### Enzyme Assisted Chemical Refining of Vegetable Oils

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**Shirdi Sai Nutraceuticals Pvt Ltd**

**Bengaluru**

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#### Refining Techniques: Chemical vs Physical

<table>
<thead>
<tr>
<th>Chemical Refining</th>
<th>Physical Refining</th>
</tr>
</thead>
<tbody>
<tr>
<td>More tolerant to quality of crude oil</td>
<td>Sensitive to quality of crude oil-solutions in place</td>
</tr>
<tr>
<td>Very good shelf stability</td>
<td>Very good shelf stability-extra care needed</td>
</tr>
<tr>
<td>Very low color</td>
<td>Extra cost to produce low color</td>
</tr>
<tr>
<td>Higher price of fatty acid distillate</td>
<td>Lower price of fatty acid distillate</td>
</tr>
<tr>
<td>Higher price of fatty acid distillate</td>
<td>Lower price of fatty acid distillate</td>
</tr>
<tr>
<td>Trans fat could be an issue</td>
<td></td>
</tr>
<tr>
<td>Higher price of fatty acid distillate</td>
<td></td>
</tr>
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<td></td>
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</tbody>
</table>
REFINING TECHNIQUES
CHEMICAL VS PHYSICAL

- CHEMICAL REFINING
  - LOSS OF NEUTRAL OIL
  - LOSS OF FATTY ACIDS TO LOW QUALITY ACID OIL
  - HIGH EFFLUENT GENERATION
  - ENVIRONMENTAL POLLUTION
  - HIGH PHOSPHOROUS IN NEUTRAL OIL
  - HIGH SOAP IN NEUTRAL OIL

- PHYSICAL REFINING
  - HIGHER YIELD OF NEUTRAL OIL
  - RECOVERY OF HIGH QUALITY FATTY ACIDS
  - MINIMUM EFFLUENT & POLLUTION
  - CLEANER ENVIRONMENT

WE PROPOSE AN **ENZYMATIC SOLUTION** THAT REDUCES IMPACT OF NEGATIVE FACTORS OF REFINING
REFINING TECHNIQUES
CHEMICAL VS PHYSICAL

• CHEMICAL REFINING
  • LOSS OF NEUTRAL OIL
  • LOSS OF FATTY ACIDS TO LOW QUALITY ACID OIL
  • HIGH EFFlUENT GENERATION
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• PHYSICAL REFINING
  • HIGHER YIELD OF NEUTRAL OIL
  • RECOVERY OF HIGH QUALITY FATTY ACIDS
  • MINIMUM EFFlUENT & POLLUTION
  • CLEANER ENVIRONMENT
  • BETTER QUALITY & STABILITY

ENZYME ASSISTED CHEMICAL REFINING
PROCESS DIAGRAM

CRUDE OIL

ENZYME SOLUTION TANKS

HIGH SHEAR MIXER

STOICHIOMETRIC CAUSTIC DOSING

SOAP STOCK

NEUTRAL OIL

GENTLE MIXER

HAZ Dosing

ENZYMATIC GLUM HYDROLYSIS

STOICHIOMETRIC CAUSTIC DOSING

SOAP STOCK

NEUTRAL OIL

GENTLE MIXER

HAZ Dosing

ENZYMATIC GLUM HYDROLYSIS
ENZYME ASSISTED CHEMICAL REFINING DELIVERABLES

• NEUTRAL OIL PARAMETERS:
  • PHOSPHOROUS: 3-4 ppm
  • SOAP CONTENT: ~250 ppm
  • FFA: 0.08 %
  • SOAP STOCK: ALMOST FREE FROM GUMS- CAN BE SPLIT AT 80⁰ C ADJUSTING pH to 4.5.

RESULTS OBTAINED ON WATER DEGUMMED SOY OIL WITH
ROHALASE PLXTRA & ROHALASE F – PHOSPHOLIPID HYDROLYSING ENZYMES OF AB ENZYMES GmbH

ENZYME ASSISTED CHEMICAL REFINING BENEFITS

• INCREASED YIELD OF NEUTRAL OIL UP TO 0.4 %
• LOWER NEUTRALIZATION LOSS
• NO SAPONIFICATION LOSS
• REDUCED OR NIL EMULSIFICATION LOSS
• NO SOAP ADSORBANT
• LOWER CONSUMPTION OF BLEACHING EARTH
• HIGH QUALITY OIL RECOVERED FROM SOAP
• REDUCED EFFLUENT GENERATION

• HIGHER PROFITABILITY
ENZYME ASSISTED WATER DEGUMMING

THANK YOU FOR YOUR ATTENTION
&
LET’S JOIN HANDS
Enzymatic Degumming in vegetable Oil Processing

Remco Muntendam
Ghent
22-09-2016

- Introduction DSM
- Enzymes in General
- Enzymes in Degumming
- Potential benefits of EDG gums
- Tech Lessons Learned
DSM Life Sciences and Material Sciences company

active in: nutrition health materials

We create solutions to bring healthier, better performing and more sustainable products to the lives of people today and for generations to come.

<table>
<thead>
<tr>
<th>Net sales</th>
<th>about € 10,000m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workforce</td>
<td>25,000</td>
</tr>
</tbody>
</table>
100 years of successful transformation

Biomaterials / Biologics
Life Science Products
Performance Materials
Petrochemicals
Fertilizers
Coal

#1 position in the Dow Jones Sustainability World Index material industry sector; see www.sustainability-index.com
Building on an impressive history

Hoffman La Roche’s Vitamins (1930s)

Gist-Brocades (1869)

DSM (1902)

Chemical synthesis & biotechnology

Life Sciences

Biotechnology

Materials Sciences

Energy, chemistry & polymer technology

Vitamins
Omega’s
Carotenoids
Premixes for food & feed
Enzymes
Minerals
Cultures & Yeasts
Nutraceuticals
Pharmaceuticals
Cellulosic bioethanol
Biomedical materials
Bio-plastics
High Performance Plastics
Coating Resins
Functional Materials
Solar - advanced surfaces
Biotechnology is everywhere...
Enzymes are biocatalysts

Accelerate reaction rate by lowering energy threshold:
- Specific, resulting in desired conversion
- Lower temperatures, natural environment and less chemicals
Main constituents of crude vegetable oil?

CRUDE OIL comprises

OIL (TAG, DAG) + IMPURITIES (FFA, PL and lyso-PL)

Free Fatty Acid (FFA)
Lyso-Phospholipid (LPL)
Phospholipid (PL)

Amounts & types of impurities in crude oil are determined by:

- Growing conditions
- Seed storage & handling
- Oil extraction method
- Other impurities (metals, tocopherols, carotenoids, chlorophylls)

Page 6
Phospholipid Impurities Make A Difference

- Hydration increases with polarity and correlates with emulsifying strength
- Formation of metal salts reduces polarity and results in “non-hydratable” phospholipids

Phospholipids (PLs) are emulsifiers.

- PLs reduce surface tension between water and oil.
- Oil is emulsified (& trapped) in the gum fraction.
- Different PLs have different properties - Hydratable or Non-hydratable.

Oil losses are caused by intact phospholipids:

- Phospholipids (PLs) are emulsifiers.
- PLs reduce surface tension between water and oil.
- Oil is emulsified (& trapped) in the gum fraction.
- Different PLs have different properties - Hydratable or Non-hydratable.
Water Degumming: Separation of Phospholipids

- Basic process to remove phospholipids falls between extraction & refining
- Process designed to minimize emulsion
- Target water wash degummed oil specification of < 200 ppm residual P
- Basic measurement of step yield AND little control of process
- Attention to “quality” only if coupled with caustic refining
Phospholipase Enzymes Break Emulsion

- Phospholipase enzymes uncouple oil & water-soluble parts of phospholipids
- Reaction requires contact between enzyme and phospholipid (i.e. emulsion)
- Enzyme action on phospholipids breaks down emulsion and releases entrained oil
- Reaction products create additional value

Disclaimer: more water added in favor of demonstrated effect
High Industrial potential for Purifine based enzymatic degumming

- Enzymatic degumming provides extra oil
- Less oil entrapment after enzymatic degumming
Commercial Degumming Enzymes

PLA₁

PLA₂

PLC

GLYCEROL

P

Polar head group

1,2 - Diacylglycerol (DAG)

OIL

WATER

Polar head group

Polar head group

Polar head group

123

234

567

890

glycerophosphate

Lysophospholipid

Phosphate ester

Lecitase® Ultra
(Novozymes)

Rohalase® MPL (AB Enzymes)
LysoMax® Oil (Danisco)
Purifine PLA2 (DSM)
Purifine® PLC and Purifine® 3G (DSM)

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(1) Gramatikova, S., US Patent 7,226,771
Purifine vs. PLA compared

For crude rapeseed oils

- 600 ppm P rapeseed oil
  - Purifine PLC: 0.75
  - Single PLA solutions: 0.3
  - Purifine 3G: 1.1

For crude soybean oils

- 1200 ppm P soybean oil
  - Purifine PLC: 1.5
  - Single PLA solutions: 0.6
  - Purifine 3G: 2.25

- 600 ppm P rapeseed oil
  - Purifine PLC
  - Single PLA solutions
  - Purifine 3G

- 1200 ppm P soybean oil
  - Purifine PLC
  - Single PLA solutions
  - Purifine 3G

- FFA
  - Purifine PLC
  - Single PLA solutions
  - Purifine 3G

- Oil
  - Purifine PLC
  - Single PLA solutions
  - Purifine 3G
Basic principles of Enzymatic Degumming

Simple modification of standard degumming

<table>
<thead>
<tr>
<th>Variable</th>
<th>Implementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Oil Temperature</td>
<td>Cooled for Emulsion stability + Enzyme activity</td>
</tr>
<tr>
<td>Process Control</td>
<td>Enzyme &amp; water dosing, steady process flow</td>
</tr>
<tr>
<td>Reaction Interface</td>
<td>High-shear emulsion maintained by agitation</td>
</tr>
<tr>
<td>Reaction Time</td>
<td>Plug-flow reactor of sufficient size</td>
</tr>
<tr>
<td>Effective Separation</td>
<td>Heat to break emulsion &amp; optimized stack-disk centrifuge</td>
</tr>
</tbody>
</table>

Maximize Reaction - Minimize Losses
Rapeseed oil processes:

- Oil seeds
  - press
  - Press cake
  - Hexane extraction
  - Meal

Multiple or flexible processing options

- Expeller oil
  - 250 - 350 ppm P

- Extracted oil
  - 1000 - 1500 ppm P
  - Processed separately

- Crude oil
  - 600 - 800 ppm P
  - Processed combined
Rapeseed oil processes:
multiple or flexible processing options

Oil seeds

Press cake

Hexane extraction

Meal

Expeller oil
250 - 350 ppm P

Extracted oil
1000 - 1500 ppm P

Crude oil
600 - 800 ppm P
Rapeseed oil processes: multiple or flexible processing options

- **Expeller oil**: 250 - 350 ppm P
- **Extracted oil**: 1000 - 1500 ppm P
- **Crude oil**: 600 - 800 ppm P
- **Deep or acid degumming**
- **RBD**
Introducing phospholipase-assisted degumming

**Expeller oil**
- 250 - 350 ppm P

**Extracted oil**
- 1000 - 1500 ppm P

**Crude oil**
- 600 - 800 ppm P

PL profile requires combination of PLC + PI-PLC + PLA2

Enzymatic degumming

RBD
Soy meal is the premier protein source, but:

- Soy protein levels are declining due to new varieties of seeds & farmers focus on yield
- Increased competition coming from DDGS, canola meal, synthetic amino acids & other ingredients

- Soy producers need to maintain leadership position by
  - Keeping protein levels high in meal products
  - Running plants efficiently and at capacity

- Purifine 3G EDG can help!

Sources:
Special Gum Product Creates Opportunities

Traditional outlets for gums are:
- Lecithin, meal additive, acid oil
  feedstock, fuel

Enzymatic degumming Gums have different chemical, nutritional and energy profile:
- P content 3X higher
- Energy content 25% lower
- Reaction products from PLC action on phospholipids are present at high levels and could be “natural” source of high-value ingredients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Unit</th>
<th>WDG gums</th>
<th>3G gums</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>% of oil</td>
<td>84%</td>
<td>62%</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>g/kg</td>
<td>19</td>
<td>62</td>
</tr>
<tr>
<td>Choline</td>
<td>g/kg</td>
<td>20</td>
<td>72</td>
</tr>
<tr>
<td>Inositol</td>
<td>g/kg</td>
<td>21</td>
<td>63</td>
</tr>
<tr>
<td>Lyso-PL</td>
<td>g/kg</td>
<td>None</td>
<td>13</td>
</tr>
</tbody>
</table>

¹Independent research on behalf of DSM. Research under leadership of Willem Smink of Feed Innovation Services (The Netherlands)
²Cowieson et al., 2013; Zyla et al
Enzymatic degumming vs. Lecithin Drying for Soy Crushing Installations

- Maximizing profitability calls for flexible set-up?
- Lecithin plants that are EDG capable do exist
- Future value of GM soy lecithin in EU market?
- Enzyme degumming for a soy crushing plant is an off-the-shelf option!

<table>
<thead>
<tr>
<th>Driver</th>
<th>Unit</th>
<th>2016</th>
<th>2015</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refined Soybean Oil Price</td>
<td>€/MT</td>
<td>750</td>
<td>600</td>
<td>800</td>
</tr>
<tr>
<td>GM Crude Soy Lecithin Price</td>
<td>€/MT</td>
<td>300</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>GM Soy Meal Price</td>
<td>€/MT</td>
<td>300</td>
<td>350</td>
<td>450</td>
</tr>
<tr>
<td>Enzyme Dosing</td>
<td>ppm</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Purifine 3G Price</td>
<td>€/MT</td>
<td>22,5</td>
<td>22,5</td>
<td>22,5</td>
</tr>
<tr>
<td>Total Yield</td>
<td>in %</td>
<td>2,50%</td>
<td>2,50%</td>
<td>1,60%</td>
</tr>
<tr>
<td>Lecithin Yield</td>
<td>in %</td>
<td>3,50%</td>
<td>3,50%</td>
<td>3,50%</td>
</tr>
<tr>
<td>Net profit EDG</td>
<td>€/MT</td>
<td>17,25</td>
<td>14,00</td>
<td>12,80</td>
</tr>
<tr>
<td>Net profit Lecithin</td>
<td>€/MT</td>
<td>10,50</td>
<td>7,00</td>
<td>14,00</td>
</tr>
</tbody>
</table>

2016 prices correspond with global average levels in April ‘16
Lessons From the Field: Optimizing Reaction Yields

- Enough reaction time is critical:
  - 2 Hr for simple degumming; 4-6 Hr for deep degumming

- Temperature control is critical:
  - Enzymatic degumming activities optimal between 55-60°C = (131-140°F)

- Emulsion is important:
  - Silverson or IKA high shear mixer for emulsification of the oil
  - Water dosage (1.5-3%) impacts emulsification

- Low caustic dose enhances emulsion & degumming robustness:
  - 50-200 ppm dosed in crude oil before enzyme addition
  - Dilute (15-20%) solution for accurate & safe dosing
  - Mix thoroughly in oil before enzyme addition
  - Optional: acid injection before separator for increase separation robustness.

DSM provides on site support for maximizing reaction conditions
Lessons From the Field: Driving for Low P

- **Ensure availability & removal of NHP:**
  - Citric is preferred to phosphoric acid (30-60 min)
  - Make sure oil is at 85-90°C during acid treatment

- **Maximize reaction:**
  - 4-6 h reaction time needed.
  - Bottom feed tanks, & ensure effective agitation to maintain emulsion
  - IKA-type HS mixer
Lessons From the Field: Visual Clues

- Visual observations can provide clues that a reaction is occurring

Emulsion after HS mixer

Particles agglomerate, Cloudlike appearance

Gums exiting centrifuge

Liquid, flowing EDG

Lumps of gums WDG

To stable, bad separation

VS

VS
• Prevent high losses in acid degumming or physical refining

• Enzymatic degumming is the enabling technology for physical refining on soy oil

• Other innovations (eg double scrubber) potentially increases overall value
## Deep EDG: Comparison of Process Options

<table>
<thead>
<tr>
<th></th>
<th>PLA1</th>
<th>PLC+PLA1 (sequential)</th>
<th>Purifine 3G*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Separator P</td>
<td>&lt; 20 ppm</td>
<td>&lt; 20 ppm</td>
<td>&lt; 30 ppm</td>
</tr>
<tr>
<td>2 Separator P</td>
<td>&lt; 5 ppm</td>
<td>&lt; 5 ppm</td>
<td>&lt; 10 ppm</td>
</tr>
<tr>
<td>Est. Oil Yield Gain†</td>
<td>1.2%</td>
<td>2%</td>
<td>2.5%</td>
</tr>
</tbody>
</table>
| Status                 | • Validated on industrial scale  
                        | • 10-15 Plants running       | • Industrial trialing in progress |
| Specific Process       | 4-8 h reaction time  
                        | Acidic conditions  
                        | Acid to prevent salts in separator | 2-6 h reaction time |
| Requirements           | • 4-8 h reaction time  
                        | Acidic conditions  
                        | Acid to prevent salts in separator | 2-6 h reaction time |
| Comments               | • Low enzyme dose in good oils  
                        | • Much higher dose in poor oils  
                        | • Significant increase in FFA  
                        | • Lowest yield potential | • Low PLA1 dose in good oils, may need higher in poor oils  
                        | • Complicated engineering  
                        | • 2 Enzyme suppliers required  
                        | • Mid yield potential | • Highest yield potential  
                        | • Drop in for PLA1 plants |

*Based on preliminary industrial trials
†Compared with caustic refining assuming 4.5% losses & excluding FFA recovery
Take home message

• Purifine® solutions in enzymatic degumming provides higher yield due to less entrapment of oil and release of diacylglycerol with marginal fatty acid increase.

• Low P solution possible with minor process adaptation.

• Deliver technical field support from industrial experts to get process up and running efficiently.

• Purifine®: More oil more profit!
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