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DESIGNING NOVEL HARDSTOKS BY ENZYMATIC INTERESTERIFICATION

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DESIGNING NOVEL HARDSTOKS BY ENZYMATIC INTERESTERIFICATION

"Optimized" edible oil processing is required to meet "new oil" quality parameters

OIL QUALITY

"STANDARDS" → **OIL CONCERNS**

"NEW"

Organoleptic properties/oxidative stability

- Bland taste, odourless
- Light and brilliant colour
- Good oxidation parameters (PV and An-V)
- Good shelf life
- Good cold stability

HEALTH CONCERNS

Nutritional qualities

- Balanced FA composition
- High vitamin content (tocos …)
- Low or zero trans FA
- No contaminants
- No fat degradation products

ENVIRONMENTAL ISSUES

- No effluent; no waste
- No chemicals; no solvent
- Low risk
- Lower oil losses

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DESIGNING NOVEL HARDSTOKS BY ENZYMATIC INTERESTERIFICATION

Low or zero *trans* FA

- Negative health effects of *trans* FA and *un-natural cis* isomers are clearly identified

  (adverse effects on plasma lipoprotein levels, inhibitory effect on prostaglandin synthesis ….)

- Stricter labelling and legislations regarding to max. *trans* FA in fat based foods

- Objective is: - Low *trans* products: < 5% on fat basis
  - Zero *trans* products: < 0.5 % on fat basis

IMPROVED/MODIFIED EDIBLE OIL PROCESSING

- Necessity for optimized refining conditions (deodorization/de-acidification):
  - lower temperature / shorter time / higher vacuum

- Alternatives of partial hydrogenation:
  - dry fractionation, interesterification (CIE or EIE), full hydrogenation

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CHEMICAL versus ENZYMATIC INTERESTERIFICATION

CIE

PRO:

- Highly reproducible and cost effective process.
- Easily operated as batch -> “flexible” for frequent feed changes (large number of small batches - relatively low cross-contamination).

CON:

- Hazardous chemical catalyst (sodium methoxide) : safe handling is required.
- Relatively high temperature processing.
- Side reactions require proper post-treatment (post-bleaching/deodorization).
- Risk of flavour reversion and reduced oxidative stability (anti-oxidant losses).
- Relatively high oil losses.

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CHEMICAL versus ENZYMATIC INTERESTERIFICATION

EIE

PRO:

- Cost effective when running continuously on bulk fats.
- Simple, clean and safe process (low temperature, “bio-catalyst”).
- No side reactions, no post-bleaching (less oil losses).
- Better quality of the EIE processed fat.
- Lower capital investment cost compared to CIE.

CON:

- Enzyme sensitivity: importance of “purity” of the feed oil.
- Less easy if frequent feed changes (cross contamination).

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CHEMICAL versus ENZYMATIC INTERESTERIFICATION

Chemical interesterification

Pretreatment of oil → Reaction Catalyst: NaOCH3 → Deactivation water / acid → Post-bleaching → Deodorization

Pref. Batch Process

Enzymatic interesterification

Pretreatment of oil → Reaction Catalyst: Lipase → Deodorization

Pref. Continuous process

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ENZYMATIC INTERESTERIFICATION

- **Stepped -up interest for EIE:**

- The production of low or zero *trans* margarines, shortenings and fat based food.
- The design of other innovative food fats.

- **Enzymatic processing includes:**

- **Reactions of an ester with another ester (ester-ester exchange).**
- **Reactions of an ester with a fatty acid (acidolysis).**
- **Reactions of an ester with an alcohol (alcoholysis).**
- **Reactions of an ester with glycerol (glycerolysis).**

- **The ester-ester exchange modifies the physico-chemical properties of fats by rearranging the distribution of the fatty acids on the glycerol backbone.**
This can be done *randomly* or *regio-specifically*, depending on the used enzyme.

- **Random EIE is applied:**

- To modify the overall melting profile of a blend.
- To enhance the compatibility in the solid state of selected fats.
- To improve the plasticity of the fat blends.
- To combine properties of mixed oils.

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RANDOM ENZYMATIC INTERESTERIFICATION

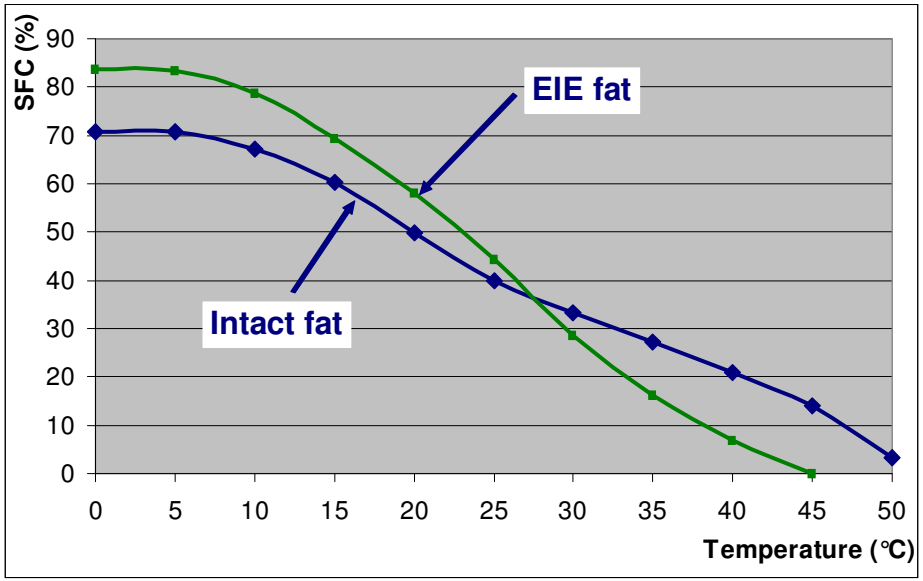
NON-SELECTIVE ENZYME: Lipozyme TL IM, a lipase from *Thermomyces lanuginosus* (Novozymes)

Re-arrangement of FA over the glycerol backbone according to law of probability

ACA + BBB → RANDOM → AAA, BBB, CCC + ABA, BBB, BAC + AAB, BBC, ABA, ACA + BBC, BBA, BAB, BCB + CCA, CCB, CAC, CBC

A, B and C: FA on glycerol

Solid fat Content Profile (SFC) by p-NMR



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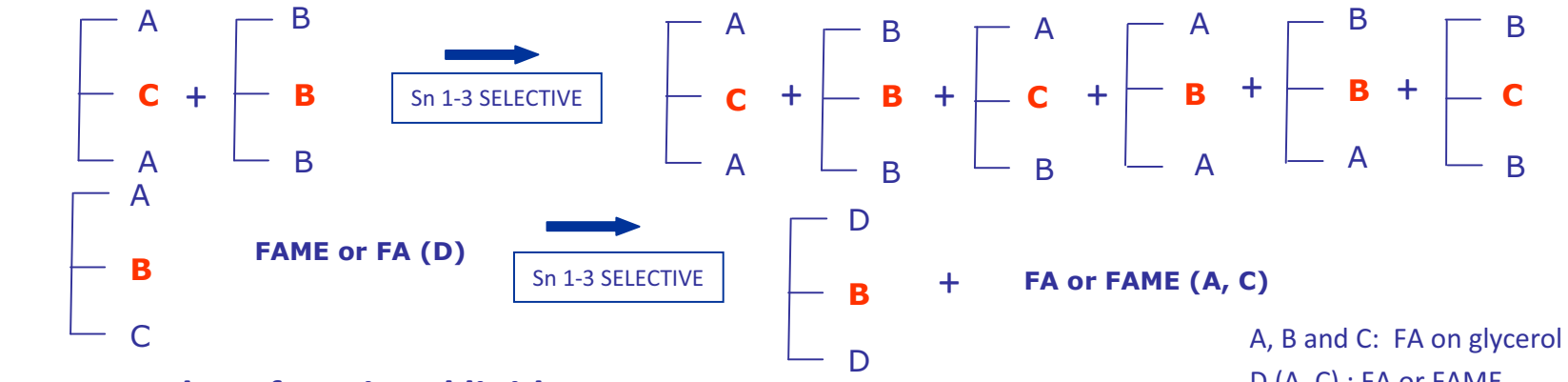
REGIO-SELECTIVE ENZYMATIC INTERESTERIFICATION

REGIO-SELECTIVE ENZYME: Lipozyme RM IM, a lipase from *Rhizomucor miehei* (Novozymes)

Sn 1-3 regio-selective re-arrangement of FA over glycerol backbone

Ester-ester exchange/acidolysis



A, B and C: FA on glycerol

D (A, C) : FA or FAME

- **To produce <u>functional lipids</u>:**

- High StUSt Fats (CBE).

- High OPO Fats (Human Milk-like Fat).

- Anti Fat Bloom Fats etc.

- **Combined with other modification technologies to improve compositional properties.**

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RANDOM ENZYMATIC INTERESTERIFICATION

Quality parameters of the feed oil in order to ensure a sufficient lifetime to the enzyme

FFA: < 0.1%	
Moisture: 0.02 %min.	
Moisture and impurities: 0.1% max.	
Soaps: 1 ppm max.	AnV: < 5
P: < 3 ppm	PV: < 1 meqO_2/kg
Fe: < 0.1 ppm	pH of water extract : 6-9
Ni: < 0.2 ppm	Citric acid: < 25 ppm
Cu: < 0.01 ppm	

Novozymes specifications

For high, long and consistent enzyme activity, fully refined (RBD) oils are preferred.

High attention with respect to:

- **Oxidation parameters, that must be kept as low as possible.**
- **"Acidity" of the oil in terms of pH of water extract: > 6 and < 9.**

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RANDOM ENZYMATIC INTERESTERIFICATION

- **Oxidation parameters**: covered by Good Manufacturing Practices
  -> freshly refined oil and/or adequate storage.
- **pH of water extract**: sensitivity of the enzyme in relation to oil "acidity"

[residual acids from refining: citric acid, phosphoric acid, some acids released from ABE …]

Lab-scale procedure for testing oil "acidity":
Water washing of the oil at a 3/1 oil/water ratio.
30 gr of oil, 70°C, high shear mixing, 30 second.
Distilled water with neutral pH.
Centrifugation.
pH of the water phase (water extract).

Novozymes procedure

- **MBA tests** (performance test in multi-batch reaction)

Lab-scale procedure used to evaluate the performance of the immobilized enzyme by statistical analysis of
1) the average production rate (kg EIE oil/kg enzyme.hr),
2) the volume-based half-time V1/2 (kg EIE oil/kg enzyme),
3) the initial reaction rate k-0 (1/hr).
Based on SFC determination by p-NMR of EIE oil as function of time.

Novozymes procedure

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RANDOM ENZYMATIC INTERESTERIFICATION

MBA tests on the same oil with modified peroxide values and pH of water extract

Combination of low/high peroxide value and low/good pH of water extract

PV meqO_2/kg	pH	Av. Prod. (kg/kg.h)	V1/2 (kg/kg)
0.90	4.2	1.38	909
0.90	6.4	10.43	2214
8.14	4.8	0.42	316
7.85	6.2	1.65	481

Novozymes Data

Good oxidation parameters (PV < 1meqO_2/kg) combined with low oil "acidity" (pH of water extract ~6) significantly improve MBA numbers and give assurance of will longer enzyme activity.

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RANDOM ENZYMATIC INTERESTERIFICATION

- **Enzyme productivity (kg EIE oil/kg enzyme) (enzyme consumption):**

  Largely depnds on feedstock quality:

  Oxidation parameters

  Oil “acidity” (pH of water extract)

  Must be high in order to keep operating cost competitive.

  Productivity: ~ 2.5 ton EIE oil/kg enzyme.

- **Enzyme activity - flow rate (kg EIE oil/kg enzyme.hr):**

  Continuous process.

  Constant but rather slow flow rate : 1-2 kg EIE oil/kg enzyme.hr.

  “ Slow converting”oils: lauric fats (palm kernel, coconut).

- **Cross contamination:**

  Between batches of different oils.

  Oil losses due to cross contamination estimated at ~ 3.5 * the quantity of enzyme in the reactors.

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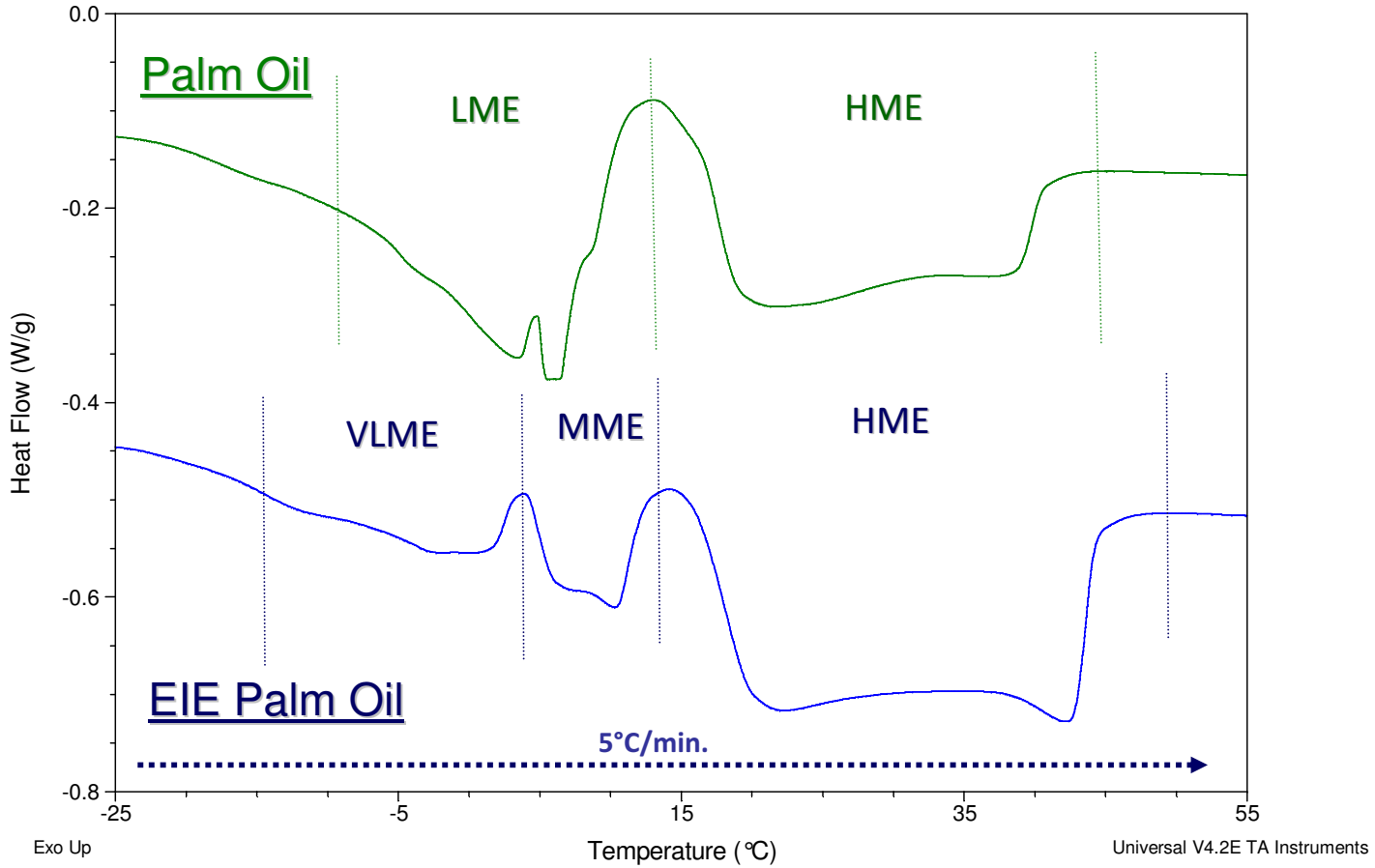
RANDOM ENZYMATIC INTERESTERIFICATION

DSC (differential scanning calorimetry) melting profiles of Palm Oil an EIE Palm Oil



De Clercq N. et al., submitted to JAOCS

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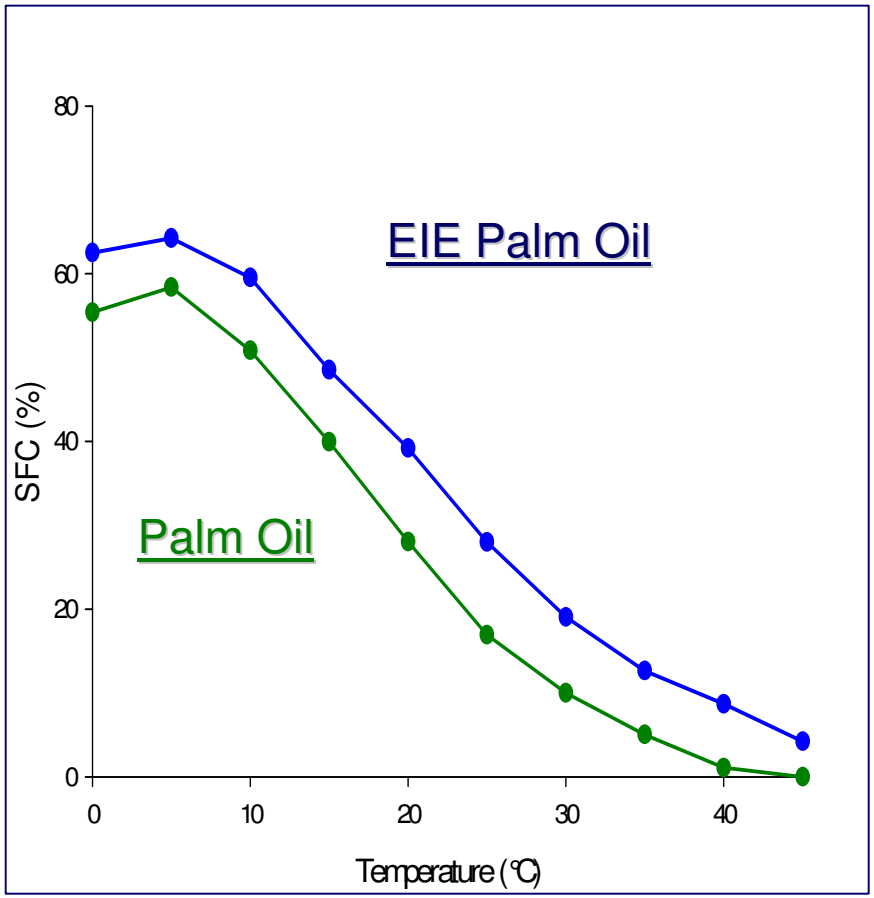
RANDOM ENZYMATIC INTERESTERIFICATION

SFC profiles of Palm Oil and EIE Palm Oil



Composition of Palm Oil and EIE Palm Oil

[St: saturated FA; U: unsaturated FA on TAGs]

Palm Oil

- StStSt: 7%
- StUSt: 46 %
- StUU: 40%
- UUU: 7%

EIE Palm Oil

- StStSt: 12% (+++)
- StUSt: 38 % (- -)
- StUU : 37 % (-)
- UUU: 12% (+++)

De Clercq N. et al., submitted to JAOCS

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RANDOM ENZYMATIC INTERESTERIFICATION

Calculation of the degree of interesterification during batch operations: D.I.

D.I. calculated from TAG composition (HPLC):

D.I. (%) = 100 * [(A/B) $_{BL}$ – (A/B) $_{EIE}$] / [(A/B) $_{BL}$ – (A/B) $_{Rand}$]

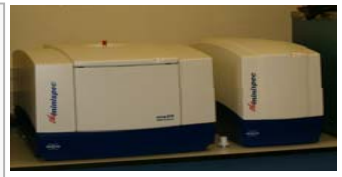
A and **B** are the TAGs decreasing and increasing the most during batch EIE

BL is the initial TAG composition of the fat blend

EIE is the TAG composition of enzymatically interesterified

Rand is the theoretical random TAG calculation

D.I. calculated from SFC profile (p-NMR):



D.I. (%) = 100 * [(SFCt0 – SFCtx) / (SFCt0 – SFCt∞)] -> f (temperature)

SFC t0 is the SFC at time 0 (BL)

SFC tx is the SFC at EIE batch reaction time

SFC t∞ is the SFC at the equilibrium stage (CIE)

SFC profile by p-NMR: the best tool to evaluate the D.I. during the EIE reaction

Calculation of the D.I. from TAG composition is questionnable mainly because positionnal isomers (formation of SSU at the expense of SUS) can not be quantified by the classical reverse-phase HPLC method.

De Clercq N. et al., submitted to JAOCS