
Men (n = 53)	4.09[a] ± 0.93	4.40[b] ± 0.69	0.3
Women (n = 91)	4.33 ± 0.72	4.30 ± 0.82	-

Consumer group 1 = general public; RPO: red palm olein; SFO: sunflower oil

[ab] Means with different superscripts in a row, differ significantly ($p<0.05$)

effect / practical significance: d=0.2 : small effect; d=0.5 : medium effect; d=0.8 : big effect

(no practical significance indicated when statistical significance lack)

* 0-1 = extremely unacceptable; 4-5 = Extremely acceptable

Table 5 represents the acceptance scores and consumption intent of consumers in group two for rusks. SFO rusks scored statistically higher for appearance (4.55 ± 0.58; p=0.000), colour (4.51 ± 0.61; p=0.000), texture (4.42 ± 0.63; p=0.042) and overall acceptability (4.49 ± 0.52; p=0.000) than RPO rusks. However, the practical significance of these statistical differences generally ranged from small (d≤0.3) to medium (d≤0.6) in effect size. RPO rusks still received high scores (≥4 on the 5-point scale) on all the evaluated sensory attributes. Consumers intended to eat rusks baked with RPO as often as those baked with SFO, namely one per day (4.0 ± [4.0-5.0]).

Table 5 Evaluation by consumer group 2 of acceptance and consumption intent of rusks containing either red palm olein (RPO) or sunflower oil (SFO).

	Rusk formulas		
	RPO	SFO	Practical
Acceptance*	**Mean ±SD**	**Mean ±SD**	**significance (d)**
Appearance	$4.13^{a} \pm 0.65$	$4.55^{b} \pm 0.58$	0.6
Colour	$4.03^{a} \pm 0.78$	$4.51^{b} \pm 0.61$	0.6
Texture	$4.21^{a} \pm 0.84$	$4.42^{b} \pm 0.63$	0.3
Taste	4.34 ± 0.75	4.48 ± 0.66	-
Overall acceptability	$4.18^{a} \pm 0.65$	$4.49^{b} \pm 0.52$	0.5
Consumption intent#	**Median (25-75 percentiles)**	**Median (25-75 percentiles)**	
	4.0 ± (4.0-5.0)	4.0 ± (4.0-5.0)	-

Consumer group 2 = subjects in nutrition study
RPO: red palm olein; SFO: sunflower oil
[ab] Means with different superscripts in a row, differ significantly ($p<0.05$)
d = effect / practical significance: d=0.2 : small effect; d=0.5 : medium effect; d=0.8 : big effect
(no practical significance indicated when statistical significance lack)
* 0-1 = extremely unacceptable; 4-5 = Extremely acceptable
# 0-1 = Will never eat 1-2 = Will eat only when no other food is available
2-3 = Will eat once a week 3-4 = Will eat often (1/day)
4-5 = Will eat very often (3/day)

Gender distribution in general was less equal in the first consumer group (men=36.5% vs women=63.5%) than in the second (men=56.7% vs women=43.3%), but it must be taken into consideration that consumers were recruited on a basis of availability and willingness to participate. The gender distribution of preference for the different muffin and rusk samples is given in Table 6. Gender and age (results not shown) had no significant effect on preference for any set of samples in either of the consumer groups. In the first group, the majority of consumers preferred the SFO muffin to the RPO muffins (45.3% vs 29.7%; p=0.03), whereas the minority (25.0%) indicated no preference at all. Although not significant, in both the cases of muffins and rusks, the majority of consumers in the second group expressed no preference for any of the samples evaluated. Regarding the total number of consumers that have indeed indicated some preference for muffins in this group, no statistical difference was found between RPO and SFO. In contrast, SFO rusks were significantly preferred to RPO rusks (p=0.01).

Table 6 Frequency of preference for muffins and rusks in the different consumer groups according to gender.

	No preference	RPO	SFO
Consumer group 1: muffins			
Men	13	12	29
Women	24	32	38
Total (n=148)	37[a] (25.0%)	44[ab] (29.7%)	67[c] (45.3%)
Consumer group 2: muffins			
Men	17	10	11
Women	16	4	9
Total (n=67)	33[a] (49.25%)	14[b] (20.90%)	20 (29.85%)
Consumer group 2: rusks			
Men	16	7	15
Women	14	4	11
Total (n=67)	30[a] (44.78%)	11[b] (16.42%)	26[ac] (38.81%)

[abc] Frequencies with different superscripts in a row, differ significantly ($p<0.05$);
Consumer group 1 = general public; Consumer group 2 = subjects in nutrition study;
RPO: red palm olein; SFO: sunflower oil

Discussion and conclusion

While consumers are clearly receptive to the concept of improving their health and well-being through diet, the realisation of this is far from straightforward (Young, 1998). The study of consumer perception of food quality and acceptability is complex and interdisciplinary, encompassing scientific disciplines including, amongst other, food science and technology, nutrition, psychology and physiology (Imram, 1999). No matter how nutritious a food product may be, it would have no health benefit unless its sensory attributes are acceptable to such an extent that the product is consumed repeatedly. It was thus necessary to ensure as far as possible by means of sensory evaluation, that subjects in the planned nutrition study accept and optimally consume the product containing the test oil, in order to test the hypothesis regarding RPO successfully.

According to results of the sensory evaluation by the first group (general consumers), consumers were not prepared to eat three muffins per day. This mainly encouraged the researchers to consider and include an additional vehicle for the test oils, namely rusks, in a subsequent sensory evaluation among the subjects recruited for the nutrition intervention study. Subjects would probably also comply better with the intake of two products, than with three servings of the same product per day. Although the SFO

muffins and rusks scored significantly higher than the RPO products in several of the evaluated attributes, the practical significance thereof was small to medium in effect size. Nevertheless, RPO muffins and rusks still scored ≥4 on the 5-point acceptability scale, thus resulting in a very high score for overall acceptability. When the two groups were compared, consumers of the second group rated the RPO muffins significantly more favourable in most cases. It could be speculated that these consumers, who are probably more health conscious as they regularly attend the Lipid Clinic (PU for CHE) and receive dietary advice, were generally more positively orientated towards the potential health benefits of RPO. Although they were not specifically aware of the test products' composition during sensory evaluation, they were, however, informed about RPO in a preceding information session as they were due to take part in the clinical intervention trial with RPO. A study by Cardello *et al.* (1985) provided unambiguous evidence that product information has greater facilitative effects on consumer acceptance (and likely purchase and use) for new foods (as in the current case of RPO muffins) than it does on familiar foods. It is proposed that this is due to product information reducing the discrepancy between expectancies and experiences for novel (new) foods, but having a minimal effect on familiar foods (Cardello *et al.* 1985). In one of the few other studies that were done on consumer acceptance of red palm oil products, Van Stuijvenberg *et al.* (2000) found that the taste and appearance of biscuits baked with red palm oil were well accepted among school children. RPO, like refined, bleached and deodorised palm olein (POL), is very stable towards free radical-induced oxidation as it contains very small amounts of polyunsaturated fatty acids (Edionwe & Kies, 1993), and relatively large amounts of natural antioxidants, namely β-carotene, tocopherol and tocotrienol (Rukmini, 1994). Consequently, the formation of harmful oxidized products during processing and cooking is negligible compared with that of other polyunsaturated oils (Ong & Goh, 2002). This may be considered an advantage regarding shelf life (Edionwe & Kies, 1993) and consequently also the taste of products baked with red palm oil, as oxidative rancidity will be limited to a great extent. Although taste and texture have specifically shown to play a profound role in sensory evaluation, the effect of visual sensations (colour) should not be underestimated (Imram, 1999; Manorama & Rukmini, 1992). Manorama and Rukmini (1992) investigated the sensory attributes of several Indian food products, including cake, prepared with RPO. It was

found to be well accepted in these preparations in which the reddish-brown colour, imparted by RPO, blended well with the natural colour of the Indian foods. In the present study, the dark brown colour of the high fibre content of the muffins and rusks masked the reddish-brown colour of red palm oil to some extent, but not totally. It may be speculated, however, that the colour of the RPO products still influenced consumers' expectations, and thus their overall evaluation of acceptability, as it has been well established that colour and appearance can have a halo effect which modifies subsequent flavour perception and food acceptability (Imram, 1999). While fresh crude (red) palm oil has been described by an analytical sensory panel as having a sweet, pleasant, caramel-like flavour, regular crude palm oil was described as being carroty, sourish and fruity (Idris *et al.,* 1992). A positive result regarding this relatively new commercial cooking oil used in the current study, namely Carotino® (RPO), is the fact that in neither consumer group there was a significant difference in taste between any of the RPO and SFO products, while both were well accepted. Land (1988) provides a basis for measurement with his definition that acceptability of a product is the level of continued purchase or consumption by a specified population. Michicich *et al.* (1999) reviewed several sensory studies and also came to the conclusion that there is a positive relationship between liking and consumption. In the present study, the consistence with which consumers favourably accepted the sensory attributes of the muffins and rusks was also demonstrated in their intended consumption thereof, as consumers in group one intended to eat muffins baked with RPO and SFO often (1/day). Consumers in group two also intended to eat at least one RPO muffin and rusk a day, and even three SFO muffins a day. It would be interesting to evaluate the effect of prolonged consumption of these baked products on sensory parameters, as well as to compare such results with the "short–term" acceptability and preference evaluations of the current study.

It can be viewed in a positive light that the majority of consumers in the second group expressed no preference for any of the muffin or rusk formulas, as this may be indicative of the fact that the RPO and SFO products were considered equal in sensory characteristics by the majority. Furthermore, although consumers in the first group preferred the SFO muffins, those in group two, who would actually be consuming these

products in the intervention study, did not express a significant preference for SFO muffins to RPO muffins.

It may be concluded that, although not preferred to those baked with SFO, high-fibre muffins and/or rusks baked with RPO were well accepted among consumers of both groups, while they also intended to consume them often. The authors are of the opinion that a combination of muffins and rusks baked with RPO may ensure optimum intake of the test oil as well as adequate compliance in the subsequent clinical trial. We strongly suggest that intervention studies in which the health benefits of potential functional foods are tested, inevitably be preceded by sensory evaluation of the products to ensure optimal compliance with and acceptance of the test products. Furthermore, the fact that these new, potential functional foods, containing RPO and being good sources of β-carotene and fibre, were well accepted and consumers intended to eat them daily, could be of great value to the food industry.

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CHAPTER 4

THE EFFECT OF RED PALM OLEIN AND REFINED PALM OLEIN ON LIPIDS AND HAEMOSTATIC FACTORS IN HYPERFIBRINOGENAEMIC SUBJECTS

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Abstract

Dyslipidaemia and a hypercoagulable state are known risk factors for cardiovascular disease, while diet plays an important role in the risk prevention thereof. The effects of palm oil (PO) on lipids and haemostasis is inconsistent, while few studies have reported on the effects of red palm olein (RPO) on these parameters. In this study, the effects of palm olein (POL) and RPO compared to sunflower oil (SFO), were examined on lipids, haemostatic factors and fibrin network characteristics (FNC) in hyperfibrinogenaemic volunteers.

Fifty-nine free-living, hyperfibrinogenaemic volunteers participated in this randomized, controlled, singleblind parallel study. After 4 weeks of run-in, during which subjects received baked SFO products, they were paired and randomly assigned into three intervention groups receiving baked products containing 25g/day of either RPO, POL or SFO for another 4 weeks. Anthropometric measurements, blood samples and dietary intakes were measured before run-in, as well as before and after intervention.

The difference in changes in total serum cholesterol response between POL and RPO (+0.59 vs +0.18mmol/L; p=0.053), and between POL and SFO (+0.59 vs -0.003mmol/L; $p \leq 0.01$) was significant. The low-density lipoprotein cholesterol response in the POL- and SFO-groups also differed significantly (+0.42 vs -0.11mmol/L; $p \leq 0.01$). Tissue plasminogen activator antigen decreased significantly in the RPO-group (p=0.01). No effects were found in other haemostatic variables. POL and RPO had no independent effect on FNC. In conclusion, compared to POL, RPO had favourable effects on the lipid profile and fibrinolysis. As a result of its nutritional benefits, RPO may play an increasingly important role in the food industry.

Sponsorhip: Palm Oil Research Institute of Malaysia (PORIM) (sponsoring the study, providing the refined and red palm olein, as well as analyses of oil, carotene and vitamin E content of test products).

Short running title Palm and red palm olein, lipids and haemostasis

Key Words: Red palm olein, refined palm olein, hyperfibrinogenaemic men and women, serum lipids, haemostatic factors, fibrin network structure

Introduction

Palm oil (PO), a rich source of saturated fatty acids (SFA), specifically palmitic acid (16:0; 44.3 %), as well as mono-unsaturated fatty acids (MUFA) (18:1; 39.0%; Ong & Goh, 2002), is notably growing in popularity and use in the food industry for several functional reasons (e.g. inherent stability and resistance to oxidation) (O' Holohan, 1997). Dry fractionation of PO yields palm olein (POL, liquid fraction: 70-80%) and palm stearin (solid fraction: 20-30%). POL contains less SFA, and more PUFA than PO, thus having a P/S ratio of 0.3 versus the 0.2 ratio of PO. The effects of PO and POL on lipids and lipoproteins have been well studied, but as indicated by the reviews of Ng (1994) and Sundram (1997), results are highly inconsistent, ranging from hypocholesterolaemic or neutral, to hypercholesterolaemic. Since red palm olein (RPO, mildly or unrefined palm oil) became available commercially, its role as an excellent natural source of antioxidant vitamins, namely tocopherols and tocotrienols, as well as β-carotene became all the more evident (Manorama *et al.,* 1999). Although controversial, these antioxidant components, specifically tocotrienols, have shown beneficial effects on lipids and haemostatic profiles in several controlled intervention studies (Qureshi *et al.*, 1991; Qureshi *et al.*, 1995; Tan *et al.,* 1991). To our knowledge, the number of studies on the effect of RPO on lipids is very limited.

Although it is known that diet influences the haemostatic process, the relationship of the total diet, as well as specific foods and nutrients, with the different haemostatic variables is far from clear (Vorster *et al.,* 1997). Controlled dietary experiments have indicated that plasma activities of coagulation and fibrinolytic parameters may be affected by the fatty acid composition of the diet (Marckmann, 1995; De Bosch *et al.*, 1996), but few studies have been performed to establish the specific effects of individual fatty acids on the haemostatic system (Hunter *et al.*, 1999). Comparison of results are, furthermore, complicated by different study designs. In several studies the effect of PO on some haemostatic variables, such as platelet aggregation, bleeding time, fibrinogen, plasminogen and thromboxane B_2 (TXB_2) production has been investigated (Pereira *et al.,* 1991; Rand *et al.*, 1988; Salomaa *et al.*, 1997; Temme *et al.*, 1998), finding sometimes contradictory, but not the same detrimental effects, as seen with other oils high in saturated fatty acids (De Bosch *et al.,* 1996).

The end product of the coagulation system is the stable fibrin network, which is also the target of the fibrinolytic enzymes. The quality of the fibrin network structure may also contribute to the risk for cardiovascular diseases (CVD) and thrombosis (Fatah *et al.,* 1992; Fatah *et al.,* 1996). A change in any of the constituents of plasma could influence the characteristics of fibrin networks via its effect on metabolism, possible direct steric effects or altered fibrinogen conversion. Atherogenic, tight and rigid fibrin clots have been associated with CVD risk factors such as increased fibrinogen and serum lipids (low density lipoprotein [LDL], very low density lipoprotein [VLDL]) (Fatah *et al.*, 1992; Fatah *et al.*, 1996). The effect of POL on the fibrin clot, as well as the effect of RPO on specific haemostatic variables and the fibrin clot has not been investigated before. It is thus unknown what effect, if any, the unique fatty acid composition and the rich antioxidant content of especially RPO may have on these parameters.

After the products which will serve as vehicles for the test oils were found very acceptable in a preceding consumer study conducted by the authors (Scholtz & Bosman, unpublished observations), a randomized, controlled, single blind, parallel study design was used to compare the effect of POL and RPO to that of sunflower oil (SFO) on lipids, haemostatic profiles and fibrin network characteristics (FNC) of hyperfibrinogenaemic subjects.

Subjects and methods

Fifty nine (37 men, 22 women) free-living hyperfibrinogenaemic, motivated volunteers who regularly attend the Lipid Clinic, Potchefstroom University for Christian Higher Education (PU for CHE) and who signed consent forms for participation in clinical studies in the Lipid Clinic, were recruited on a basis of availability. The ethics committee, PU for CHE approved dietary intervention studies on patients in the Lipid Clinic, PU for CHE (HHK 3M3-92). Inclusion criteria for this study were: men and women; adults (>18 years) and plasma fibrinogen >3.0g/L. Subjects using lipid lowering medication were asked to maintain their usual dosage throughout the study. Exclusion criteria were: familial hypercholesterolaemia; diabetes or other chronic diseases, pregnant/lactating women, triacylglycerol (TG) >4mmol/L. Of the initial 59 subjects, aged 21-59 years (mean 48.29 years) 56 were included in the study, of which 36 were men and 20 were women. Mean [95% confidence intervals (CI)] values at the beginning of the run-in are presented in **Table 1.**

TABLE 1 Baseline characteristics of subjects (n=56).

Variables	Before run-in					
	Men (n=36)			Women (n=20)		
	Mean	*95% CI*		*Mean*	*95% CI*	
Age (years)	48.8	46.0	51.6	47.4	42.7	52.1
Weight (kg)	91.0	86.9	95.1	75.1	67.8	82.4
BMI (kg/m^2)	29.0	27.8	30.3	28.1	25.1	31.2
Systolic BP (mmHg)	131.8	127.7	135.9	128.5	122.6	134.4
Dystolic BP (mmg/Hg)	86.5	83.0	90.1	82.0	77.4	86.6
Fibrinogen (g/L)	4.0	3.6	4.3	3.8	3.3	4.2
Haematocrit (%)	45.9	45.0	46.7	41.0	39.3	42.6
Haemoglobin (mmol/L)	10.7	10.2	11.2	9.7	8.9	10.5
TC (mmol/L)	5.1	4.8	5.4	5.1	4.7	5.6
TG (mmol/L)	1.9	1.6	2.2	1.5	1.1	1.8
HDLC (mmol/L)	0.9	0.8	1.0	1.3	1.1	1.6
LDLC (mmol/L)	3.3	3.0	3.6	3.1	2.6	3.6

CI: confidence interval; BMI: Body mass index; BP: Blood pressure; TC: Total cholesterol; TG: Triacylglycerol; LDLC: Low-density lipoprotein cholesterol; HDLC: High-density lipoprotein cholesterol

Study design and materials

A randomized, controlled, single-blind, parallel study design was used. The study was done under free-living conditions. Subjects underwent a run-in period of four weeks during which they received baked SFO products on a weekly basis. The purpose of the run-in period was, firstly, for subjects to familiarize themselves with the intervention and, secondly, to exclude possible independent effects of SFO. After the run-in period, subjects were paired according to gender, plasma fibrinogen level, age and body mass index (BMI). They were then randomized into three groups receiving either RPO (a mildly refined palm olein in the form of the cooking oil, Carotino®) or refined, bleached and deodorized POL, both provided by the Palm Oil Research Institute of Malaysia (PORIM), or SFO (pressed from locally produced sunflower seed) products on a weekly basis for another four weeks. There were no significant differences between the three groups for any of the baseline variables (results not shown).

In both the run-in and intervention phases, 25g/day of oil was given in the form of one high-fibre muffin (60g), providing 7.5g of the test oil, and 70g of rusks, providing

17.5g of the test oil per day. Rusk is a typical South African, bread-like product which is dried at a low temperature (± 100 °C) for several hours after being baked, thus having a crisp and brittle texture. Being advised by the researchers, subjects exchanged carbohydrate foods used in their habitual diet (for example breakfast cereal and bread with margarine) for the muffins and rusks. These baked products were chosen as "vehicles" for the oils because they are well known and acceptable to most South Africans (Bosman, 1998) and vitamins in RPO are also better preserved when baked than when fried (Manorama & Rukmini, 1992; Manorama & Rukmini, 1991). The fatty acid composition of the oils used in this study is shown in **Table 2**. As analysed by gas chromatography by the Palm Oil Research Institute of Malaysia (PORIM), RPO and POL provided 15mg and 10mg, respectively, of tocotrienols per 25g oil/day. RPO, furthermore provided 16.8mg total carotenoids, 7.1mg beta-carotene and 6.8mg alpha-carotene per 25g oil/day. SFO is a rich source of alpha-tocopherol and provided 14.12mg of alpha-tocopherol per 25g oil/day.

TABEL 2 Fatty acid profiles of the test oils.*

Fatty acid	Palm olein	Red palm olein	Sunflower oil
	Percent distribution (g/100g)		
12:0	0.3	0.3	Nd
14:0	1.0	0.9	0.2
16:0	40.9	36.2	6.3
18:0	4.3	3.7	4.9
18:1n-9	41.7	46.7	25.7
18:2n-6	11.3	12.8	62.1
18:3n-3	0.2	0.4	0.1
20:0	0.3	nd	0.2

* Analyzed by the Palm Oil Research Institute of Malaysia

The subjects were asked to maintain their habitual life-styles, alcohol consumption and physical activity for the duration of the study, as well as to keep a diary of illnesses and medication used (if absolutely necessary) for the duration of the study. Fasting blood samples and anthropometric measurements were taken before the run-in period, as well as before and after the intervention period. Body mass index (BMI) (kg/m^2) was calculated.

Dietary intake and nutrient analysis

Except for the exchange of carbohydrate rich food products for the test products, subjects were advised to follow their habitual diets throughout the study. Dietary intakes were estimated by a 24-hour dietary recall at the beginning of the run-in, as well as at the beginning and end of the intervention. The computer programme, Foodfinder 2 (Medical Research Council of South Africa, 2000), based on the South African food composition tables (Langenhoven *et al.*, 1991), was used to analyse nutrient intakes.

Blood sampling

Fasting venous blood samples, taken before the run-in, as well as before and after the intervention period, were collected by a qualified nursing sister using a 21-gauged scalp infusion set. Samples were drawn with minimal stasis between 07:00 and 10:00 to avoid effects of diurnal variation. Five ml blood was drawn for the preparation of EDTA blood, that was used for the analysis of haematocrit and haemoglobin. For the lipid analysis 10ml blood was drawn and left to clot. For the determination of coagulation factors and fatty acids ±10ml citrated blood was drawn. The clotted and citrated blood was centrifuged at 3660*g* for 15 minutes at 10°C to yield serum and plasma, respectively. For the determination of fibrin network structure another 50 ml of citrated blood (3.8 % citrate) with added 10 000 KIU/ml Trasylol® (35 µl / 10 ml blood) was drawn. This citrated blood was centrifuged twice at 3660*g* for 15 minutes at room temperature to yield platelet poor plasma. Plasma was divided for compaction, permeability and turbidity analyses. Aliquots of serum and plasma were stored at -84°C until the analyses were done.

Analytical methods

Fatty acids from total plasma were determined with the chloroform/methanol extraction method (Smuts, 1992). All coefficients of variance were produced in the local laboratory during analyses, and between-run figures are based on the same samples. Haematocrit was measured with the capillary tube method and haemoglobin with a colorimetric method (Boehringer Mannheim, Cat.no. 124729). Serum total cholesterol (TC) and TG were measured using enzymatic methods (Randox, Antrim, UK, TC: Cat.no. CH200, (CV) =4.2%; TG: GPO-PAP method, Cat.no. TR210, CV=6.2%). Serum high-density lipoprotein cholesterol (HDLC) was

measured using a precipitant method (Randox, Antrim, UK, Cat.no. CH204, CV=4.4%) and serum low-density lipoprotein cholesterol (LDLC) was calculated using the Friedewald formula (Friedewald,1972).

Plasma thrombin-antithrombin complex (TAT), plasmin-antiplasmin-complex (PAP), tissue plasminogen activator antigen (tPA_{ag}) and D-dimer were all measured using ELISA-methods (TAT: Enzygnost® TAT micro, Behring, Cat.no. OWM G15, within-run CV=1.9%, between-run CV=2.6%; PAP: Enzygnost® PAP micro, Cat.no. OQBM G11, within run CV=7.1%, between run CV=8.5%; tPA_{ag}: Imulyse™ tPA, Biopool, Umeå, Sweden, Cat.no. 101005, within-run CV=5.6%, between-run CV=18.3%; D-dimer: Enzygnost® D-Dimer micro, Behring, Cat.no. OQBC G11, within-run CV=11.4%, between-run CV=24.3%). Plasminogen activator inhibitor-1 activity ($PAI\text{-}1_{act}$) was measured using an indirect enzymatic method (Spectrolyse pL PAI-1, Biopool, Umeå, Sweden, Cat.no. 101201, within-run CV=7.9%, between-run CV=11.1%). FNC measurements were: permeability (K_S) [(Fatah *et al*, 1992); within-run CV=11.1%; between-run CV=17.68%]; compaction [(Dhall *et al.*, 1976); within-run CV=3.9%; between-run CV=10.2%]; mass-length-ratio (MLR) [turbidimetric method, (Nair *et al.*, 1991); within-run CV=7.8%; between-run CV=15.3%] and fibrinogen (Clauss method) using a coagulation auto-analyser [ACL-200] and reagents from Instrumentation Laboratory, Italy, Milan; CV=4.5%.

Statistical analyses

The computer software package Statistica® was used for the analyses of the data. The statistical analysis was done in five steps. Firstly, the variables were tested for normality using the Shapiro-Wilk's W-test. Secondly, descriptive statistics were done. Data that were normally distributed are expressed as mean [95% confidence intervals (CI)]. Data that are not normally distributed are expressed as median [25, 75 percentiles]. Thirdly, changes within groups, from baseline to end, were tested for significance by using the *t*-test for dependent samples in the case of parametric data and the Wilcoxon matched pairs test fin the case of non-parametric data. Fourthly, differences between the three groups were determined by using the ANOVA for parametric data and the Kruskall Wallis ANOVA for non-parametric data. Lastly, when significance was indicated with the ANOVA, the Tukey honest significant difference test for unequal N for parametric data, and the Mann-Whitney U test

together with Bonferroni adjustments for non-parametric data were used to determine between which groups the differences occurred. Significance was set at $p \leq 0.05$. Spearman's correlation coefficient for non-parametric data was used for correlations between FNC and fatty acids, lipids and/or haemostatic variables.

Results

The effects on the different variables were in the same direction when analysed separately for men and women than when combined. The data are therefore reported for men and women combined.

Compliance and dropouts

Mean compliance, as determined by collecting left over muffins and rusks, was 98.8±3.2%. The results of one subject from the POL group were discarded due to lack of compliance (subjects were considered compliant when they had ingested >80 % of the total fatty acid dose). Two subjects from the RPO group dropped out due to allergic symptoms and illness, respectively.

Dietary intakes and BMI

During intervention, BMI increased slightly but significantly (p=0.05) with a median of 0.2 BMI points in the RPO group (Median [25-75 percentiles]: 27.2 [25.2-32.6] to 27.3 [25.3-32.6]). BMI did not change significantly in either the POL or SFO group (POL: 29.5[27.2-31.7] to 29.3[26.8-32.4] and SFO: 27.5[24.8-31.9] to 27.8[25.0-32.1]). Strict control over carbohydrate and fat exchanges, especially when subjects themselves are responsible for making exchanges between experimental products and foods in their normal diets, should prevent any changes in BMIs during intervention.

The intake of selected macronutrients, as calculated from the 24-h-recall dietary questionnaires, are presented in **Table 3**. No significant changes were found in most macronutrient intakes (total energy, protein, carbohydrate, sugar, total fat, MUFA and SFA expressed as percentage of total energy [%En]) or dietary cholesterol intake in any group during the study (not all results shown). After intervention, median dietary cholesterol intakes were moderate, averaging from 207.5mg to 224mg per day, while median fat intake averaged from 33.01 %En to 37.83 %En.

The changes that occurred in nutrient intakes were expected, based on the contents of the different oils. Linoleic acid (C18:2) and polyunsaturated fatty acids (PUFA) as %En were the only macronutrients that increased significantly in all groups during the run-in period. Linoleic acid remained elevated in the SFO group, while it decreased in the POL and RPO groups during the experimental period. Palmitic acid (C16:0) intake increased significantly during the experimental period in both the POL and RPO groups.

Concerning the intake of selected micronutrients present in the test oils (**Table 4**), the alpha, beta and total carotene intake increased significantly, as expected, in the RPO group. Due to the high content of vitamin E in all three oils, dietary intake thereof increased significantly during the run-in period and remained increased for the duration of the study. Total tocopherol intake increased significantly during the run-in in all groups. It remained increased in the SFO group, and decreased significantly in the other groups. As POL and RPO are good sources of tocotrienol, the intake thereof was increased during intervention in both these groups.

TABLE 3 Intake of selected macronutrients during intervention.

Variable		Palm olein (n=18)			Red palm olein (n=18)			Sunflower oil (n=20)		
		Median	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***
Total fat (%En)	B	34.22	28.64, 38.51	0.88	34.24	28.61, 38.54	0.90	36.80	30.11, 39.04	0.39
	M	32.99	28.71, 40.53		34.20	29.83, 41.52		37.71	36.00, 40.24	
	E	33.01	27.31, 38.82		33.55	30.01, 37.84		37.83	31.22, 42.98	
SFA	B	11.29	9.33, 13.25	0.12	10.79	8.96, 12.61	0.50	11.97	9.85, 14.08	0.54
(%En)	M	9.54	7.78, 11.31		10.41	8.45, 12.38		10.62	9.29, 11.95	
	E	11.73	10.46, 13.01		11.71	10.38, 13.05		11.42	9.62, 13.22	
C16:0 (g)	B	12.13[a]	9.15, 15.11	0.0016	13.23	9.74, 16.72	0.036	15.88	11.79, 19.98	0.85
	M	10.83[b]	7.79, 13.87		11.80[a]	9.29, 14.30		14.56	10.71, 18.41	
	E	18.12[a, b]	14.94, 21.29		16.83[a]	14.19, 19.47		14.56	10.57, 18.54	
MUFA (%En)	B	11.31	9.42, 13.21	0.34	10.28	8.68, 11.88	0.26	11.32	9.09, 13.55	0.95
	M	10.66	8.36, 12.96		10.50	8.84, 12.17		11.69	10.20, 13.19	
	E	12.58	10.82, 14.34		11.89	10.38, 13.42		11.62	10.13, 13.12	
C18:1 (g)	B	20.36	17.25, 28.15	0.19	23.11	17.08, 30.92	0.46	27.19	17.66, 34.05	0.89
	M	16.31	12.29, 31.93		21.37	13.74, 28.50		20.60	15.68, 39.30	
	E	25.38	16.66, 34.67		23.43	19.01, 31.43		23.50	15.84, 34.55	
PUFA (%En)	B	5.73[a]	4.42, 9.22	0.00001	6.21[a]	5.43, 7.57	0.00001	5.93[a1, a2]	4.54, 8.04	0.0001
	M	11.61[a]	9.43, 12.74		11.43[a]	8.46, 13.01		12.67[a1]	9.31, 14.54	
	E	5.35	4.90, 6.58		5.68	4.69, 8.12		11.50[a2]	9.98, 12.88	

Variable		Palm olein (n=18)			Red palm olein (n=18)			Sunflower oil (n=20)		
		Median	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***
C18: 2 (g)	B	11.64[a]	7.63, 17.27	0.05	14.46[a1]	10.14, 20.11	0.0008	13.73[a1, a2]	10.20, 19.51	0.0025
	M	21.51[a]	13.80, 30.19		21.84[a1, a2]	16.85, 26.32		25.29[a1]	20.30, 34.73	
	E	11.06	6.24, 19.24		11.37[a2]	8.4, 16.95		24.99[a2]	15.35, 32.63	
Cholesterol	B	201	95, 291	0.65	212	101, 274	0.64	232	132, 405	0.89
(mg)	M	166	114, 244		215	155, 283		219	161, 378	
	E	208	144, 252		213	148, 335		224	140, 377	

B: Baseline (before run-in); M: Middle (before intervention); E: End (after intervention); SFA: Saturated fatty acids; MUFA: Mono-unsaturated fatty acids; PUFA: Polyunsaturated fatty acids; CHO: Carbohydrates. Medians with the same symbol differed significantly within groups: [a; a1; a2]: $p \leq 0.05$; [b]: $p \leq 0.01$.

TABLE 4 Intake of selected micronutrients during intervention.

Variable		Palm olein (n=18)			Red palm olein (n=18)			Sunflower oil (n=20)		
		Median	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***
Alpha-Carotene (µg)	B	74.5	19.5, 3011.5	0.28	43.5[a]	2.0, 1374.0	0.00001	86.0	9.0, 145.0	0.26
	M	55.0	9.0, 2480.0		83.0[b]	8.0, 723.0		64.5	25.0, 84.0	
	E	244.5	75.0, 1264.0		5072.0[a,b]	5048.0, 5125.0		169.5	35.5, 2794.0	
Beta-Carotene (µg)	B	1884.0	459.0, 7105.5	0.96	1346.0	457.0, 5472.0	0.018	658.0	364.0, 1747.0	0.28
	M	1829.0	904.0, 6966.0		944.0[a]	239.0, 2274.0		827.5	415.0, 2427.0	
	E	1877.5	459.0, 5760.0		5599.0[a]	5415.0, 6084.0		1412.0	654.5, 7636.5	
Total Carotene (µg)	B	1924.5	550.5, 8618.0	0.94	1778.5[a]	496.0, 6502.0	0.0001	661.0	454.0, 1822.0	0.19
	M	2067.5	936.0, 8066.0		1109.0[b]	325.0, 3363.0		1456.5	481.0, 3366.0	
	E	2078.5	484.0, 6426.0		12966.0[a,b]	12654, 13467		1539.5	793.0, 9068.5	
Vit A (µg)	B	763.5	355.0, 1565.5	0.91	647.5	343.0, 1480.0	0.23	480.0	370.0, 767.0	0.36
	M	686.0	464.0, 1641.0		453.0	274.0, 701.0		608.0	379.0, 959.0	
	E	626.5	408.0, 1971.0		357.0	252.0, 605.0		671.5	451.5, 1816.0	
Vit E (mg)	B	11.8[b1, b2]	6.6, 19.0	0.031	14.6[a, b]	9.3, 19.1	0.014	12.2[a1, a2]	10.2, 20.3	0.0045
	M	22.8[b1]	15.2, 28.3		20.2[a]	16.6, 22.1		23.0[a1]	20.3, 29.2	
	E	21.2[b2]	18.5, 30.6		21.6[b]	18.4, 27.7		25.7[a2]	17.1, 33.4	
Total Tocopherol (mg)	B	2.3[b]	1.8, 3.9	0.00001	3.1[b1]	2.3, 7.5	0.00001	2.4[b1, b2]	2.1, 4.0	0.00001
	M	13.1[b]	12.5, 16.3		15.3[b1, b2]	13.0, 16.8		14.5[b1, b2]	13.5, 20.6	
	E	6.8[b]	5.4, 9.6		6.2[b2]	4.4, 8.9		16.6[b1]	13.0, 22.8	
Total Tocotrienol (mg)	B	0.1[b]	0.0, 0.2	0.00001	0.2[c]	0.0, 0.3	0.00001	0.1	0.1, 0.2	0.17
	M	0.2[c]	0.1, 0.3		0.2[b]	0.1, 0.4		0.2	0.1, 0.3	
	E	7.5[b, c]	7.4, 7.8		11.0[b, c]	10.9, 11.3		0.2	0.1, 0.3	

B: Baseline (before run-in); M: Middle (before intervention); E: End (after intervention); Medians with the same symbol differed significantly within groups: [a; a1; a2]: p≤0.05; [b; b1; b2]: p≤0.01; [c]: p≤0.001

Plasma fatty acids and lipids

The changes within and between groups in plasma fatty acids and lipids during the experimental period are presented in **Tables 5** and **6**. Only results of relevant fatty acids are reported. The high oleic acid (C18:1) content of POL and RPO led to a significant increase in the plasma levels thereof in both these groups. Consequently, POL and SFO differed significantly in their effects on plasma C18:1. In the POL group, C18:2 was significantly decreased, while a slight, non-significant decrease was observed for this variable in the RPO group. In contrast to the increase in dietary intake levels, the plasma levels of C16:0 decreased significantly with the intake of POL, but not with the intake of RPO. Furthermore, plasma palmitoleic acid (C16:1), the metabolite of C16:0, increased significantly with intake of POL, but not with the intake of RPO. Myristic acid (C14:0) decreased significantly with the intake of RPO and SFO. The above mentioned increase in plasma oleic acid level in the POL and RPO groups, as well as the increase in linoleic acid level in the SFO group during the run-in (data not shown), is an indication of good compliance by the subjects.

TC and LDLC increased significantly with 7% and 13%, respectively, in the POL group. Relative to RPO and SFO, TC increased significantly with the intake of POL. Compared to SFO, LDLC was also significant higher after POL intake. Although HDLC levels increased equally in all three groups, it only reached significance in the RPO-group (7%). The TC/HDLC ratio decreased significantly only with the intake of SFO, resulting in a significant difference compared to the change in POL. Serum TG levels did not change significantly during the study.

TABLE 5 Changes within and between groups in plasma fatty acids during intervention.

Variable		Palm olein (n=18)			Red palm olein (n=18)			Sunflower oil (n=20)			
		Mean	***95% CI***	***p-value***	***Mean***	***95% CI***	***p-value***	***Mean***	***95% CI***	***p-value***	***p-value between groups***
C18:1 (n-9)	B	20.40	18.96, 21.84	0.01	20.91	19.72, 22.10	0.02	20.31	19.01, 21.60	0.62	0.76
(%w/w)	A	22.60[a]	21.09, 24.12		21.92	20.77, 23.07		20.02[a]	18.67, 21.36		0.04
	Δ	2.20[b]	0.72, 3.67		1.01	0.16, 1.85		-0.29[b]	-1.48, 0.90		0.01
C18:2 (n-6)	B	31.38	29.22, 33.54	0.03	32.50	30.76, 34.24	0.18	32.78	30.53, 35.03	0.54	0.58
(%w/w)	A	29.54[a]	27.35, 31.73		31.56	29.67, 33.45		33.56[a]	30.94, 36.18		0.02
	Δ	-1.83	-3.41, -0.26		-0.94	-2.35, 0.47		0.78	-1.83, 3.40		0.15
		Median	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***	***p-value between groups***
C14:0	B	0.82	0.67, 0.99	0.33	0.79	0.69, 1.07	0.02	0.80	0.68, 1.14	0.02	0.99
(%w/w)	A	0.83	0.58, 0.98		0.68	0.61, 0.84		0.70	0.50, 0.82		0.30
	Δ	-0.06	-0.16, 0.06		-0.09	-0.23, -0.04		-0.18	-0.35, -0.01		0.22
C16:0	B	24.24	21.80, 25.38	0.05	22.84	21.80, 23.77	0.21	23.86	21.88, 26.41	0.02	0.51
(%w/w)	A	22.51	21.78, 24.64		22.64	21.24, 23.45		21.62	20.40, 23.29		0.48
	Δ	-0.77	-1.84, 0.10		-0.52	-1.85, 0.47		-2.67	-4.98, 0.08		0.11
C16:1 (n-7)	B	1.86	1.60, 2.64	0.04	2.25	1.38, 2.80	0.88	1.50	1.22, 2.76	0.68	0.49
(%w/w)	A	2.53	1.62, 3.33		1.81	1.59, 2.94		1.97	1.22, 2.77		0.25
	Δ	0.47	-0.14, 0.71		-0.12	-0.58, 0.41		0.28	-0.54, 0.55		0.25

B: Before intervention; A: After intervention; Δ = change; C14:0: myristic acid; C16: palmitic acid; C16:1: palmitoleic acid; C18:1: oleic acid; C18:2: linoleic acid; Means and medians with the same symbol differed significantly between groups: [a]: $p \leq 0.05$; [b]: $p \leq 0.01$

TABLE 6 Changes within and between groups in plasma lipids during intervention.

Variable		Palm olein (n=18)			Red palm olein (n=18)			Sunflower oil (n=20)			
		Mean	***95% CI***	***p-value***	***Mean***	***95% CI***	***p-value***	***Mean***	***95% CI***	***p-value***	***p-value between groups***
TC (mmol/L)	B	4.98	4.57, 5.39	0.0003	5.12	4.73, 5.52	0.084	4.98	4.56, 5.39	0.98	0.78
	A	5.57	5.16, 5.98		5.31	4.96, 5.66		4.97	4.51, 5.44		0.07
	Δ	0.59[a, b]	0.31, 0.86		0.18[a]	-0.03, 0.39		-0.003[b]	-0.25, 0.24		0.00
LDLC (mmol/L)	B	3.14	2.71, 3.57	0.01	3.33	2.91, 3.75	0.56	3.37	2.95, 3.80	0.30	0.69
	A	3.59	3.12, 3.96		3.39	3.00, 3.79		3.27	2.83, 3.70		0.53
	Δ	0.42[b]	0.13, 0.72		0.065	-0.16, 0.29		-0.11[b]	-0.32, 0.10		0.01
		Median	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***	***Median***	***25, 75 Percentiles***	***p-value***	***p-value between groups***
TC/HDLC	*B*	5.22	3.95, 6.07	0.23	5.37	4.59, 8.35	0.45	5.85	4.27, 6.62	0.02	0.58
	A	5.16	4.05, 6.04		5.66	3.71, 7.31		4.66	3.60, 6.20		0.60
	Δ	0.10[a]	-0.18, 0.70		-0.16	-0.94, 0.38		-0.57[a]	-1.54, 0.08		0.02
TG (mmol/L)	B	1.54	1.19, 2.40	0.08	1.63	1.19, 2.12	0.40	1.25	0.89, 1.86	0.57	0.41
	A	1.60	1.32, 2.79		1.76	1.37, 2.38		1.25	0.74, 1.68		0.09
	Δ	0.13	0.03, 0.47		0.21	-0.14, 0.40		0.007	-0.34, 0.10		0.16
HDLC (mmol/L)	B	0.97	0.77, 1.12	0.21	0.88	0.72, 1.01	0.03	0.90	0.76, 1.12	0.15	0.52
	A	1.07	0.99, 1.29		0.93	0.78, 1.15		0.97	0.87, 1.43		0.39
	Δ	0.05	-0.03, 0.19		0.05	-0.03, 0.12		0.05	-0.05, 0.21		0.98

B: Before intervention; A: After intervention; Δ = change; Means and medians with the same symbol differed significantly between groups: [a]: p≤0.05; [b]: p≤0.01; [c]: p≤0.001; TC: Total cholesterol; LDLC: Low density lipoprotein cholesterol; TG: Triacylglycerol; HDLC: High density lipoprotein cholesterol.

Haemostatic variables

Changes in the haemostatic profile of subjects are presented in **Table 7.** The intake of RPO had a possible beneficial effect by significantly decreasing tPA_{ag}. tPA_{ag} was significantly increased by the intake of SFO, thus causing a significant difference in change between RPO and POL, and RPO and SFO. Plasma $PAI\text{-}1_{act}$, TAT, PAP, D-dimer and fibrinogen were not affected by the intake of POL or RPO, while fibrinogen and D-dimer was decreased significantly by SFO. MLR increased significantly in the POL group and this increase was significantly larger than that in the RPO group. MLR also tended to increase in the SFO group (p=0.06). Compaction increased significantly in all three groups. K_S tended to increase in the RPO group (p=0.06). Furthermore, the following correlations were found, using values of the different variables before intervention, as these could be considered normal physiological correlations: negative correlations were found between MLR and plasma C16:0 (R= -0.3, p=0.04), and K_S and fibrinogen (R= -0.4, p=0.006), while the correlation between MLR and total unsaturated fatty acids was positive (R=0.34, p=0.02).

Discussion and conclusion

This study on middle aged, mildly hypercholesterolemic men and women with elevated fibrinogen levels, is unique in the sense that it revealed several important new facts about a field not researched before, namely the effect of RPO on both lipid and haemostatic profiles. Results regarding the haemostatic variables should, however, be interpreted with caution, as the study was somewhat underpowered for these variables due to practical considerations regarding costs and recruitment of hiperfibrinogenaemic subjects. Although having comparable fatty acid compositions (Table 1), subjects in the RPO group did not show the same hypercholesterolemic effect as did those in the POL group. RPO furthermore had a beneficial effect on fibrinolytic risk markers for CVD by decreasing tPA_{ag} levels.

In the current study, POL showed increased cholesterol levels similar to other studies reviewed by Sundram (1997) in hypercholesterolaemic subjects or subjects with abnormal cholesterol metabolism. In contrast to this, neutral effects were found especially in normocholesterolaemic subjects and C16:0 even caused hypo-cholesterolemic responses relative to C12:0 + C14:0 in other studies (as reviewed by Khosla & Sundram, 1996; Ng, 1994; Sundram, 1997).

TABLE 7 Changes within and between groups in haemostatic variables during intervention.

Variable		Palm olein (n=18)			Red palm olein (n=18)			Sunflower oil (n=20)			*p-value between groups*
		Median	*25, 75 Percentiles*	*p-value*	*Median*	*25, 75 Percentiles*	*p-value*	*Median*	*25, 75 Percentiles*	*p-value*	
tPA_{ag}	B	8.51	1.72, 14.00	0.10	7.19	5.60, 11.63	0.01	6.46	3.31, 9.70	0.04	0.65
(µg/L)	A	9.27	4.18, 16.34		6.30	4.23, 9.12		8.19	5.00, 13.72		0.34
(3-10 µg/L)#	Δ	2.49[a]	-0.82, 2.20		-1.02[ab]	-2.19, -0.41		4.68[b]	-1.12, 5.53		0.02
*PAI_{act} (U/mL)	B	19.21	14.67, 23.75	0.85	16.09	11.49, 20.69	0.81	14.62	9.83, 19.41	0.36	0.33
(12.8±12.1 U/mL)#	A	19.64	15.23, 24.04		15.68	11.24, 20.11		15.90	10.92, 20.89		0.38
	Δ	0.43	-4.16, 5.02		-0.41	-3.96, 3.13		1.29	-1.57, 4.14		0.79
TAT (µg/L)	B	5.89	5.65, 8.93	0.61	5.85	5.66, 6.12	0.09	5.80	5.63, 8.86	0.44	0.90
(1.0-4.1 µg/L)#	A	6.34	5.76, 8.89		6.12	5.74, 7.25		6.27	6.02, 10.59		0.56
	Δ	0.02	-2.55, 1.80		0.15	-0.06, 1.53		0.18	-1.36, 0.90		0.72
PAP (µg/L)	B	146.05	108.66, 183.10	0.31	124.09	109.38, 151.07	0.53	141.07	112.41, 169.19	0.28	0.63
(99-368 µg/L)#	A	133.07	106.91, 199.93		122.66	107.45, 144.73		128.30	113.12, 181.25		0.66
	Δ	-11.25	-19.08, 14.59		-3.33	-15.09, 7.70		-1.30	-24.32, 7.92		0.52
DD (µg/L)	B	16.57	15.76, 19.04	0.79	15.04	14.06, 20.10	0.76	16.07	14.28, 20.31	0.01	0.36
(7-78 µg/L)#	A	16.31	15.96, 17.92		16.75	14.06, 20.51		15.50	13.84, 17.38		0.43
	Δ	0.19	-2.64, 1.08		-0.03	-2.02, 0.72		-0.36	-1.89, -0.08		0.18
Fibrinogen (g/L)	B	3.42	2.93, 3.18	0.53	4.03	3.25, 4.32	0.50	3.97	3.25, 4.25	0.04	0.71
(2-4 g/L)#	A	3.23	4.23, 4.25		3.78	2.98, 4.59		3.48	2.77, 4.02		0.61
	Δ	-0.13	-0.79, 0.56		-0.05	-0.41, 0.28		-0.19	-1.21, 0.12		0.98

Variable		Palm olein (n=18)			Red palm olein (n=18)			Sunflower oil (n=20)			*p-value between groups*
		Median	*25, 75 Percentiles*	*p-value*	*Median*	*25, 75 Percentiles*	*p-value*	*Median*	*25, 75 Percentiles*	*p-value*	
MLR (dalton/cm x 10^{-12})	B	28.93	25.04, 29.64	0.0008	30.80	27.76, 34.62	0.91	31.97	26.49, 36.72	0.06	0.13
	A	34.62	30.10, 37.39		30.25	29.05, 34.05		35.63	33.45, 39.34		0.12
	Δ	6.05[a]	1.55, 10.15		0.11[a]	-3.00, 3.70		7.72	-1.00, 10.38		0.02
*K_S (cm^2 x 10^{-9})	B	5.15	4.65, 5.66	0.41	5.72	4.79, 6.99	0.06	5.87	4.88, 6.87	0.48	0.46
	A	5.53	4.77, 6.30		6.47	5.67, 7.34		6.21	5.43, 7.00		0.20
	Δ	0.38	-0.56, 1.32		0.75	-0.02, 1.51		0.34	-0.64, 1.31		0.77
Compaction (%)	B	46.58	43.9, 48.6	0.0002	49.13	44.80, 50.20	0.0010	45.25	43.50, 47.30	0.0001	0.16
	A	50.95	47.63, 53.40		54.10	49.80, 57.00		54.20	50.25, 57.15		0.08
	Δ	4.88	4.10, 6.40		6.34	2.50, 11.80		8.34	5.00, 13.00		0.06

Reference ranges as given by the producers of the diagnostic kits (see analytical methods). * Given as means [95% confidence interval].
B: Before intervention, A: After intervention; Δ = change; tPA_{ag}: Tissue plasminogen activator antigen; PAI-1_{act}: Plasminogen activator inhibitor 1; TAT: Thrombin-antithrombin complex; PAP: Plasmin-antiplasmin complex; MLR: Mass-length-ratio; K_s: Permeability; Means and medians with the same symbol differed significantly between groups: [a]: $p \leq 0.05$; [b]: $p \leq 0.01$

In the current study, the total dietary fat intake was not abnormally high at the end of the intervention period (POL = 33.0 %En and RPO = 33.6 %En), natural test oils were presented in whole, solid foods, and the median dietary cholesterol intakes of the POL and RPO groups were not very high (Hunter, 2001; Sundram, 1997). Other factors that could have influenced results are the fact that subjects were free-living, middle-aged and half of them were mildly hypercholesterolaemic with rather high BMIs (Hunter, 2001). The effects of RPO on lipid levels have, however, only been examined to a very limited extent. Wood *et al.* (1993) found that crude palm oil reduced LDLC significantly.

It is speculated whether the significant increases in alpha, beta and total carotene levels, as well as tocopherol and tocotrienol levels in the RPO group, may have contributed to its more neutral effect on lipids and favourable haemostatic profile compared to POL. The tocotrienol-enriched fraction from palm oil (palmvitee) has been shown to decrease TC and LDLC in previous studies (Qureshi *et al.*, 1995; Qureshi *et al.*, 1991; Tan *et al.*, 1991) partly through inhibiting HMG-CoA-reductase. The effect of tocotrienol, however, seems to be dose-dependant (Qureshi *et al.*, 1991). The tocotrienol-content of RPO in the current study was probably insufficient to have a substantial hypocholesterolaemic effect.

To our knowledge, the only published study in which the effect of crude palm oil, amongst others, were investigated on haemostatic variables (thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha,}$) is that by Wood *et al.* (1993), but crude palm oil showed no effect on these parameters. High tPA_{ag} and low tPA_{act} reflects decreased fibrinolytic activity and in several cross-sectional and case-control studies (Fukao *et al.*, 1992; Hellsten *et al.*, 1992; Smith *et al.*, 1995; Yamada *et al.*, 1996; Yamauchi *et al.*, 1992), as well as a meta-analysis (Vorster *et al.*, 2000), the relationship of high tPA_{ag} and low tPA_{act} with primary and secondary CVD events has been confirmed. The decreased tPA_{ag}-levels caused by RPO in the current study may thus be seen as a beneficial effect. Few studies have specifically examined the effects of beta-carotene (of which RPO is an excellent source), on haemostasis. In the Swedish MONICA Study, a cross-sectional population study, tPA_{act} correlated directly and $PAI\text{-}1_{act}$ correlated inversely with plasma β-carotene. However, when adjusted for confounders these correlations were not significant any more (Eliasson *et al.*, 1995). Van Poppel *et al.* (1995) examined the effects of 20mg/day of beta-carotene on male

smokers for 14 weeks on haemostatic variables, including fibrinogen, tPA_{ag} and fibrin degradation products. Beta-carotene had no influence on the measured haemostatic indicators. Animal and human studies that examined the effect of PO or its constituting fatty acids found inconsistent results on fibrinogen (De Bosch *et al.*, 1996; Pereira *et al.*, 1991; Tholstrup *et al.*, 1994b), tPA_{ag} or tPA_{act} (Tholstrup *et al.*, 1994a), $PAI\text{-}1_{act}$ and $PAI\text{-}1_{ag}$ (De Bosch *et al.*, 1996; Tholstrup *et al.*, 1994a; Tholstrup *et al.*, 1994b), platelet aggregation (Mensink *et al.*, 1999; Pereira *et al.*, 1991; Rand *et al.*, 1988) and TXB_2 production (Mensink *et al.*, 1999; Temme *et al.*, 1998). Tocotrienol and tocopherol have been shown in some studies to be anti-thrombotic by decreasing TXB_2 and platelet factor 4 (PF_4) (Qureshi *et al.*, 1995; Qureshi *et al.*, 1991; Theriault *et al.*, 1999) and anti-aggregatory (Steiner & Anastasia, 1975; Qureshi *et al.*, 1991), although it had no effect on these parameters in other studies (Mensink *et al.*, 1999; Wahlqvist *et al.*, 1992).

Given the fact that humans are in post-prandial state over much of a 24-h-period and that all haemostatic variables show a post-prandial circadian variability (Hunter *et al.*, 1999), it would be worthwhile to repeat this study with subjects in a post-prandial state.

All three oils, to different degrees, caused some beneficial effects on the FNC. In this study, RPO and SFO increased compaction, while POL increased both MLR and compaction of fibrin clots. Increased MLR indicates the formation of more porous, less atherogenic clots with shorter thicker fibres (Shah *et al.*, 1985), while the increased compaction is associated with decreased cross-linking and branch points, resulting in less rigid and therefore more lysable fibrin networks (Nair & Shats, 1997). It is known that clots with thicker fibres generally have fewer branch points (Ryan *et al.*, 1999), making the clots less rigid and therefore explaining the increased compaction. MLR showed a negative correlation with C16:0 and a positive correlation with the total amount of unsaturated fatty acids. It is therefore possible that the changes in plasma fatty acid composition (decrease in saturated fatty acids and increase in total unsaturated fatty acids) resulted in the formation of healthier fibrin networks with thicker fibres. This effect may be a function of the saturation of the fatty acids, but needs to be further investigated as the molecular basis of the proposed association is not yet clear.

The decrease of plasma fibrinogen levels in the SFO group might also explain the increase (although not significant, p=0.06) in the MLR of the fibrin clots. A decrease in fibrinogen concentration leads to the formation of thicker fibres, with fewer branch points, explaining the observed increase in compaction in this group.

In conclusion, the intake of 25g of POL per day had a hypercholesterolaemic effect in this study. POL did, however, not show significant detrimental influences on haemostasis, confirming the lack of any negative effects as found in other studies. In contrast, RPO, having a richer antioxidant content but almost the same fatty acid composition as POL, did not show hypercholesterolaemic properties, while it furthermore had a possible beneficial effect on HDLC and a beneficial effect on fibrinolytic risk markers for CVD through its lowering effect on plasma tPA_{ag}. Taking into consideration these nutritional benefits related to RPO, together with the fact that it is also chemically more stable compared to other unsaturated oils, RPO may play an increasingly important role in the food industry. Future studies are, however, needed to confirm the above results, to further investigate effects of non-glyceride constituents in RPO (e.g. carotenoids, tocotrienols and sterols) and to explore the mechanisms involved.

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Authors' contributions:

Susanna C. Scholtz:

Preparation and dissemination of muffins and rusks. Responsible, together with W Oosthuizen, MJC Bosman, JC Jerling and M Pieters, for the execution of the total study. Responsible for literature searches, statistical analyses, processing of data. Main author of the paper.

Marlien Pieters:

Responsible, together with SC Scholtz, W Oosthuizen, MJC Bosman and JC Jerling, for the execution of the total study. Execution of laboratory analyses, statistical analyses and compilation of the data. Part of own Ph.D study.

Welma Oosthuizen:

Study co-ordinator, responsible for the execution of the total study, laboratory analyses, compilation of the data, literature searches and contributing to writing of the paper.

Johann C. Jerling:

Design, planning and approval of final protocol. Responsible, together with W Oosthuizen, SC Scholtz and M Pieters, for the execution of the total study and writing of the paper.

Magdalena J.C. Bosman:

Co-ordination, preparation and dissemination of muffins and rusks to subjects. Involved in execution of the total study.

Hester H. Vorster:

Design, planning, approval of final protocol.

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CHAPTER 5

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

CHAPTER 5

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

1. INTRODUCTION

In this final chapter, a summary of the main findings from the two studies reported in this thesis will be given. As the results of each study are discussed, interpreted, elucidated and compared to the relevant literature in the preceding chapters, only general conclusions will subsequently be made. This will be followed by general recommendations regarding this study, as well as recommendations to the food industry and health professionals as deduced from these findings.

2. SUMMARY OF MAIN FINDINGS

2.1 Consumer acceptance of RPO products

- The sensory evaluation by a *general consumer group* showed that, although not preferred to the high-fibre muffins baked with sunflower oil (SFO), high-fibre muffins baked with red palm olein (RPO) were found very acceptable overall by these consumers as all sensory attributes scored higher than 4 on a 5 point scale.
- Consumers in this group intended to eat high-fibre muffins baked with RPO often (one per day), but as at least 3 muffins should have been consumed in order to include the optimum amount of 25g of test oil per day in the subsequent nutrition intervention study, an additional vehicle or carrier for the oil had to be considered and evaluated.
- Within the *second consumer group* (subjects from the nutrition intervention trial), high-fibre muffins baked with RPO and SFO were rated equally acceptable on all the evaluated sensory attributes.
- No significant difference was found in preference for, or consumption intent of muffins baked with either RPO or SFO in this group.
- Consumers in the second group rated RPO muffins significantly higher on acceptability of several of the sensory attributes compared to the general consumer group.

- RPO rusks, the additional vehicle chosen by the researchers for including the RPO, were also found very acceptable on all sensory attributes by this group, while consumers furthermore intended to eat rusks baked with RPO as often as those baked with SFO, namely one per day.

2.2 Effect of POL and RPO on lipids and haemostatic factors

- Results from the dietary intervention study showed that the intake of 25g of RPO per day by hyperfibrinogenaemic subjects did not increase serum total cholesterol (TC) and low density lipoprotein cholesterol (LDLC) levels as seen with intake of palm olein (POL).
- Although high density lipoprotein cholesterol (HDLC) was increased in all three groups, the increase was only significant in the RPO group (7% increase).
- Tissue plasminogen activator antigen (tPA_{ag}) was decreased in the RPO-group compared to the POL and SFO groups, possibly having a beneficial effect on risk markers for CVD.
- None of the other investigated haemostatic variables [plasma fibrinogen, D-dimer, plasminogen activator inhibitor-1 activity ($PAI\text{-}1_{act}$), thrombin-antithrombin complex (TAT) and plasmin-antiplasmin complex (PAP)] were influenced by either POL or RPO.
- Regarding the fibrin network structure, RPO increased compaction, while POL increased both mass-length-ratio (MLR) and compaction of fibrin clots, being beneficial in both cases as increased compaction is associated with decreased cross-linking and branch points, resulting in less rigid and therefore more lysable fibrin networks, while increased MLR indicates the formation of more porous, less atherogenic clots with shorter thicker fibres. Furthermore, increased compaction was also seen with the intake of SFO.
- MLR showed a negative correlation with plasma C16:0 and a positive correlation with the total amount of unsaturated fatty acids (UFA) in plasma. RPO and POL did not have independent effects on the fibrin network characteristics (FNC), but it seems as though decreased saturated fatty acids (SFA) and increased plasma UFA may have influenced FNC.

3. CONCLUSIONS

Recent developments in functional food science highlighted the inevitable necessity of interdisciplinary cooperation between several fields in academia and the food industry. Although consumer testing has already evolved as an essential element in the overall decision making process with regard to the likelihood for new product success from the development stage, through to market consolidation, product launch and commercialization, it is clear from the literature that sensory evaluation by consumers has not yet received much or enough attention prior to especially intervention trials with newly developed functional foods. The major contribution of this thesis is thus considered by the author as the reinforcement of the importance of close collaboration between especially food science, sensory evaluation and nutrition, as these fields are lately considered all the more interdependent and complementary to each other. The significance of the sequential nature of the two current studies in foods and nutrition, respectively, is made clear by the important findings obtained. After results from the first consumer group indicated the need for an additional vehicle for the test oils during the subsequent nutrition intervention trial, small adaptations could by made to the study design of the latter. Although RPO products were found very acceptable in general, by comparing evaluation results of the two consumer groups in the first study, it seemed that those in the second group (recruited for the intervention study), were even more positively inclined towards RPO products. Furthermore, consumers' good overall rating of acceptability and consumption intent of the baked products containing the test oils, could be seen as a potential indication of optimal consumption of these products during the intervention trial, which was indeed revealed by the excellent compliance during the latter study. Good compliance with test products is an inevitable requirement for successful and reliable results in any intervention study.

As indicated by results of this intervention study, 25g/day of RPO did not have any negative effects on the lipid and haemostatic profiles of this hyperfibrinogenaemic group of subjects. Furthermore, RPO may have a beneficial effect on risk markers for CVD because of its lowering effect on plasma tPA_{ag}. While POL increased TC and LDLC, it at least did not have any negative effects on the haemostatic profile. The changes in certain plasma fatty acid concentrations (decreased SFA and increased UFA) that resulted from the inclusion of the different oils in this study may

have contributed to healthier, more lysable fibrin clots. This effect needs to be examined in another study with the appropriate study design.

The combination of the above mentioned and earlier discussed health characteristics of RPO reinforce the fact that it can be considered an excellent oil for household human consumption and may thus also encourage increased use thereof in the food industry. Its suitability for a wide range of food applications (as previously described in Chapter 2) together with its health profile, justifies the claim that POL, and soon perhaps also RPO, could indeed be regarded as the most versatile of all natural fats. Furthermore, the development of innovative lipid-based functional foods, like the high-fibre baked products used in the current study, will improve consumer perception, economic value and market share of healthy food products. However, no matter how nutritious a food product is or how many functional benefits it may have, there is no use in developing such products if it does not taste good, consumers do not find it acceptable overall and have the intention to buy and consume it repeatedly.

4. RECOMMENDATIONS

- The author strongly recommends that intervention studies in which the health benefits of potential functional foods are tested, inevitably be preceded by sensory evaluation of the products to ensure optimal compliance with, and acceptance of the test products.
- It would be worthwhile to evaluate the effect of prolonged consumption of functional foods on sensory parameters, as well as to compare such results with "short –term" acceptability and preference evaluations as it may influence or alter compliance during clinical trials.
- It would be recommendable to advise subjects about the necessity of strict control concerning the intake and exchange of carbohydrates and fats when subjects themselves are responsible for making exchanges between experimental products (like those used in this study) and foods rich in carbohydrates and fats in their normal diets, to ensure that body mass indexes do not change during intervention.

- Since the effects of RPO on lipids and haemostatic profiles have only been investigated in a very limited number of studies, the current findings need to be confirmed and/or verified by future research.
- Given that humans are in the post-prandial state over much of a 24h-period and that the processes of atherogenesis and thrombosis may be accelerated as a result of circadian variation and thus changes in the haemostatic system which occur during nutrient absorption, the author suggest that a similar study be repeated under post-prandial conditions.
- The formation of healthier fibrin networks with thicker fibres may be a function of the degree of saturation of fatty acids. This hypothesis needs to be tested and further investigated in future studies.
- Taking into consideration its health benefits, more studies on the development and sensory evaluation of products containing RPO should be undertaken to expand the consumers' available choice of commercial products. Thus, the food industry could be encouraged to use RPO more often in new product developments.
- In the light of its functional and health benefits (high carotene content and favourable effects on lipid and haemostatic profile), attempts should be made by industry and/or government to make RPO more readily accessible and affordable to South African consumers.
- Nutritionists and food scientists should be encouraged to inform and educate consumers on the important health benefits of RPO, as well as to clear away any misconceptions or confusion regarding the health aspects and differences between POL and palm kernel oil.

CONSUMER QUESTIONNAIRE: STRICTLY CONFIDENTIAL

QUESTIONNAIRE NO. | | | |

*** Mark the appropriate square with a cross (X)**

SECTION A
DEMOGRAPHIC INFORMATION

1. SEX

For office use only

Male	1
Female	2

2. AGE

Between 21 and 30 years	1
Between 31 and 40 years	2
Older than 40 years	3

SECTION B

SENSORY EVALUATION OF THE ACCEPTABILITY OF MUFFINS / RUSKS WITH RESPECT TO VARIOUS CRITERIA

- **Evaluate the acceptability of the muffins / rusks (in the specified order) with respect to the given criteria by marking the appropriate square with a cross.**

		EXTENT OF ACCEPTABILITY					Office use	
Criteria	Sample codes	5 Extremely acceptable	4 Acceptable	3 Neutral	2 Not acceptable	1 Extremely unacceptable	Code	
General Appearance	1							
	2							
Colour	1							
	2							
Texture	1							
	2							
Flavour	1							
	2							

- **You have now evaluated both samples for acceptability according to certain criteria**

	Yes	No	
Is one of the samples more acceptable in total?			
If yes, which one? Write the code in the square.			

- **Please indicate how often you will be willing to eat the muffins / rusks. Choose only one option under each code.**

		Sample Code	
		plk	svg
5. I will eat it very often(3/day).	5		
4. I will eat it often (1/day).	4		
3. I will eat it occasionally (1x/week).	3		
2. I will only eat it when no other food is available.	2		
1. I will never eat it.	1		

plk	svg

24-HOUR DIETARY RECALL

SUBJECT:_____

DATE:_____

TIME	FOOD	PREPARATION METHOD	AMOUNT	CODE

How does weekend eating pattern differ from week days:

_____

_____

_____

_____

_____

CALENDER

SUBJECT:_____

DAY	APRIL	☒	MAY	☒	JUNE	☒
Mo	2					
Tue	3		1			
We	4		2			
Thu	5		3			
Fr	6		4		1	
Sa	7		5		2	
Su	8		6		3	
Mo	9		7		4	
Tue	10		8		5	
We	11		9		6	
Thu	12		10		7	
Fr	13		11		8	
Sa	14		12		9	
Su	15		13		10	
Mo	16		14		11	
Tue	17		15		12	
We	18		16		13	
Thu	19		17		14	
Fr	20		18		15	
Sa	21		19		16	
Su	22		20		17	
Mo	23		21		18	
Tue	24		22		19	
We	25		23		20	
Thu	26		24		21	
Fr	27		25		22	
Sa	28		26		23	
Su	29		27		24	
Mo	30		28		25	
Tue			29		26	
We			30		27	
Thu			31		28	

☒ - Please indicate daily whether experimental food was consumed or not (✓ or ×).

- Information session.

- Days on which blood samples are drawn.

- Please fill in the 24-hour dietary recall.

- Run-in period (4 weeks).

Intervention period (4 weeks).

- Days on which experimental food must be collected.