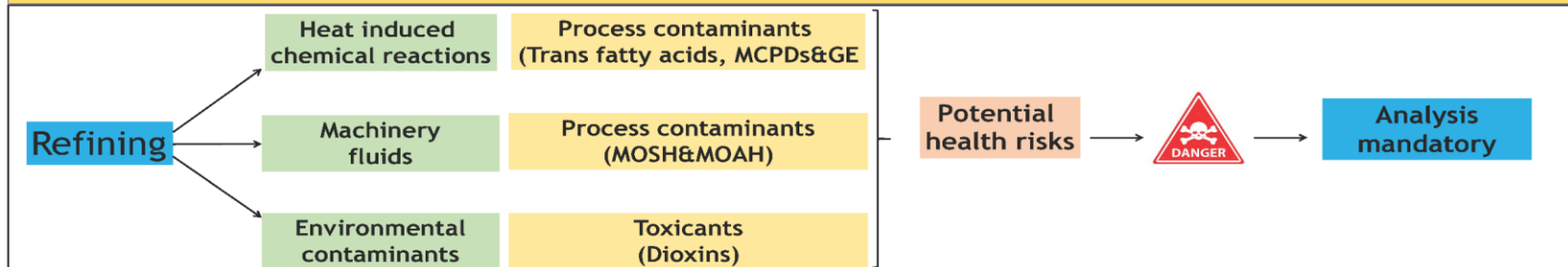# Detection and Quantification of Process Contaminants and Toxins in Rice Bran Oil

Objectives: To establish (1) Trans fat analysis by GC-FID
(2) MCPDs & GE analysis by GC-MSMS
(3) MOSH & MOAH analysis by column chromatography/HPLC-UV-GC-FID
(4) Dioxins analysis by GC-MSMS

Rice Bran Oil- Process Contaminants and Toxins

Refining →
- Heat induced chemical reactions → Process contaminants (Trans fatty acids, MCPDs&GE)
- Machinery fluids → Process contaminants (MOSH&MOAH)
- Environmental contaminants → Toxicants (Dioxins)

Process contaminants → Potential health risks → DANGER → Analysis mandatory

Trans fatty acids

Before Hydrogenation — linoleic, linoleic, oleic
Partial Hydrogenation
After Hydrogenation — stearic, elaidic, oleic; stearic, oleic, oleic

LIMITS — TFAs-NMT 2%

MCPDs & GE

Mono(acyl)glycerides / Di(acyl)glycerides / Tri(acyl)glycerides
> 200 °C, −H₂O/-FA
> 200 °C, + HCl*, −H₂O/-FA
"natural sources e.g. FeCl₃, chlorinated phytising/ungoxides*"
bound glycidol — Glycidyl fatty acid esters — mono esters — e.g. Glycidylpalmitate
bound MCPD — 2- & 3-MCPD fatty acid esters — 1-/2- mono esters & 1,2-/1,3- di esters — e.g. 3-MCPD-1,2-bis-palmitoyl ester

3-MCPD-2500 µg/kg , GE-1000 µg/kg

MOSH & MOAH

MOSH   MOAH

MOAH-2 mg/kg

Dioxins

Polychlorinated dibenzo-p-dioxins (PCDDs) → 7 toxic congeners
Polychlorinated dibenzo furans (PCDFs) → 10 toxic congeners
Dioxin like Polychlorinated biphenyls(dl-PCBs) → 12 dl-PCBs

0.75 pg/g fat

Detection & Quantification of Process Contaminants & Toxins

Trans fatty acids-GC analysis

Seeds &Nuts, margarine & spreads, Food ready to eat, Milk &milk products, Cheese
Soxhlet Extraction, Solvent Extraction, Acid Digestion, Base Digestion, Acid & Base Digestion → Crude Fat
Fatty Acid Methyl Esters (Fatty Acids & Trans Fatty Acids):
Accurate Measurement of samples — Methylation Process — Separation of aqueous and solvent phase — Analysis of FAME by GC
GC-FID chromatogram of Rice Bran Oil for Trans Fatty Acid Estimation

MCPDs & GE-GC-MSMS analysis

Ester cleavage — acidic/alkaline: 3-16h
2-MCPD, 3-MCPD, 3-MCPD or 3-MXPD, glycidol transformation product
Matrix removal (liquid) extraction)
Derivatisation (e.g. HFBA/Acetone/PBA)
GC-MSMS
3-MCPD, 2-MCPD, glycidol as 3-MXPD
Chromatogram displays up to 3 core analytes, LOQ: 0.05 mg/kg
AOCS Cd 29a-13   AOCS Cd 29b-13   AOCS Cd 29c-13
Quantitative Analysis Compound Report

MOSH & MOAH-column/HPLC-GC-FID analysis

Samples to be analysed — Samples Measurement and extraction into solvent — Preparative HPLC system for the collection of MOSH & MOAH fractions
Quantitative determination of hydrocarbons attributed to mineral oils — GC-FID system for the peak separation & identification
GC-FID chromatogram of mix standard (C₇ - C₄₀ n-alkane)
GC-FID chromatogram of Rice Bran Oil mineral oil hydrocarbons

Dioxins-GC-MSMS analysis

Sample Preparation
Advantages over manual system: Low time requirement, Column packing inaccuracies are minimized, Lower solvent requirement
Edible oil — Critical step — Step-1 Cleanup — FMS EZPrep — Step-2 Concentration — Nitrogen evaporator
Fractions are evaporated to dryness using nitrogen evaporator to be concentrated to the range of 25-200 µL to achieve typical analytical levels.
Analyzed using GC-MS/MS

Conclusions
We have established and validated methods to analyse the process contaminants and toxins in rice bran oil. This way, we are helping rice bran oil industry to modify their process to reduce the contaminants and toxins to minimal levels. So that, rice bran oil industry could produce safe and quality rice bran oil for consumers.