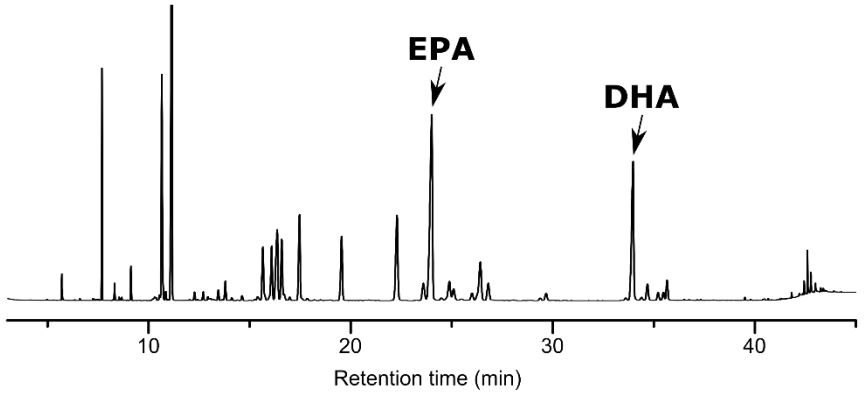
The New Zealand Institute for Plant & Food Research Limited

Plant & Food RESEARCH
RANGAHAU AHUMARA KAI



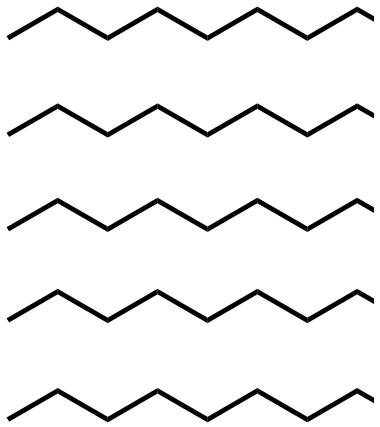
Commercial fish oil products in NZ: Raman spectroscopy to protect our image?

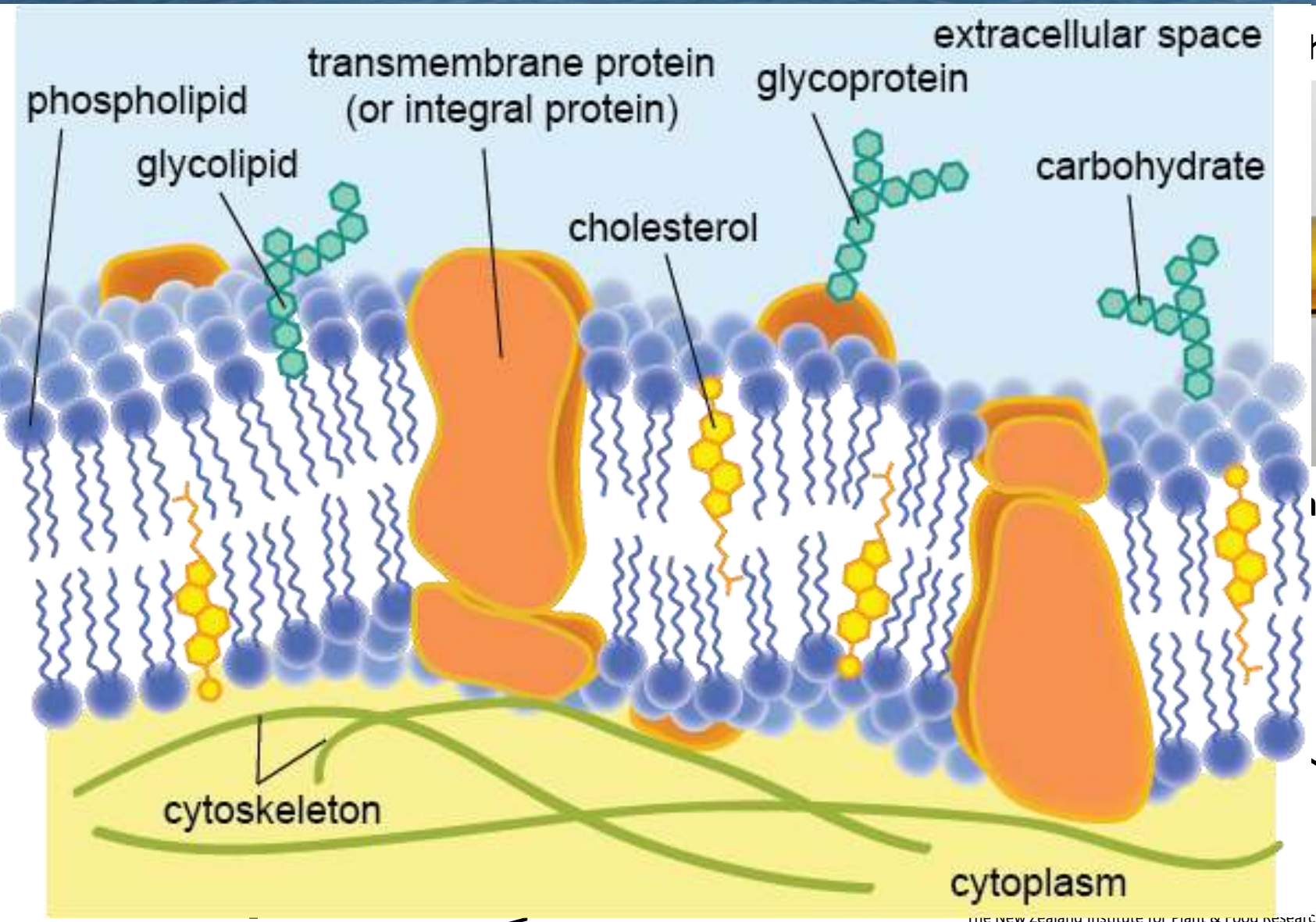
DP Killeen*, SN Marshall, EJ Burgess, KC Gordon, NB Perry

Dietary fats

Animal fats (

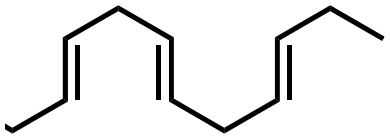
Saturated





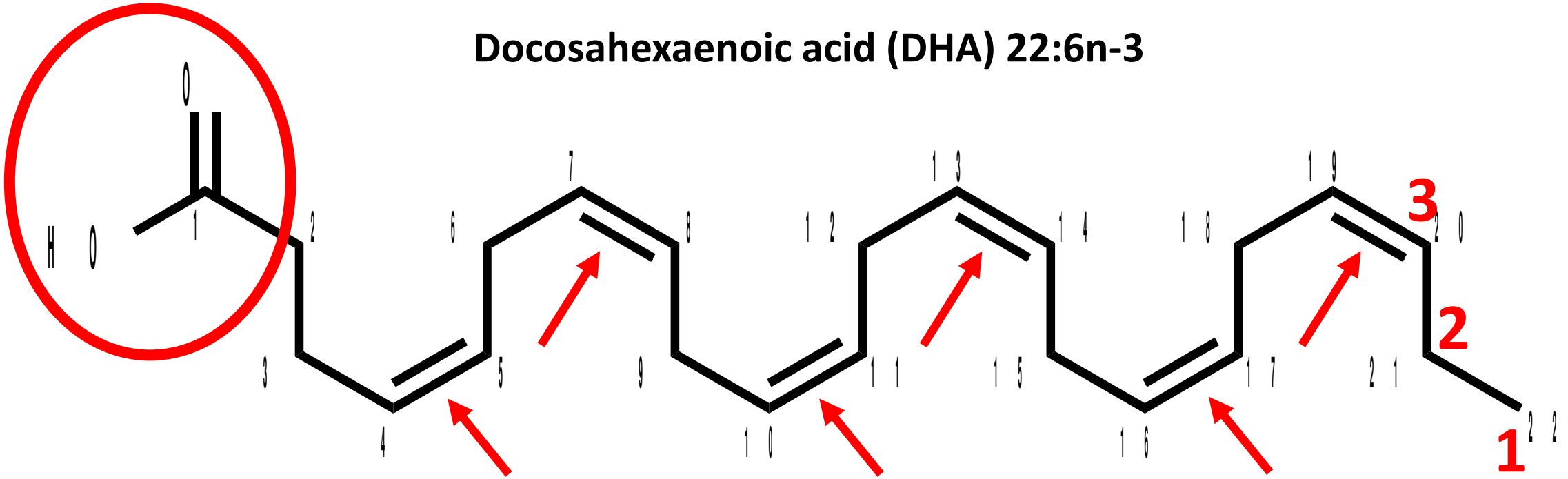
h oils

ıted fatty acids



Long chain omega-3 polyunsaturated fatty acids

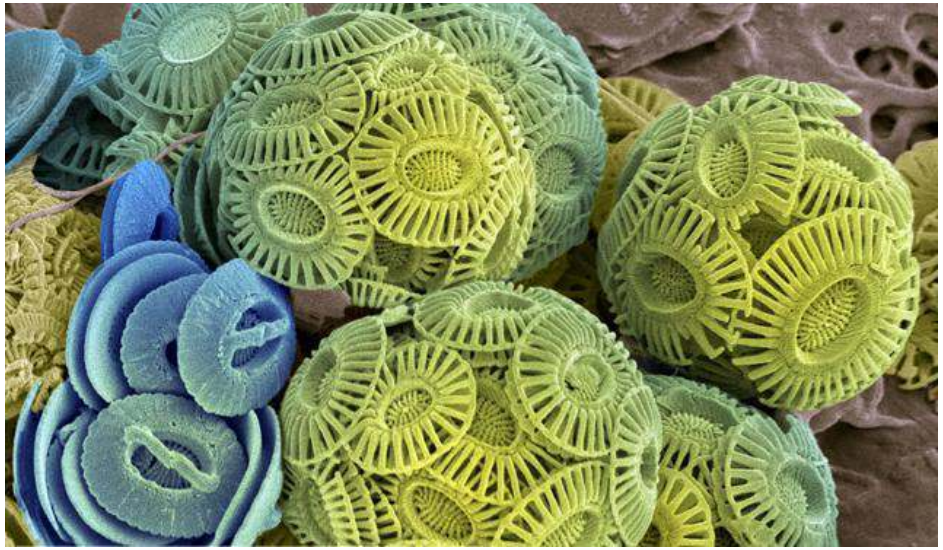
Docosahexaenoic acid (DHA) 22:6n-3



- **Long chain: 22 carbons**
- **Omega-3**
- **Polyunsaturated**
- **Fatty acids**

Humans are very bad at biosynthesising these compounds
Dietary sources are important

Marine ecology: omega-3 origins



Fish oil processing

Fishmeal

6 Million tonnes p. a.

Fish oil

1 Million tonnes p. a.

Marine oil uses

Shepherd, C.J., and A. J. Jackson. “Global fishmeal and fish – oil supply: inputs, outputs and markets.” *Journal of fish biology* 83.4 **(2013)**: 1046-1066

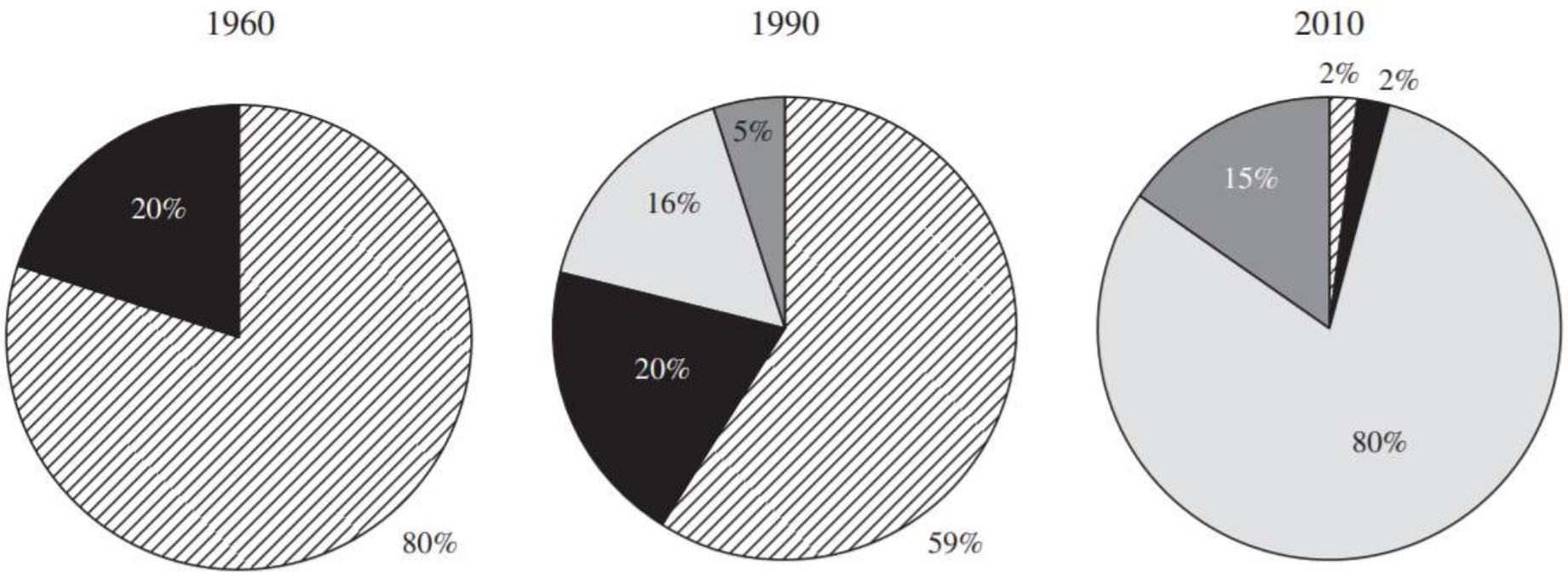


FIG. 9. Comparison of world consumption of fish-oil by market segment (▨, hardened edible; ■, industrial; □, aquafeed; ▩, refined edible) for 1960, 1990 and 2010 (Note that fish-oil which has been ‘hardened’ by hydrogenation consists of saturated fats and is solid at room temperature, hence suitable for making margarine; by contrast, fish-oil used for direct human consumption is a refined liquid containing unsaturated fats).

Fish oil in the press

NOTED

CURRENTLY CULTURE MONEY HEALTH DINING LIFE DISTRACTIONS

THE LISTENER NORTH&SOUTH Metro Paperboy RNZ

Can your fish oil cause you harm?

by Donna Chisholm / 28 September, 2016

Dramatic new research from Auckland scientists revealing the potential harm of "off" fish oil is the latest in a string of bad news stories causing a multi-million dollar headache for the former golden child of the supplements industry.

The rat babies are dying, quickly and in numbers. It is midwinter 2014, and for Auckland-based Liggins Institute scientist Dr Ben Albert, "it feels like the sky is

MOST REA

Deadly cu
infection f
Whanganu
killed me
by Gareth E

PM admits

Fish oil quality: omega-3 content

SCIENTIFIC REPORTS

OPEN

Fish oil supplements in New Zealand are highly oxidised and do not meet label content of n-3 PUFA

SUBJECT AREAS:
HEALTH CARE
MEDICAL RESEARCH

Received

Benjamin B. Albert[1], José G. B. Derraik[1], David Cameron-Smith[1], Paul L. Hofman[1], Sergey Tumanov[2], Silas G. Villas-Boas[2], Manohar L. Garg[3] & Wayne S. Cutfield[1]

nutrients

MDPI

Article

Australian and New Zealand Fish Oil Products in 2016 Meet Label Omega-3 Claims and Are Not Oxidized

Peter D. Nichols [1,*], Lalen Dogan [2] and Andrew Sinclair [3,4]

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2 DSM Nutritional Products Asia Pacific, 30 Pasir Panjang Road, Mapletree Business City, #13-31, Singapore 117440, Singapore; lalen.dogan@dsm.com
3 School of Medicine, Deakin University, Geelong, VIC 3220, Australia; andrew.sinclair@deakin.edu.au
4 Department of Nutrition & Dietetics, Monash University, Clayton, VIC 3800, Australia
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Received: 22 September 2016; Accepted: 1 November 2016; Published: 5 November 2016

Abstract: We provide new fish oil product results to assist industry in Australia and New Zealand and, ultimately, consumers in understanding the high product quality assurance protocols in place, together with the high product quality that has been determined by both industry and independent laboratories. Fish oil capsule products common to Australia and New Zealand were purchased in May 2016 in Richmond, Victoria, Australia. Products were from two groups; five standard fish oil products and five fish oil concentrates. Noting Therapeutic Goods Administration (TGA) requirement for use of standard methods, for all analyses undertaken a laboratory was selected that met the TGA

SCIENTIFIC REPORTS

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Omega-3 Long-Chain Polyunsaturated Fatty Acid Content and Oxidation State of Fish Oil Supplements in New Zealand

Received: 20 December 2016
Accepted: 22 March 2017
Published online: 03 May 2017

Gerard Bannenberg[1], Craig Mallon[2], Holly Edwards[3], Derek Yeadon[4], Kevin Yan[5], Holly Johnson[6] & Adam Ismail[1]

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Raman Spectroscopy of Fish Oil Capsules: Polyunsaturated Fatty Acid Quantitation Plus Detection of Ethyl Esters and Oxidation

Daniel P. Killeen,*[,†] Susan N. Marshall,[†] Elaine J. Burgess,[‡] Keith C. Gordon,[§] and Nigel B. Perry[‡,§]

[†]The New Zealand Institute for Plant & Food Research Limited, 300 Wakefield Quay, Nelson 7010, New, Zealand
[‡]The New Zealand Institute for Plant & Food Research Limited, [§]Department of Chemistry, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

Supporting Information

ABSTRACT: Fish oils are the primary dietary source of ω-3 polyunsaturated fatty acids (PUFA), but these compounds are prone to oxidation, and commercial fish oil supplements sometimes contain less PUFA than claimed. These supplements are predominantly sold in softgel capsules. In this work, we show that Fourier transform (FT)−Raman spectra of fish oils (n = 5) and ω-3 PUFA concentrates (n = 6) can be acquired directly through intact softgel (gelatin) capsules. These spectra could be used to rapidly distinguish supplements containing ethyl esters from those containing triacylglyceride oils. Raman spectroscopy calibrated with partial least-squares regression against traditional fatty acid methyl ester analyses by gas chromatography−mass spectrometry could be used to rapidly and nondestructively quantitate PUFA and other fatty acid classes directly though capsules. We also show that FT−Raman spectroscopy can noninvasively detect oxidation with high sensitivity. Oils with peroxide values of as low as 10 mequiv kg^{-1}, which are on the cusp of falling outside of specification, could be readily distinguished from oils that were within specification (7 mequiv kg^{-1}).

KEYWORDS: *fish oil, omega-3, Raman, polyunsaturated fatty acids, docosahexaenoic acid, eicosapentaenoic acid oxidation, softgel capsules*

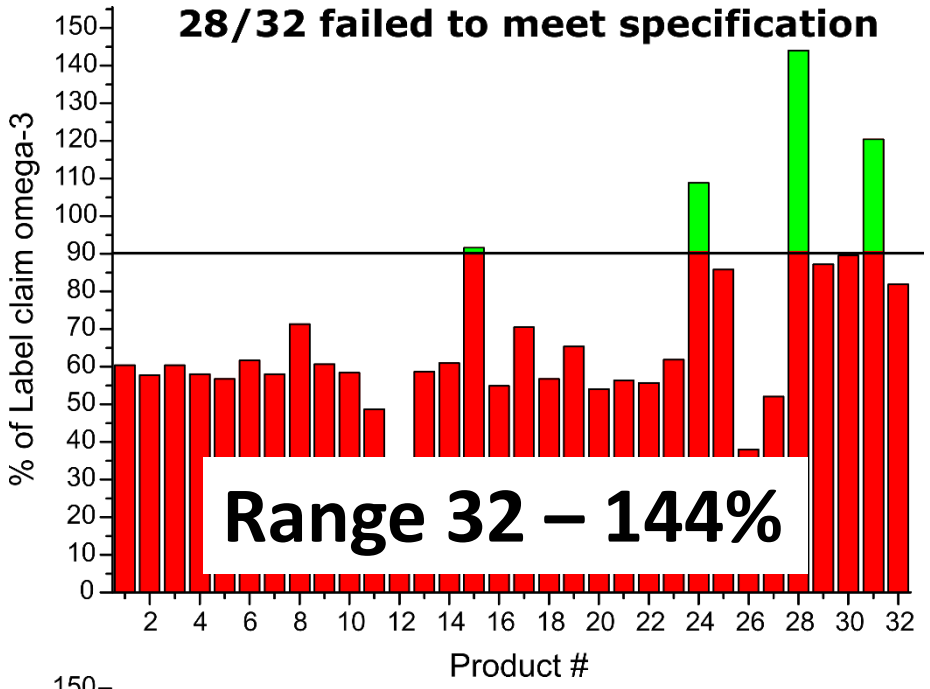
■ INTRODUCTION

The "Western diet" is deficient in long chain, ω-3

produces short chain fatty acids, peroxides, volatile aldehydes, and conjugated dienes (Figure 1), some of which have been associated with negative health impacts.[18−21] The vast majority

vere analyzed for eicosapentaenoic
xidative status in a collaborative
%, 86% and 77% complied with
z-Anisidine Value (p-AV), and
content claims. All fish oils complied
% with a calculated TOTOX value of
Pharmacopeia and the Australian
age of fish oil products reported
tent by a recently published
s for this discrepancy are evaluated

Plant & Food RESEARCH RANGAHAU AHUMARA KAI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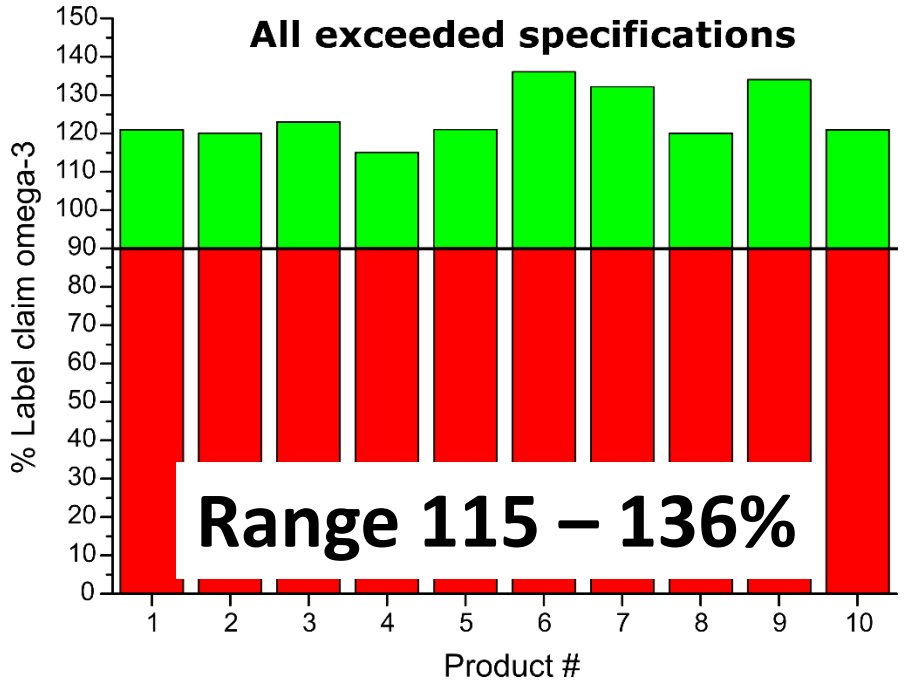
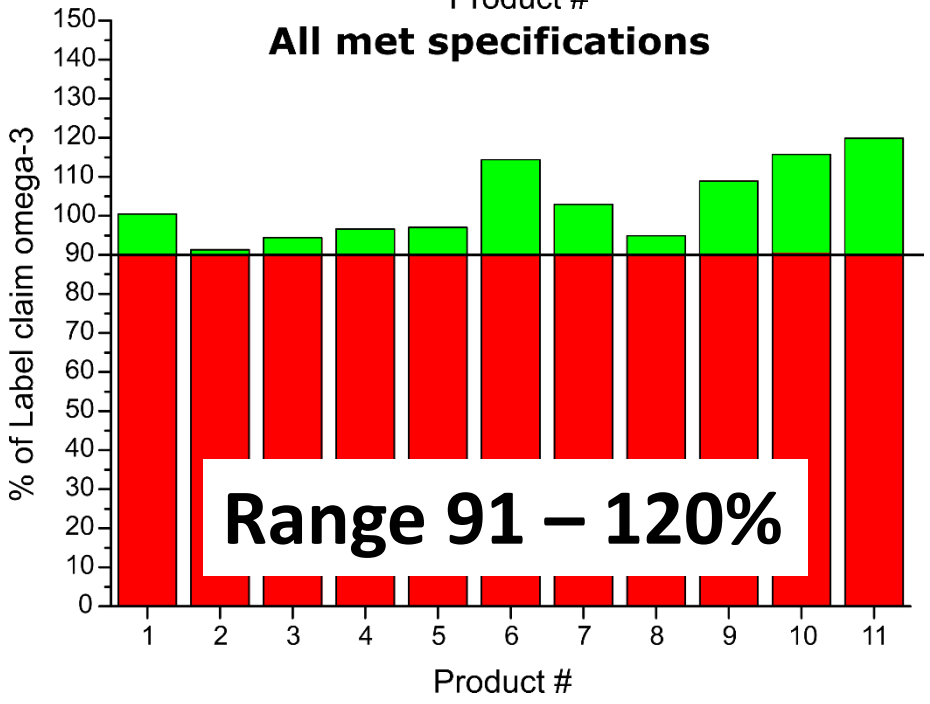
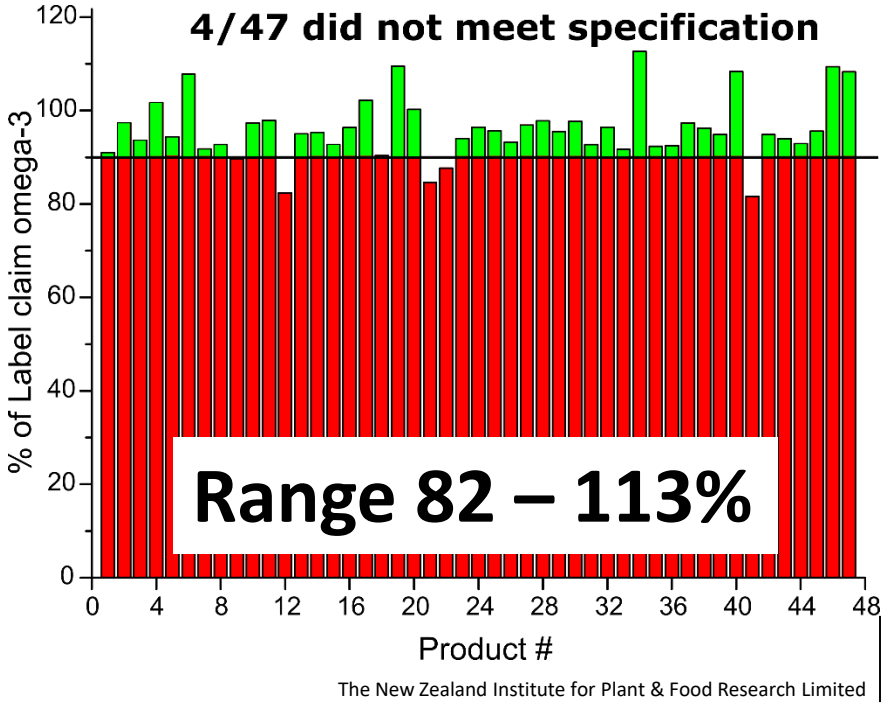
% of Label claim omega-3
Product #
Range 32 – 144%


All exceeded specifications
% Label claim omega-3
Product #
Range 115 – 136%


All met specifications
% of Label claim omega-3
Product #
Range 91 – 120%


4/47 did not meet specification
% of Label claim omega-3
Product #
Range 82 – 113%

Summary of omega-3 results

Alberts *et al.* – 13% met specification

Nichols *et al.* – 100% met specification

Killeen *et al.* – 100% met specification

Bannenberg *et al.* – 91% met specification

Fish oil quality: Oxidation

Liggins Institute paper - 2015

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
HEALTH CARE
MEDICAL RESEARCH

Received
1 October 2014

Accepted
18 December 2014

Published
21 January 2015

Correspondence and requests for materials should be addressed to W.S.C. (w.cutfield@auckland.ac.nz)

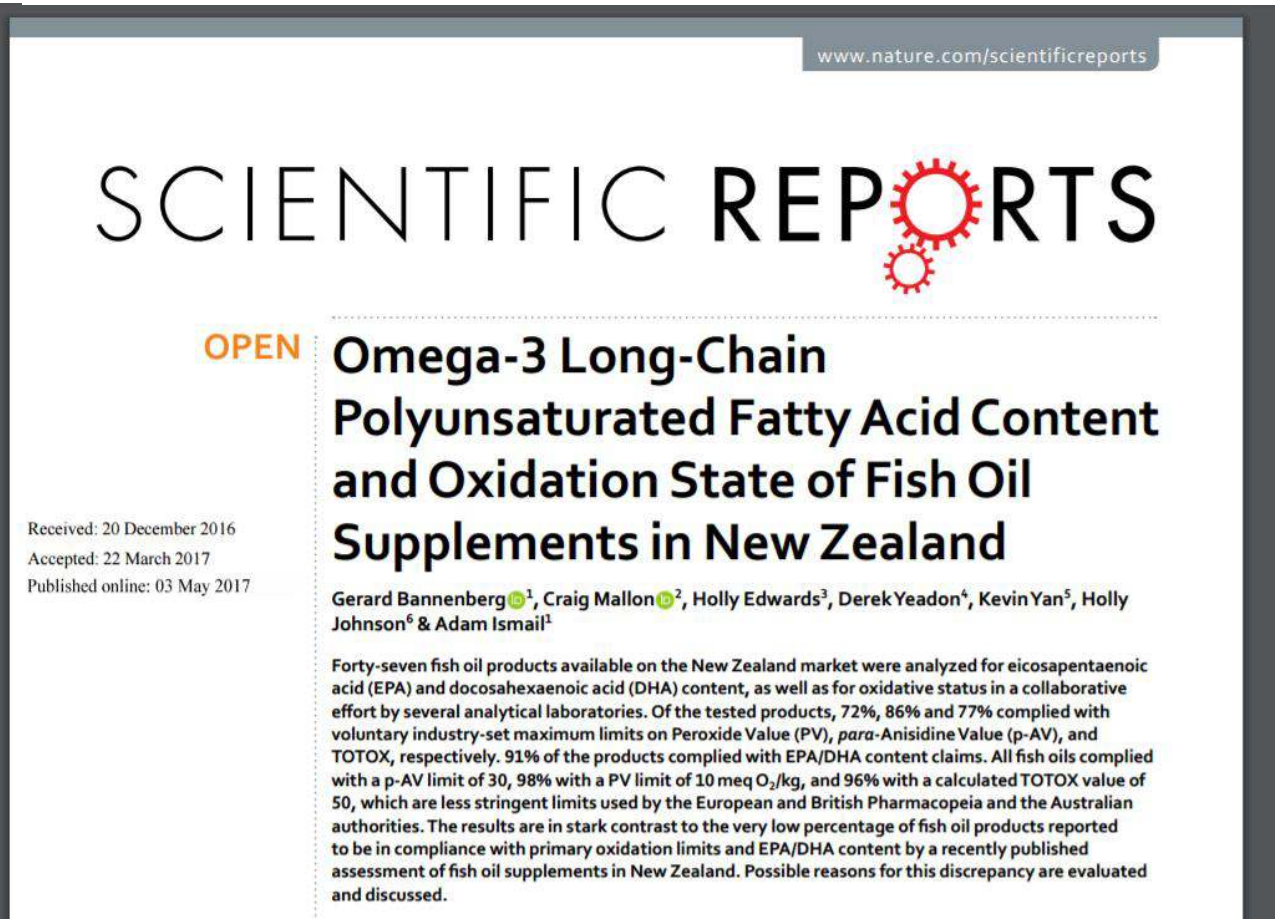
Fish oil supplements in New Zealand are highly oxidised and do not meet label content of n-3 PUFA

Benjamin B. Albert[1], José G. B. Derraik[1], David Cameron-Smith[1], Paul L. Hofman[1], Sergey Tumanov[2], Silas G. Villas-Boas[2], Manohar L. Garg[3] & Wayne S. Cutfield[1]

[1]Liggins Institute, University of Auckland, Auckland, New Zealand, [2]Centre for Microbial Innovation, School of Biological Sciences, University of Auckland, Auckland, New Zealand, [3]Nutraceuticals Research Group, University of Newcastle, Callaghan, New South Wales, Australia.

We evaluated the quality and content of fish oil supplements in New Zealand. All encapsulated fish oil supplements marketed in New Zealand were eligible for inclusion. Fatty acid content was measured by gas chromatography. Peroxide values (PV) and anisidine values (AV) were measured, and total oxidation values (Totox) calculated. Only 3 of 32 fish oil supplements contained quantities of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that were equal or higher than labelled content, with most products tested (69%) containing <67%. The vast majority of supplements exceeded recommended levels of oxidation markers. 83% products exceeded the recommended PV levels, 25% exceeded AV thresholds, and 50% exceeded recommended Totox levels. Only 8% met the international recommendations, not exceeding any of these indices. Almost all fish oil supplements available in the New Zealand market contain concentrations of EPA and DHA considerably lower than claimed by labels. Importantly, the majority of supplements tested exceeded the recommended indices of oxidative markers. Surprisingly, best-before date, cost, country of origin, and exclusivity were all poor markers of supplement quality.

GOED response paper - 2017



www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Received: 20 December 2016
Accepted: 22 March 2017
Published online: 03 May 2017

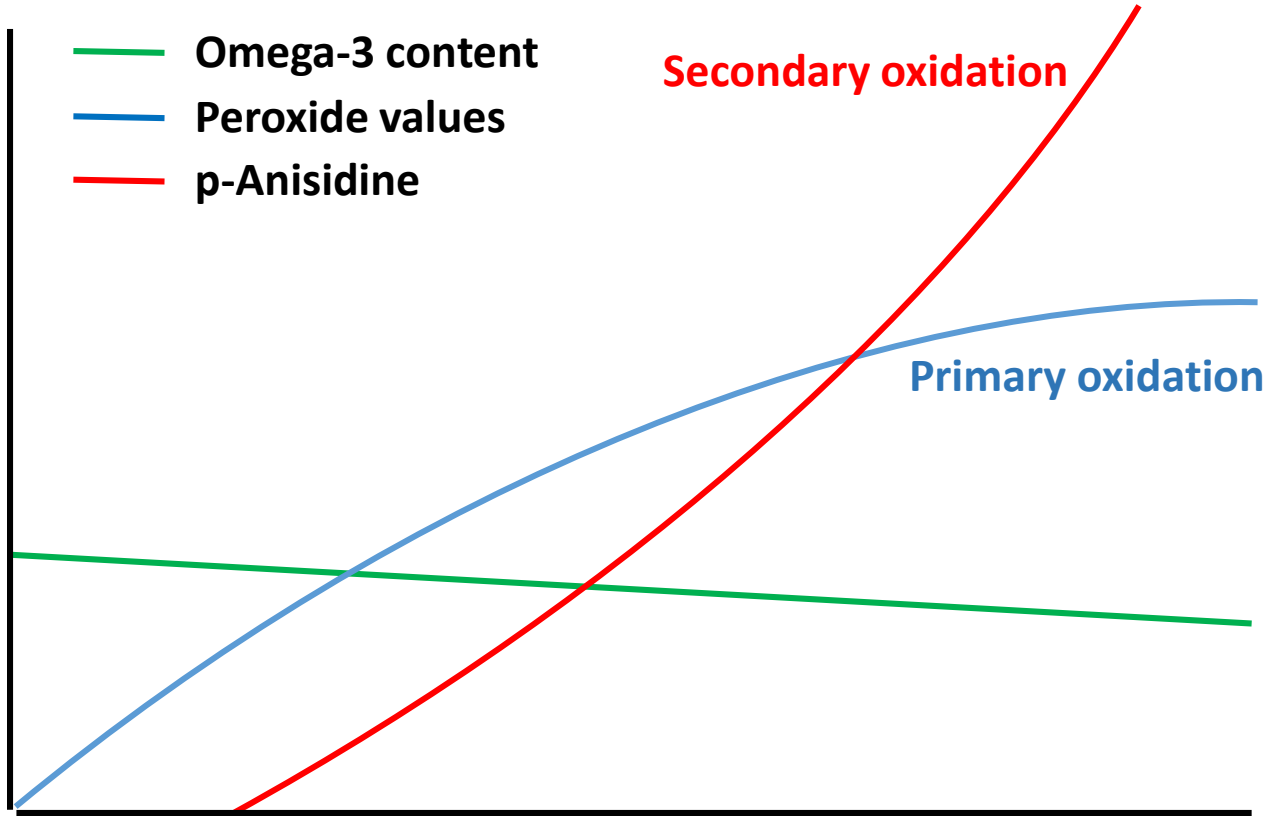
Omega-3 Long-Chain Polyunsaturated Fatty Acid Content and Oxidation State of Fish Oil Supplements in New Zealand

Gerard Bannenberg[1], Craig Mallon[2], Holly Edwards[3], Derek Yeadon[4], Kevin Yan[5], Holly Johnson[6] & Adam Ismail[1]

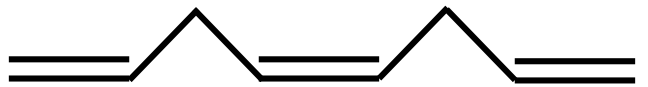
Forty-seven fish oil products available on the New Zealand market were analyzed for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content, as well as for oxidative status in a collaborative effort by several analytical laboratories. Of the tested products, 72%, 86% and 77% complied with voluntary industry-set maximum limits on Peroxide Value (PV), *para*-Anisidine Value (p-AV), and TOTOX, respectively. 91% of the products complied with EPA/DHA content claims. All fish oils complied with a p-AV limit of 30, 98% with a PV limit of 10 meq O_2/kg, and 96% with a calculated TOTOX value of 50, which are less stringent limits used by the European and British Pharmacopeia and the Australian authorities. The results are in stark contrast to the very low percentage of fish oil products reported to be in compliance with primary oxidation limits and EPA/DHA content by a recently published assessment of fish oil supplements in New Zealand. Possible reasons for this discrepancy are evaluated and discussed.

Oxidation in fish oils

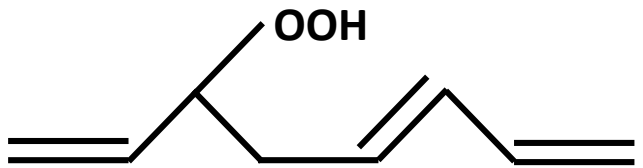
How oxidation happens



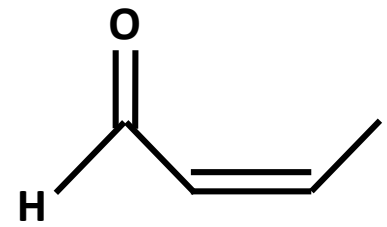
Fresh



Primary oxidation products



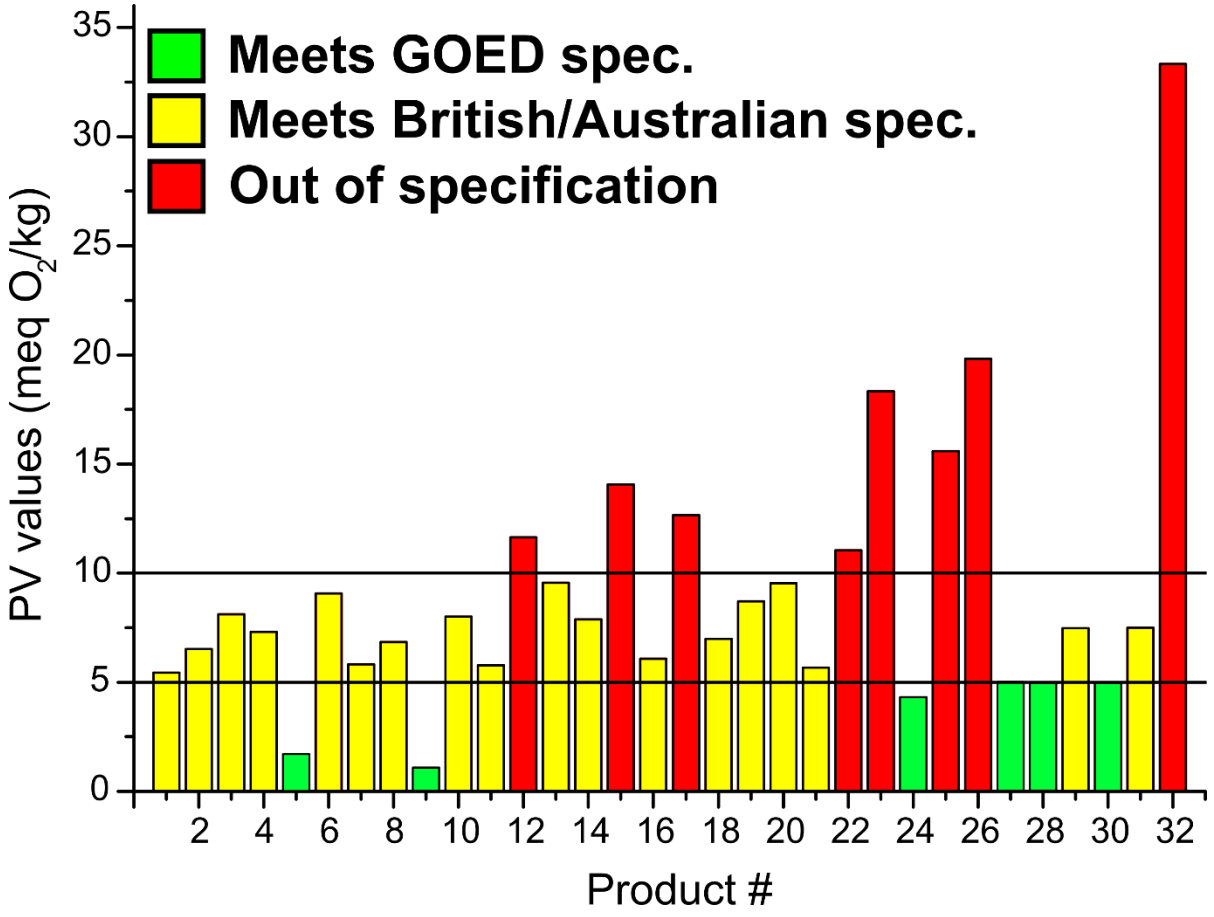
Secondary oxidation products



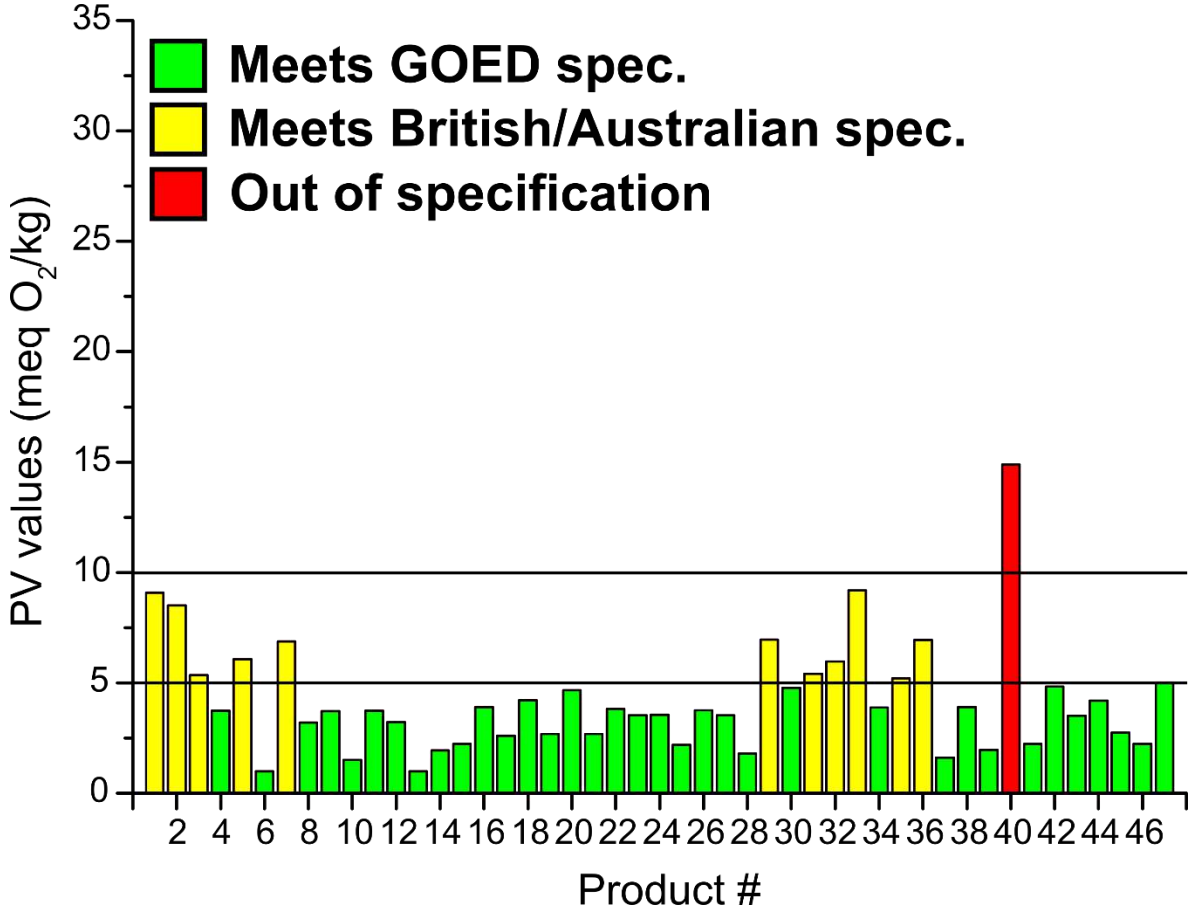
Volatile and bad odour

Primary oxidation results: PV

Liggins Institute paper - 2015

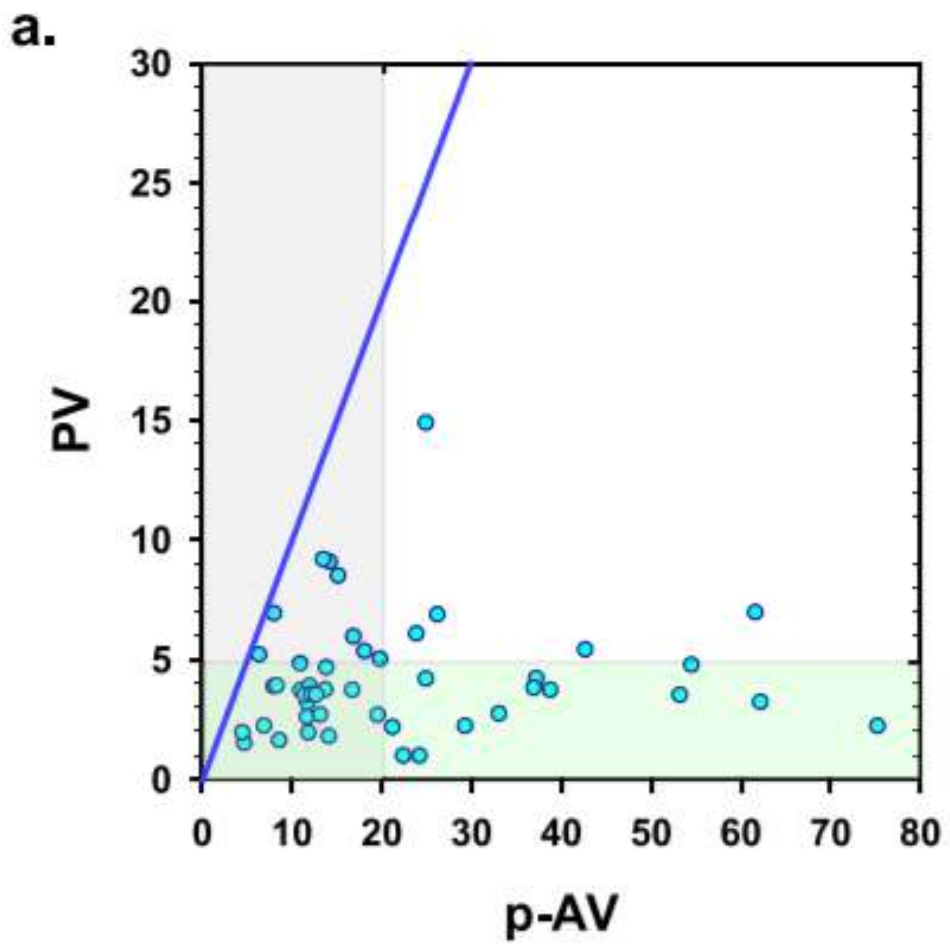


GOED response paper - 2017

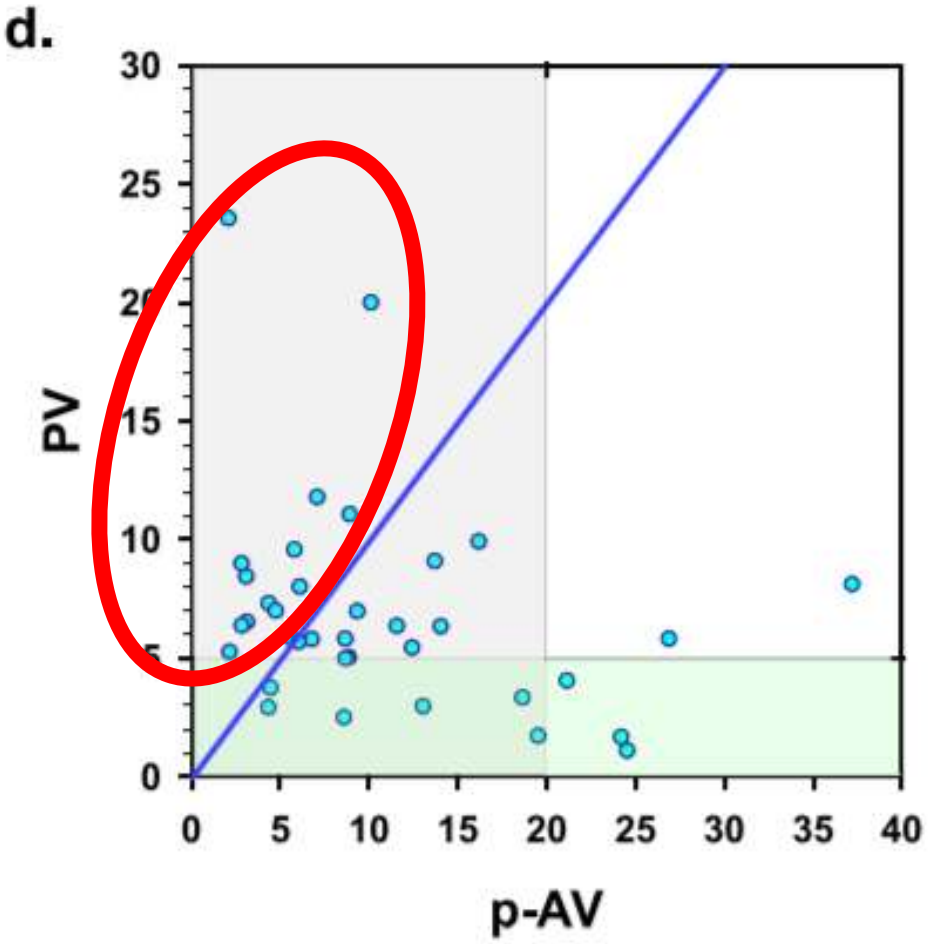


Mishandled samples?

GOED response paper - 2017



Liggins institute paper - 2015



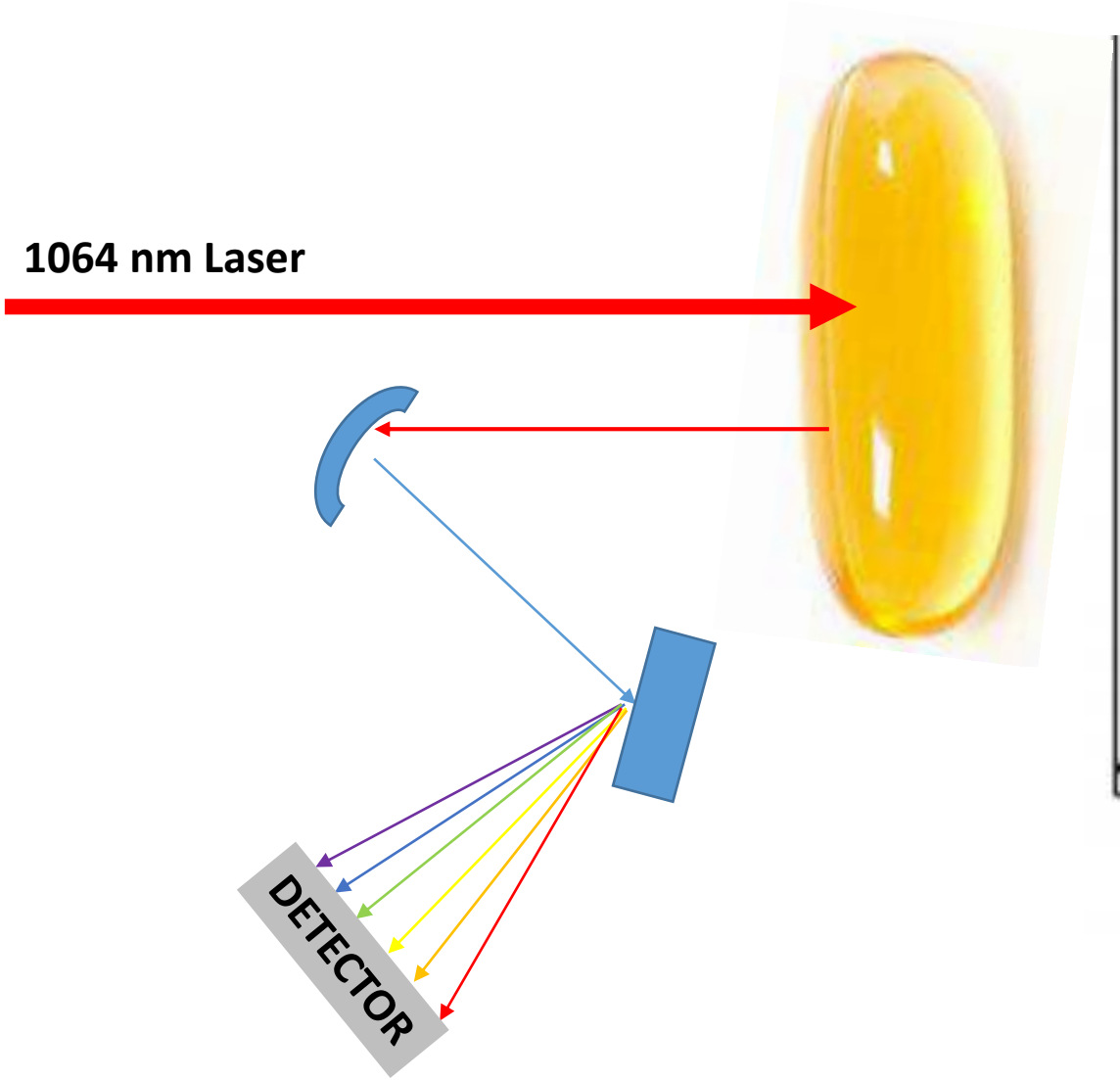
Bannenberg *et al.* **(2017)**. *Sci. Reports,* DOI:10.1038/s41598-017-01470-4

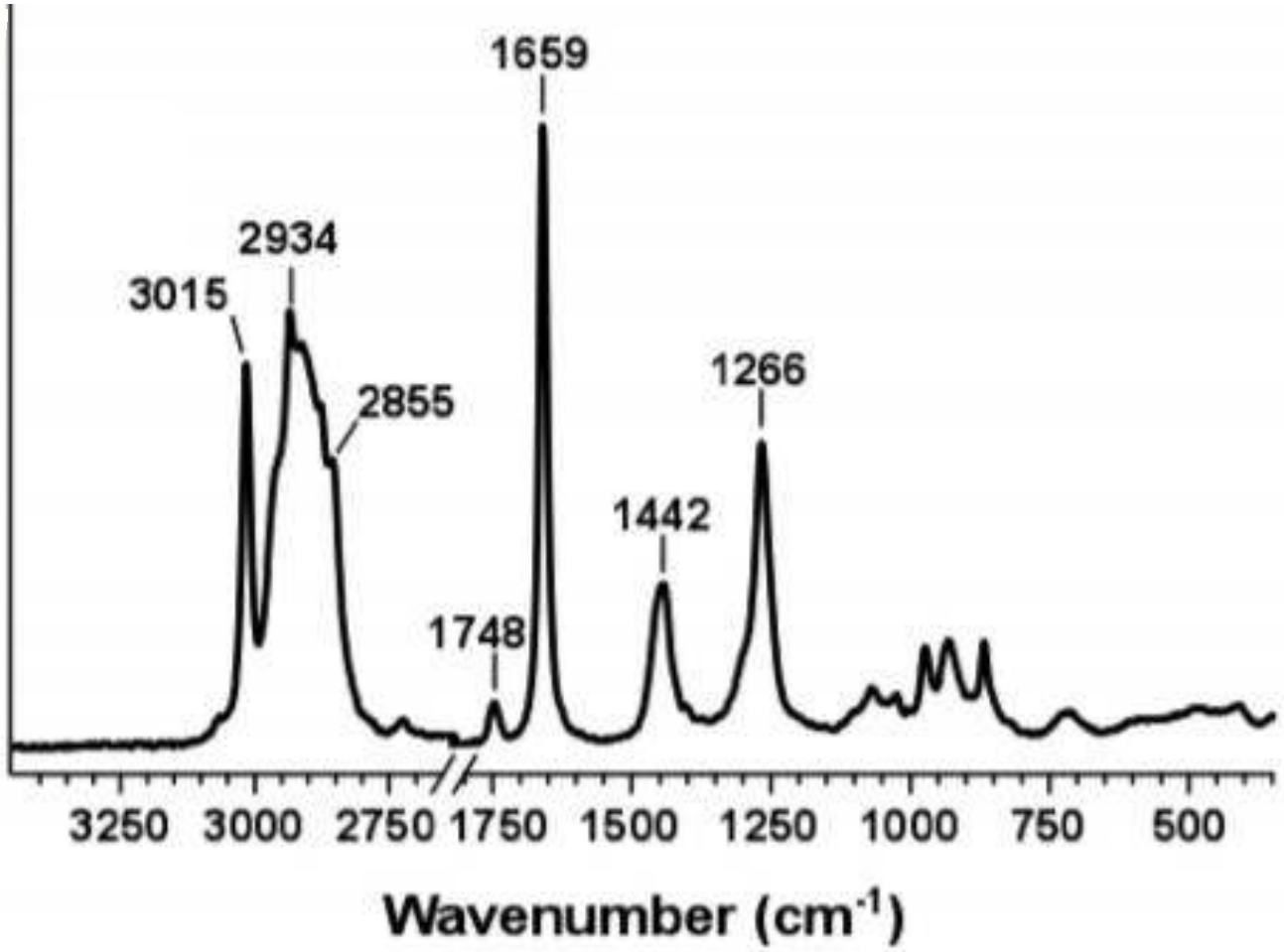
Portable analytical tools

Near infrared spectroscopy

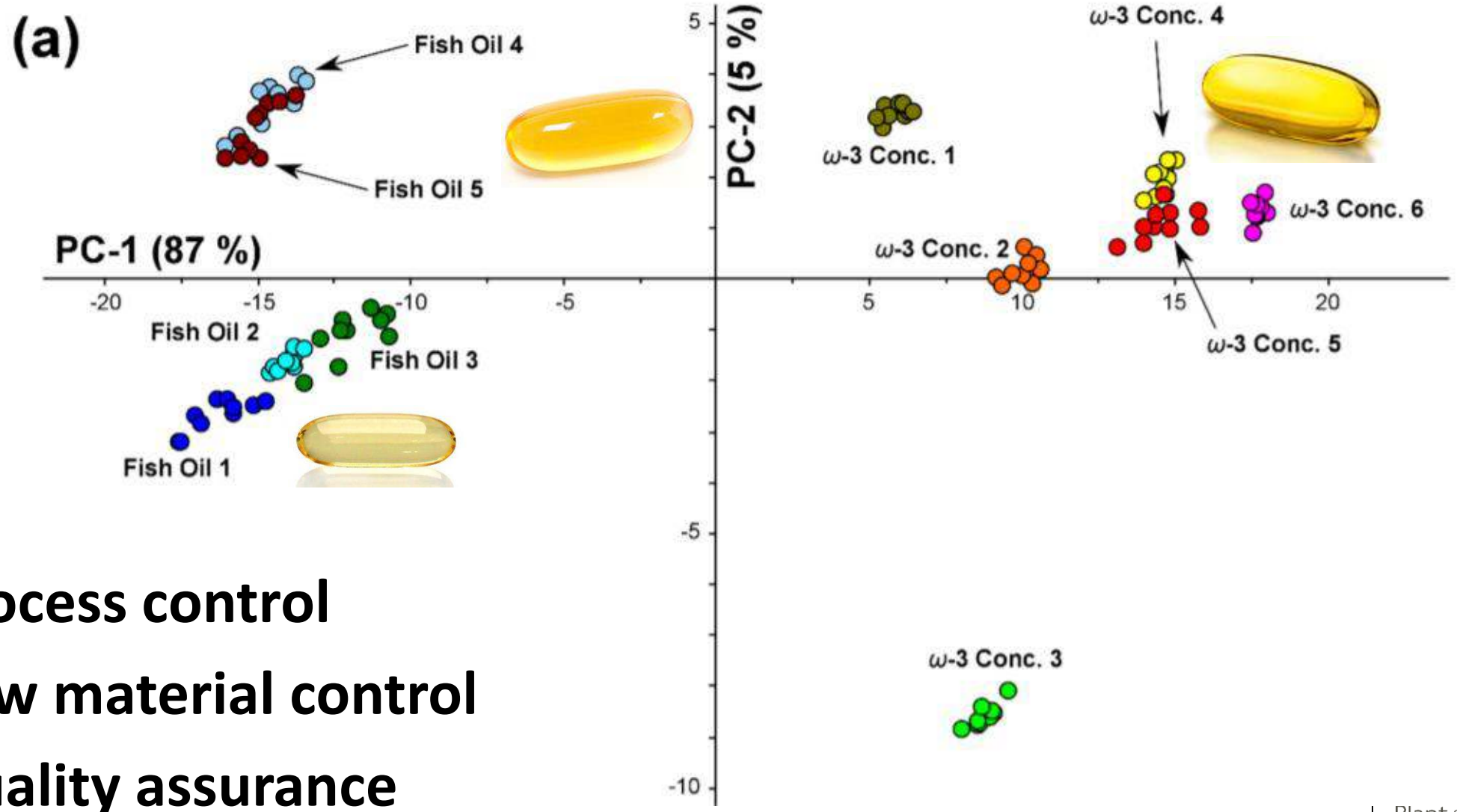
Raman spectroscopy

Raman spectroscopy of fish oil capsules





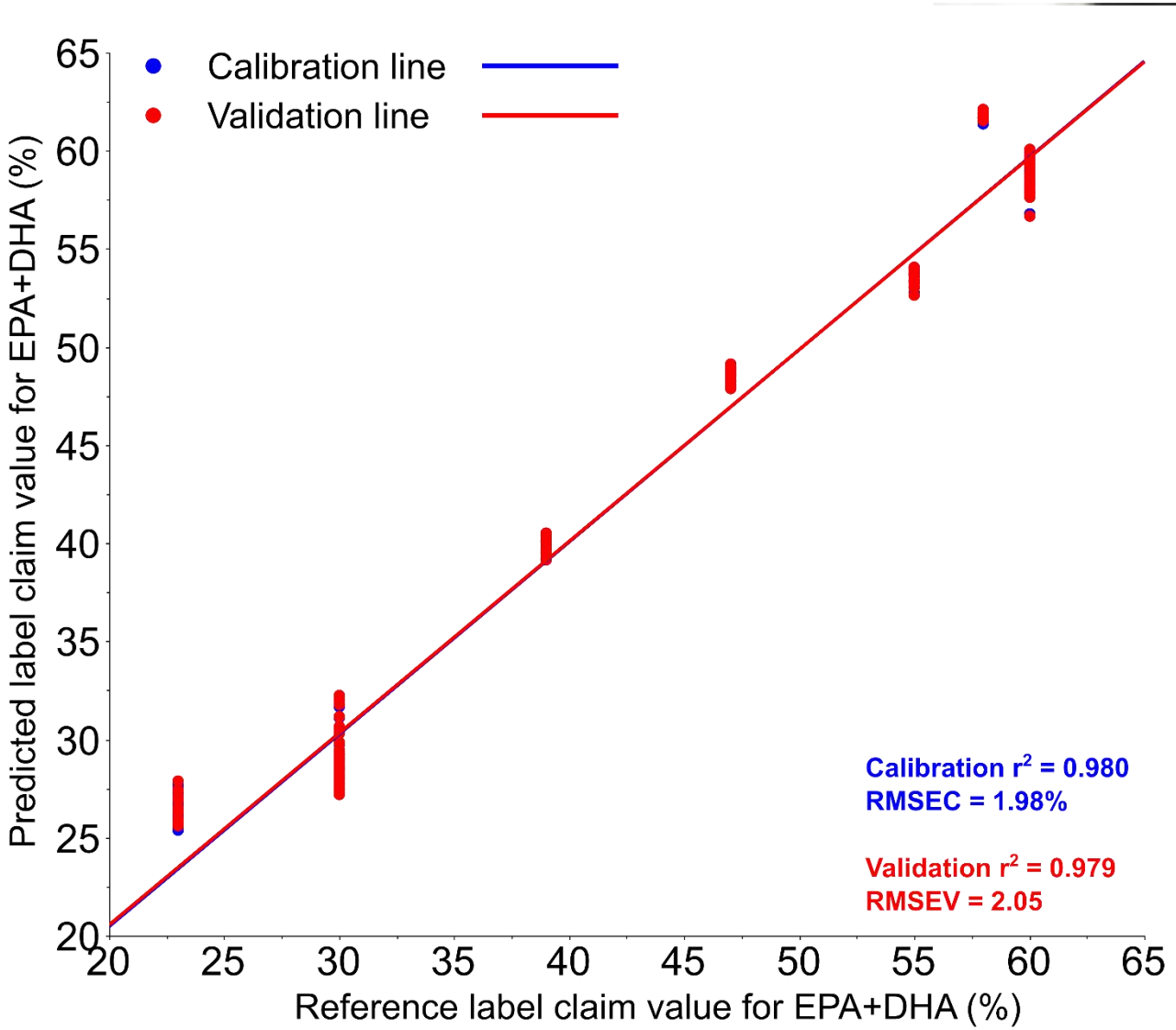
Raman spectroscopy for classification (PCA)

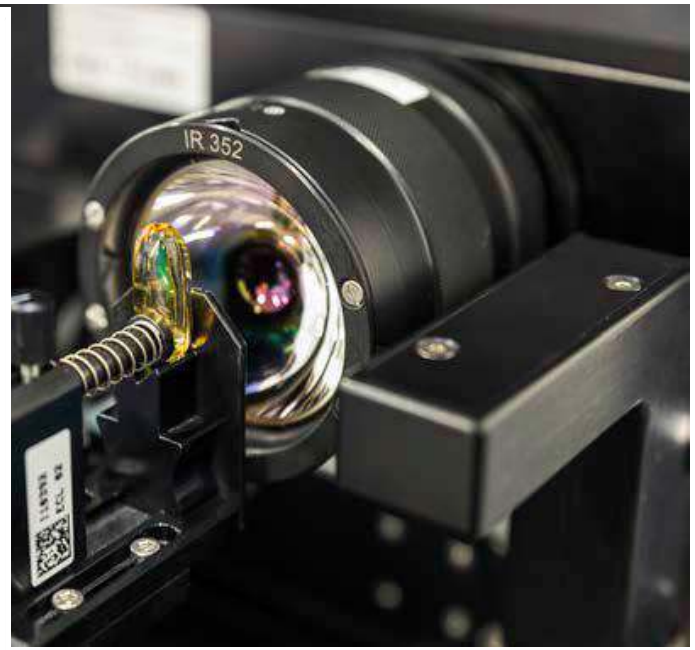


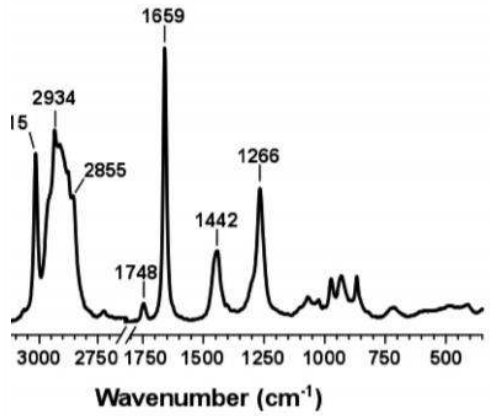
- **Process control**
- **Raw material control**
- **Quality assurance**

Killeen, Marshall, Burgess, Gordon & Perry **(2017)**. *J. Agric. Food Chem., 65*(17), 3551-3558.

Quantitative Raman spectroscopy







Raman spectroscopy for omega-3 quantitation

Table 1. Label Claim EPA and DHA Concentrations in Eleven Commercial Encapsulated ω-3 Oil Supplements Plus Determinations by GC–MS Analysis of Fatty Acid Methyl Esters and Noninvasive FT–Raman Spectroscopy of Encapsulated Oils

	label claim (%)			GC–MS determination (% of total area, $n = 2$)			FT–Raman/PLSR prediction (% of total area, average of $n = 10 \pm$ SD)		
sample	EPA	DHA	EPA+DHA	EPA	DHA	EPA+DHA	EPA	DHA	EPA+DHA
fish oil 1	14	9	23	14.2	8.9	23.1	16.1 ± 1.2	7.5 ± 1.1	24.8 ± 1.3
fish oil 2	18	12	30	17.9	9.5	27.4	16.0 ± 1.0	10.1 ± 1.0	27.7 ± 0.4
fish oil 3	18	12	30	18.3	10.0	28.3	18.1 ± 0.9	10.5 ± 0.8	30.5 ± 1.3
fish oil 4	18	12	30	18.8	10.2	29.0	18.1 ± 0.6	11.0 ± 1.0	27.1 ± 1.1
fish oil 5	18	12	30	18.3	10.7	29.1	18.4 ± 0.9	10.5 ± 1.1	26.6 ± 0.9
ω-3 concentrate 1	16	31	47	20.9	32.7	53.7	23.6 ± 1.0	31.8 ± 0.8	54.1 ± 0.5
ω-3 concentrate 2	33	23	55	34.7	22.0	56.6	32.8 ± 1.4	24.0 ± 0.8	59.5 ± 0.6
ω-3 concentrate 3[a]	36	24	60	31.0	20.1	51.1			
ω-3 concentrate 3[b]	36	24	60	34.3	22.5	56.9	34.6 ± 0.9	22.5 ± 0.7	57.7 ± 0.6
ω-3 concentrate 4	36	24	60	39.7	25.6	65.3	41.7 ± 1.4	24.7 ± 0.6	65.5 ± 0.4
ω-3 concentrate 5	36	24	60	41.7	27.8	69.4	40.0 ± 1.9	26.6 ± 1.0	65.3 ± 1.1
ω-3 concentrate 6	39	19	58	45.4	24.2	69.5	44.8 ± 0.8	25.2 ± 1.1	69.7 ± 0.2

[a]High in EEs and residual EEs in GC–MS. [b]Results from longer trans-methylation treatment; no EEs were detected in GC–MS.

Killeen, Marshall, Burgess, Gordon & Perry **(2017)**. *J. Agric. Food Chem., 65*(17), 3551-3558.

Raman spectroscopy for in-capsule oxidation?

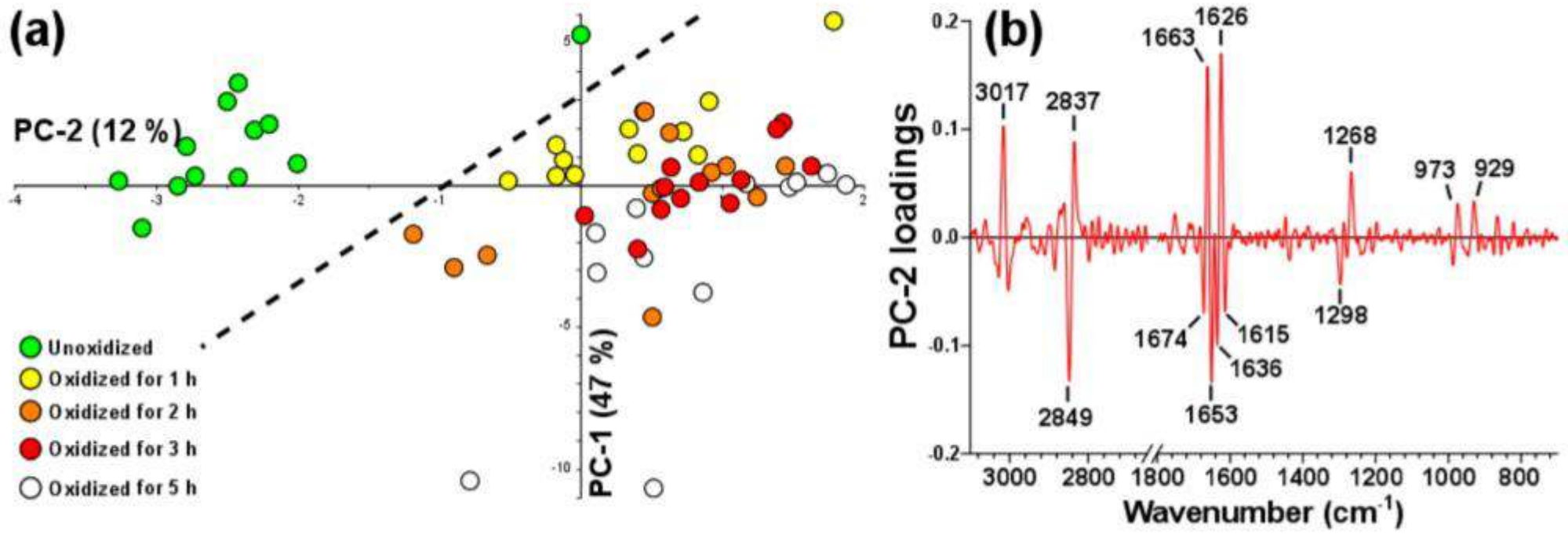


Figure 5. Principal component analysis of Raman spectra of oxidized fish oil samples (see Table 3): (a) scores plot and (b) loadings plot for PC-2.

Killeen, Marshall, Burgess, Gordon & Perry **(2017)**. *J. Agric. Food Chem., 65*(17), 3551-3558.

Cost effective? In process? In factory?