CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The phorbol esters determination by HPLC from the seeds, kernels and meals by the shaking technique is a simple method. This method was verified by evaluation of the spiked recovery; 88.10% was achieved which is considered acceptable method because the recovery was higher than 70% of matrix spiked (Kebbekus and Mitra, 1998; Mitra, 2003). Thus the extraction condition selected for prior extraction before analyzation of phorbol esters by HPLC-UV in this study was 2 g of sample per 20 ml of methanol, shaking at 300 rpm for 4 hrs.

For *J. curcas* seeds composition study, the kernel:shell ratio by weight of seeds is 63.43 : 36.57 and the average weight per seed is 0.7174 g. The seeds contain oil 36.88 %wt., crude protein 13.01 %wt. and phorbol esters 1.59 mg/g.

Even though, the solvent extraction as hexane is the ability nearly 100% recoveries and can reduce the phorbol esters from the residue meals almost 100%, this method spent the complicated work within the solvent evaporation that possibly unsuitable for the local scale. The compressing technique is more convenient and does not require any solvent, so it is easily operated by the local scale. The efficiencies of compressing and surfactant solution extraction methods were 77.03% and 78.69%, respectively. Unfortunately, the pressed meals still contained high phorbol esters, 1.45 mg/g of meals; therefore, further process of using surfactant solution to remove remained phorbol esters was introduced.

To add values of the meals, detoxified process as low as the non-toxic variety is needed. The result in this present study indicated that surfactant technology provides an opportunity to develop a promising technique for phorbol esters removal from the Jatropha meals. The results showed that nonionic surfactant solution at 40 mM of concentration was optimal to remove phorbol esters from the pressed meals for both Tween 80 and Dehydol LS9. For mixed solution, only 5 mM of anionic

surfactant (AOT) and 100 mM of NaCl with 40 mM of nonionic surfactant exhibited the similar phorbol esters removal efficiency. The optimal extraction condition was 15 min of shaking time, 0.5 g of meals per 10 ml of solution. Moreover, the reused solutions have the ability closed to the fresh ones. The removal efficiencies were 86.64% for the single T80 system; 89.94% for single LS9; 82.47% for the mixed T80 system; and 87.61% for the mixed LS9 system. The crude protein remaining from the detoxification process was 14.46%wt. for the single Tween 80 system, 15.10%wt. for the single Dehydol LS9 system, 15.21%wt. for the mixed Tween 80 system, and 16.24%wt. for the mixed Dehydol LS9. The initial crude protein in the pressed meals was 17.94%wt.

Even though the content of phorbol ester after the removal process by surfactant aqueous-based solution was still 0.2 – 0.3 mg/g which was not as low as those found in the non-toxic Mexican variety (0.11 mg/g); the highest removal efficiency is up to almost 90%. To select the most suitable formulation of the surfactant solution for phorbol esters removal, it is necessary to decide based on several criteria. Since the efficiencies of 4 formulation selected to evaluate on phorbol esters were not significantly different, other criteria was brought into final decision for the best formulation. Single nonionic solutions have higher ability for phorbol esters removal efficiency; on the other hand, they also have higher crude protein losing from residual meals. Therefore, to select only one best solution is depending on the objective of the operation as well as cost of process.

Conclusively, overall performance of the technique o using surfactant solutions to remove phorbol esters in seed meals is shown in Table 5.1. From the overall performance of 4 different surfactant solutions, the partition of phorbol esters into solid and liquid phases can be illustrated in Figure 5.1. To be optimistic, the improvement of this technique may enhance its efficiency of removing phorbol esters and subsequently able to make the residue meals can be used for further application as expected. Also, if the expected is using the meals as animal feedstock, the toxicity of the meals has to test in clear as the acceptance level.

Table 5.1 Summary of properties from the pressed meals detoxification by the surfactant solutions

Properties	Pressed meals	Detoxified meals by surfactant solutions*			
		Single T80	Mixed T80	Single LS9	Mixed LS9
Chemical properites in meals					
- Phorbol esters (mg/g)	1.45	0.19	0.25	0.15	0.18
- Crude protein (%wt.)	17.94	14.46	15.21	15.1	16.24
Detoxified efficiencies					
- %Phorbol esters removal	-	86.64	82.47	89.94	87.61
- %Crude protein loss	-	19.40	15.22	15.83	9.48
Amount of component in solution (g/100 ml)					
- Tween80	-	5.24	5.24	-	-
- Dehydol LS9	-	-	-	2.36	2.36
- AOT	-	-	0.22	-	0.22
- NaCl	-	-	0.58	-	0.58
Observation during process					
- Settling meals	-	Slower	Faster	Slower	Faster
- Filtration to separate meals and solutions	-	Difficult	Easy	Difficult	Easy

Note: * The extraction condition is 15 min-contact time, 0.5 g of meals per 10 ml of solution and one time extraction.

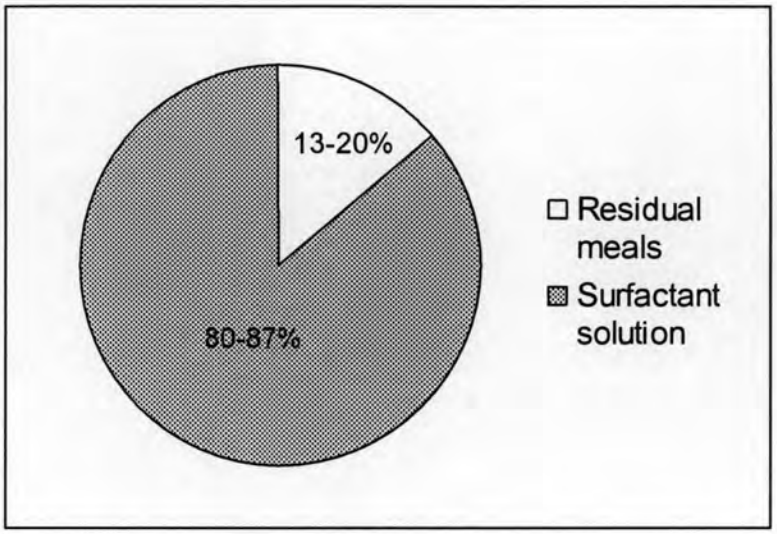

Figure 5.1 The phase of phorbol esters in the removal system by overall from the 4 surfactant solutions; Single T80 as 40 mM Tween 80, Single LS9 as 40 mM Dehydol LS9, Mixed T80 as mixed of 40 mM Tween 80 and 5 mM AOT with 100 mM NaCl, and Mixed LS9 as mixed of 40 mM Dehydol LS9 and 5 mM AOT with 100 mM NaCl

5.2 Recommendations

Since this research is not able to remove phorbol esters as low as the non-toxic variety, the improvement of this technique needs to be further investigated. Also, the by product such as waste from the detoxification process should be considered. The suggestions are drawn as in the following details.

1. An investigation of combined method i.e., with ethanol extraction should be conducted.
2. Further investigation using other surfactants mixtures may able to enhance the phorbol esters removal from meals. However, the used surfactants should be non-toxic and biodegradable.
3. Since nonionic surfactants are thermal sensitive, the study on the effect of temperature to phorbol esters removal efficiency should be conducted for both the single nonionic surfactant solutions and the mixed surfactant solutions.

4. The age of meals is important for selection removal process. Further investigation to understand the undergoing reactions of residual meals may provide answers for applications. Larger scale extractions using this technique should be carried out.
5. The feasibility study for implementation of this process should to be studied in order to decide on the economic aspect.
6. The detoxified meals should be tested the toxicity before using as feedstock; moreover, the residual surfactants that remain in the meals should be determined if it is the acceptable level.
7. Since phorbol esters itself can be used for other application, the technique to purify the phorbol esters from the surfactant solution should be carried out. In addition, the surfactants recovery for reuse in the process should be investigated.