

## OPTIMIZE THE CONDITION FOR ENZYMATIC DEGUMMING OF CRUDE SOYBEAN OIL

M Prabhakaran<sup>1\*</sup> and SK Rakshit<sup>2</sup>

<sup>1</sup>Agricultural Engineering, Faculty of Agriculture, University of Jaffna

<sup>2</sup>Professor, Food Engineering Division, School of SERD, AIT, Thailand

### ABSTRACT

High phosphatide containing oils like soybean oil, have two types of phosphatide (gum) namely, the hydratable and non-hydratable. Simple water degumming will not remove non-hydratable gums. Water degumming alone is not satisfactorily to reduce the gum content below 10ppm to enable the physical refining. Therefore enzymatic process is used together with water degumming. The enzymatic degumming process was employed to reduce the level of phosphatide (P) less than 10ppm in 10h of duration without mixing. A combined degumming process a normal mixing speedup the reduction of the gum level to less than 10ppm in 7h of mixing duration, while a combined degumming process exhibiting a normal mixing followed by initial emulsification (15min) speedup the reduction of the gum level to less than 10ppm in 6h of mixing duration. On the other hand initial emulsification (15min) with normal mixing (2000rpm) at 2ml enzyme level gave the same result 5h. Gum level of the water degummed oil (150ppm to 180ppm), which was used as a starting material for the two-step process was efficiently reduced to less than 10ppm within 4h. Initial emulsification (15min) with normal mixing (1000rpm), 1ml enzyme level the water degummed oil gave the phosphatide (P) less than 10ppm 3h. This condition is thus most preferable. Among these process the initial emulsification with normal mixing was given better result than the normal mixing, and two step process were faster than the process of combined.

### INTRODUCTION

Degumming is an important step in oil refining process as it removes phosphatide (gum) along with some other unwanted minor compounds without destroying the beneficial ones. Gums tend to produce high refining losses, foaming, settling and discoloration of oil in processing and storage (Eickhoff 2000).

Several degumming concepts have been introduced in recent years to achieve the required low phosphatide content required for physical refining, this include acid degumming (Phosphoric acid and organic acids). Unfortunately these methods have several disadvantages including corrosion of processing equipment, lower the value by-product and environmental hazards (Racicot and Handel 1983).

There are two types of phospholipids present in vegetable oils namely the hydratable and non-hydratable. Simple water degumming will not remove non-hydratable gums or pro-oxidant metals to levels which do not impair colour and oxidative stability. Hence the enzymatic degumming is very important (List *et al.* 1993).

The microbial enzyme Lecitase Novo enzymatic degumming is cost-efficient compared to chemical refining and other physical refining processes. Physical refining however requires phosphatide content to be less than 10ppm as they affect the vacuum distillation process at higher level. Enzymatic degumming process achieve this level are

required to remove the non-hydratable phospholipids (Dahlke and Buchold 1995).

### The Objectives of this study

Optimize the conditions of degumming process gum level below 10ppm using microbial enzymes (Lecitase Novo) in crude soybean oil.

### MATERIALS AND METHODS

#### Materials

Crude soy oil was obtained from Vegetable oil products Co. Ltd and enzyme Lecitase(r) Novo is an enzyme of Novo Nordisk from The East Asiatic Public Company Limited. Lecitase Novo is a carboxylic ester hydrolase produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism (Novo Nordisk 2000).

The following equipments & instruments were used. Spectrophotometer UV2-200, Shaker with water bath, mixing heads with control panel and emulsifier.

#### Methodology

Combined Process of enzymatic degumming of crude soybean oil

500ml of crude oil or degummed was taken into the reactor, which was kept at about the temperature needed for the specific reaction (40°C) The lab mixing head was turned on that the oil starts to

\*Corresponding author: [aenpraba@gmail.com](mailto:aenpraba@gmail.com)

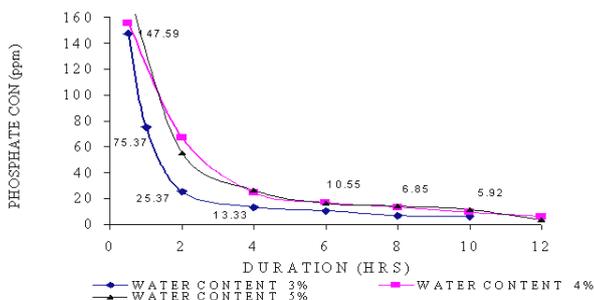
circulate from the reactor to the emulsifier and back to the reactor. The pre-treatment period starts ( $t=0$ ) with addition of 1.5% (v/v) water was added Just after this buffer solution 1.5% (v/v) were added to the system, at ( $t=1$ h) 2ml samples are drawn for phosphorus analysis and continue every 1h up to gum level less than 10ppm.

Combined process crude oil was taken and degummed as described as single step degumming process. In this experiment the potential of a combined degumming process which, using water and enzyme (Lecitase Novo) for the removal the hydratable and non-hydratable gum simultaneously. In this study, buffer solution and phospholipase A1 (Lecitase Novo) enzyme were used for hydration and hydrolysis of phospholipids respectively in soybean oil. The degumming process depends on the following operational parameters to achieve the level of phosphatide below 10ppm to enable the physical refining.

The emulsification process were performed to investigate the gum removal under the optimum conditions attained in this experiment. initial, every two-hour and every hour emulsification were further studied to be processes while compared to the normal mixing at an enzyme level of 1ml and speed of mixing levels of 500, 1000 and 2000rpm in other period (simple mixing).

Two-step process of enzymatic degumming of crude soybean oil.

This part of the studies involves water and enzymatic degumming performed separately in which 1.5% of water was added initially (an amount sufficient for the removal of hydratable gum at a considerable amount under the optimized conditions during the water degumming experiment) followed by the addition of 1.5% of water containing the buffer (0.1M citrate buffer) and enzyme (Lecitase Novo). The final amount of water was kept constant (3%). Degummed oil sample was taken for P determination. Water degumming is the simplest form of phosphatide reduction. However, only hydratable gums can be removed. Degumming were performed to investigate the gum removal under the optimum conditions attained in this experiment



**Figure 1: Water amount in the enzymatic degumming of crude oil (500ml crude oil, 1ml enzyme, normal mixing 1000rpm at 40°C)**

were percentage of water (v/v), degumming temperature (°C), speed of mixing (rpm) and duration of mixing (h)

## RESULTS AND DISCUSSION

### Enzymatic degumming of crude soybean oil (Combined process)

Simultaneous removal of hydratable and non-hydratable gum was consumed more than 10h time to reached the target level of phosphatide (Gum) less than 10ppm, in other hand stirrer mixing was involved in this process the gum removal was faster, which consumed 7h of mixing duration under the conditions of 500ml crude oil, 1ml enzyme, 3% buffer solution, mixing speed of 1000rpm at 40°C.

It was found that a 3% total water level (1.5% water and 1.5% buffer) enhanced the degumming process Fig. 1. Although it is after 9 and 10h of mixing the degumming process conducted using a total water levels of 4% and 5% provided the same phosphatide level ( $p < 10$ ppm).

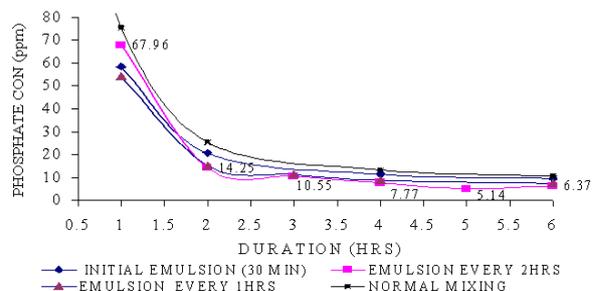
### Mode of mixing in enzymatic degumming process of crude oil

Further improvement in simultaneous gum removal was performed by stirrer mixing coupled with emulsification processes at initial stage (15min) the gum removal was faster it consumed 6h duration. (Fig. 2).

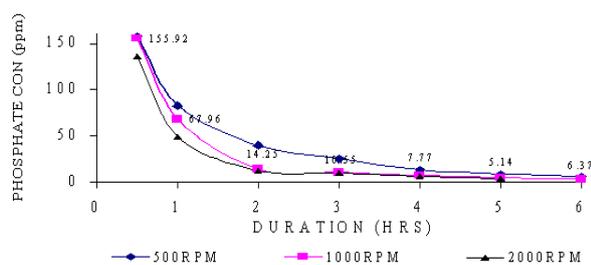
Further investigation was analyzed in various strength of enzyme (0.5, 1.0, and 2ml), mixing speeds (500, 1000 and 2000) and buffer strength (3, 4 & 5%) Fig. 3 shows that 2000rpm and 2ml enzyme level performed the gum removal were faster but the performance of gum removal was economically ineffective (Fig. 3).

### Enzymatic degumming of water degummed oil (two step process)

Combined process was compared with two step processes, the later one faster than the first one; because of enzyme only react with non-hydratable gum part. (Fig. 4)



**Figure 2: Mode of mixing in enzymatic degumming process of crude oil (500ml Crude oil, 3.0% water & buffer, 1ml enzyme, 1000rpm at 40°C)**



**Figure 3: Mixing speed and initial emulsion (15 min) in combine process** (500ml crude oil, 1.5% water, 1.5% buffer, 2ml enzyme at 40°C)

Two stage process the gum removal was employed in two stages, initially gum was removed by water up-to level of residual gum 150-180ppm, later process gum removal was more faster it consumed 4h mixing duration.

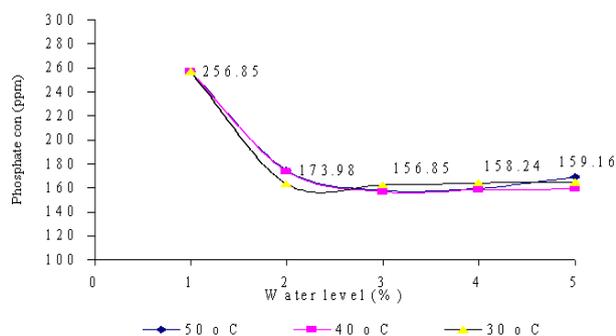
**Water degumming process**

As is presented in Fig. 5 a water level increment from 1% to 3% at 500rpm decreased the phosphate concentration of the oil up to 150 – 180ppm after 30min of mixing duration and remained almost constant afterwards. Among the temperatures tested 40°C slightly favored the water degumming process.

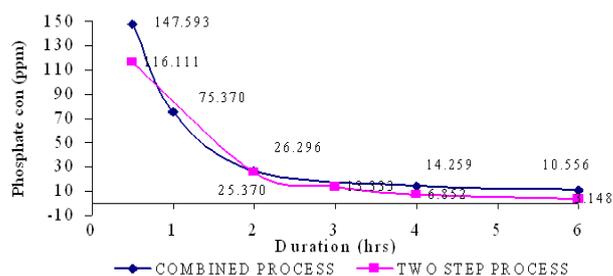
**Mixing mode in the two step enzymatic degumming process**

While determining the enzyme concentration required for the two step degumming process enzyme performed the gum removal were not significant. Studies on mixing conditions while using the two step degumming process on the other hand revealed that 2000rpm reduced phosphate to the required level faster while 1000rpm took little longer hours (Fig. 6).

Optimization conditions of enzymatic degumming of water degummed oil the gum removal was so-faster it consumed 3h of duration Fig. 6, with the conditions of initial emulsification (15min) with stirrer mixing (1000rpm) 500ml crude oil, 1ml enzyme, 3% buffer solution, at 40°C.



**Figure 5: Water level and temperature in water degumming of crude oil.** (500ml crude oil at 500rpm, 30min)



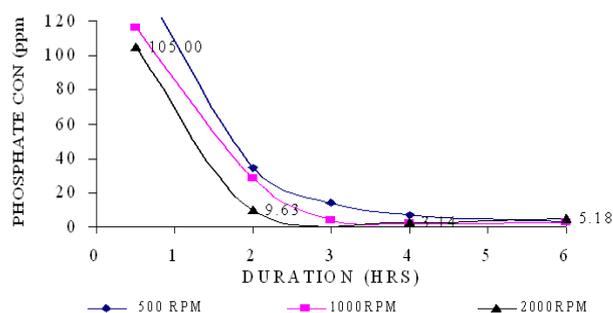
**Figure 4: Combined and two step Enzymatic degumming** (500ml oil, 1.5% buffer, 1ml enzyme, 1000rpm, at 40°C)

**CONCLUSION**

Simultaneous removal of hydratable and non hydratable gum was consumed more than 10h to reach the target level of phosphatide (Gum) less than 10ppm.

In stirrer involved mixing of degumming process, the reduction of gum was less than 10ppm at 7h of mixing duration. Normal mixing followed by initial emulsification (15min) speeded the reduction of the gum level to less than 10ppm at 6h of mixing duration. This was achieved in while 1ml enzyme was used per 500ml crude oil at 1.5% water, 1.5% buffer level at 40°C with normal mixing (1000rpm). On the other initial emulsification (15min) with normal mixing (2000rpm) at 2ml enzyme level gave the same result at 5 hours. Further investigation was analyzed in various strength of enzyme (0.5, 1.0, and 2ml), mixing speeds (500, 1000 and 2000) and buffer strength (3, 4 & 5%) Fig. 3 shows that 2000rpm and 2ml enzyme level performed the gum removal were faster but the performance of gum removal, which was economically ineffective.

During the water degumming process 3% water level, 40°C, 30min of mixing duration at 500rpm efficiently reduced the level of phosphatide. The gum level of the water degummed oil (160ppm to 180ppm), which was used as a starting material for the two-step process was efficiently reduced to less than 10ppm within 4h. This was achieved in 1ml



**Figure 6: Mixing speed in degumming of water degummed oil,** 500ml Degummed oil 1.5% buffer, 1ml enzyme, at 40°C initial emulsification(15min)

enzyme per 500ml water degummed oil in 1.5% buffer at 40°C, normal mixing (1000rpm). On the other initial emulsification (15min) with normal mixing (1000rpm) at 1ml enzyme level gave the same result at 3h. This condition is thus most preferable.

Two step process were faster than the process of combined one, because of hydratable gum part already removed in two step process so enzyme only react with non-hydratable part. Among this process the initial emulsification with normal mixing was gave better result than the normal mixing, because of emulsification process was break down the oil and gum particle into tiny pieces, so enzyme reaction was more active.

Novo Nordisk 2000 'Lecitase novo enzyme product sheet' Available online hills: [www.mynovozy.com/2001-06954-n.html](http://www.mynovozy.com/2001-06954-n.html) [down loaded: 11 2001]

Racicot LD and AP Handel 1983 'Degumming of soybean oil: Quantitative analysis of Phospholipids in crude and degummed oil 1, 2 *JA OCS* 60: 6; 1098-1101.

Edible oil refining systems providing caustic refining, bleaching' available online [www.crowniron.com/refinina.html](http://www.crowniron.com/refinina.html) [downloaded: 2. 2 2002]

Soya oil degumming available online [www.ag.uiuc.edu/archives/experts/utilization/html](http://www.ag.uiuc.edu/archives/experts/utilization/html). [down loaded: 2 2 2002]

## REFERENCES

- Carelle AAMIV and Crapiste GH 1997 'Quantitative determination of phospholipids in sun flower oil', *JAOCS*, 74:5511-514.
- Dahlke K and Buchold H 1995 'first the enzymatic degumming process' *Inform*, 6:12, 1284-1291.
- Eickhoff KP 2000 'Degumming process in edible oil Industry' World Conference Proceedings on Edible Oils and Fat Processing - Basic Principles and Modern Practices, Edited by S.C. Singhal, AOCS, Champaign, 2000.
- List GR, Avellaneda JM and TL Mounts 1993 Re-fining of soybean oil' *JAOCS*. 58: 892.