

**DEVELOPMENT OF COST EFFECTIVE PROCEDURE TO IMPROVE  
STABILITY OF PAN GREASE**

By

**Nanayakkara D.N.**  
**(02/AS/A/038)**

Thesis submitted in partial fulfillment of the requirement of the  
Degree of  
Bachelor of Science (Special) in Food Science and Technology


**Department of Food science & Technology,  
Faculty of Applied Sciences,  
Sabaragamuwa University of Sri Lanka  
Buttala (91100)**

August 2007

## DECLARATION

The work describe in this thesis was carried out by me at the Department of Food science and Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, under the supervision of Mr. M.C.N. Jayasooriya, Lecturer, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka & Mr. Hemantha Siyambalagoda, R & D Manager, Raigam marketing services (Pvt) Ltd., No 277, kiriwattuduwa. A report on this has not been submitted to any other university for another degree.


Ms. D.N.Nanayakkara  
(02-AS-A-038)

  
.....  
Signature  
Date .....

Certified by,


### External Supervisor

Mr. Hemantha Siyambalagoda  
R & D Manager,  
Raigam Marketing services (pvt.) Ltd.  
No 277,  
Kiriwattuduwa.  
Contact: 0777447212  
E-mail: [hemanthasiyambalagoda@yahoo.com](mailto:hemanthasiyambalagoda@yahoo.com)

  
.....  
Signature

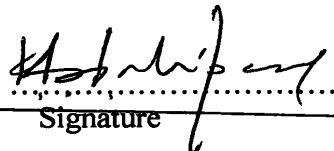
### Internal Supervisor

Mr.M.C.N.Jayasooriya  
Lecturer-Department of food science & Technology  
Faculty of Applied Sciences,  
Sabaragamuwa University of Sri Lanka  
Buttala (91100)  
Contact: 0552273518/+940773314103  
E-mail: [niroshanmcn@sab.ac.lk](mailto:niroshanmcn@sab.ac.lk)

  
.....  
Signature

Date: 28-02-2008.

Dr (Mr.) K.B.Palipana  
Head/Department of Food science & Technology,  
Faculty of Applied Sciences,  
Sabaragamuwa University of Sri Lanka,  
Buttala (91100)  
Contact: 0552273518  
E-mail:

  
.....  
Signature

Date: 28-02-2008

Affectionately  
Dedicated

To

My Parents & Teachers

## **ACKNOWLEDGEMENT**

I would like to express my sincere thanks to my internal supervisor Mr.M.C.N.Jayasooriya, Lecturer, Department of Food science and Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka for his valuable guidance, encouragement and supervisor throughout this project.

I wish to express my deepest gratitude to my external supervisor Mr. Hemantha Siyambalagoda who is the R & D manager of Raigam marketing services (pvt.) Ltd. for giving me this opportunity with their advice, encouragement and guidance through the study and for sparing their valuable time in bringing this study to a successful completion.

I express my sincere gratitude to Mrs. Deepika Priyadarshanee, Head, Department of Food science and Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, for guiding me toward a successful completion.

Finally, I also wish to express my deep sense of gratitude to my colleagues and all those who have helped me during this project for their endless encouragement and heartiest assistance.

## ABSTRACT

Raigam Pangrease is the Bakery product, produce by Raigam Marketing Services (Pvt) ltd. It is containing mixture of water, coconut oil, Polyglycerol Polyricinoleate-PGPR (emulsifier), Butylated hydroxy Anisole(BHA) and Sodium Benzoate. However it has major drawback associated with product is instability of the emulsion before the expected shelf life.

This research was directed to improve the stability of Pan Grease with low manufacturing cost. It will be mainly depended with appropriate emulsifier combination, optimization of emulsifier ratio, selection of cost effective water/oil ratio, quality control and evaluation the baking performance.

The experiment was done by using several mixtures of PGPR, Tween 20, Tween 60 and lecithin. It has been proved that mixtures of emulsifiers can provide better stability than pure surfactant. PGPR with Tween 60 provide better stability and stability was investigated by phase separation of emulsion under several freeze-thaw cycles. The emulsifier ratio is determined by considering the required HLB value of the oil phase. It shown that the optimum emulsifier ratio for W/O emulsion (long-term stability) was 20% of Tween 60 and 80% of PGPR. However high concentrations of Tween 60 given destructive effect on W/O emulsion stability.

It was shown the optimum water: oil is 4: 1. The use of  $MgSO_4$  was improve the emulsion stability and facilitates to identify the properties of W/O emulsion. According to the results of the research 0.5% is the optimum  $MgSO_4$  concentration for stability improved pan grease. But the concentration of  $MgSO_4$  use during emulsion preparation should be lower than or equal to 1%. Because it was tendency to destabilize.

The resulted sample had low yeast and mould count and total plate count with compare to standard level due to the effect of antimicrobial agent (Sodium benzoate).

Releasing property and crust colour were determined by performing sensory analysis. The P-values obtained clearly emphasize that there is a statistically significant difference between samples as coconut oil, pan grease with  $MgSO_4$ , pan grease with out  $MgSO_4$  and pan grease from another brand in releasing property and crust colour. Based on corresponding rank means and medians, it is possible to say that pan grease with out

MgSO<sub>4</sub> and pan grease from another brand are the best sample in releasing property and pan grease from another brand is the best sample in crust colour. It shown MgSO<sub>4</sub> has an influence on releasing property and crust colour. However the pan grease sample contains MgSO<sub>4</sub> was resulted on dark crust colour and stick with trays.

# CONTENTS

ABSTRACT	I
ACKNOWLEDGEMENT	III
CONTENTS	IV
LIST OF TABLES	XIII
LIST OF FIGURES	IX
LIST OF ABBREVIATIONS	X
<b>Chapter 1</b>	
<b>1.0 Introduction</b>	<b>1</b>
1.1 Background	1
1.2 Overall Objectives	2
1.3 Specific Objectives	2
<b>Chapter 2</b>	
<b>2.0 Literature Review</b>	<b>3</b>
2.1 Pan releasing agent (Pan Grease)	3
2.1.1 Ingredients used in preparation	3
2.1.1.1 Coconut oil	3
2.1.1.2 Polysorbate 60 (Tween 60)	4
2.1.1.3 Polyglycerol Polyricinoleate (PGPR)	4
2.1.1.4 Water	5
2.1.1.5 Butylated hydroxyanisole (BHA)	6
2.1.1.6 Sodium Benzoate	6
2.2 Emulsion	7
2.2.1. Definition	7
2.2.2 Structure of emulsions	7
2.3 Types of emulsions	8
2.4 Test used to identify emulsion type	9
2.4.1 Dilution test	9
2.4.2 Conductivity test	9

2.5 Properties of emulsions	9
2.5.1 Texture, plasticity and consistency	10
2.5.2 Spreadability	10
2.5.3 Viscosity	10
2.5.4 Dispersibility	11
2.5.5 Appearance	11
2.5.6 Particle size	11
2.5.7 Electrical conductivity	12
2.5.8 Ease of formation	12
2.6 Methods of emulsion preparation	12
2.6.1 English (Wet Gum) Method	12
2.6.2 Beaker Method	13
2.7 Stability of emulsions	13
2.7.1 Electrostatic stabilization	13
2.7.2 Steric stabilization	14
2.8 Emulsion instability	15
2.8.1 Creaming	15
2.8.2 Cracking	16
2.8.3 Phase Inversion	17
2.9 Preservation of emulsions	17
2.9.1 Microbiological rancidity	17
2.9.1.1 Preservatives	17
2.9.1.2 Salt	18
2.9.1.3 pH in the water phase	18
2.9.1.4 Starch	18
2.9.1.5 Pasteurization	18
2.9.1.6 Fat blend	18
2.9.1.7 Temperature	
2.9.2 Preservation from oxidation	19
2.10 Emulsifiers	19



2.10.1 Definition of Emulsifiers	19
2.10.2 Major Types of Surfactants	20
2.10.2.1 Synthetic Emulsifying Agents	20
2.10.2.2 Natural Emulsifying Agents	21
2.10.3 Properties of Emulsifiers	22
2.10.3.1 Hydrophile-lipophile Balance (HLB)	22
2.10.3.2 Solubility	23
2.10.3.3 Ionization	24
2.10.3.4 Particle charge	24
2.10.3.5 Chemical reactivity	25
2.11 Selection of emulsifiers	25
2.12 Stability testing	26
<b>Chapter 3</b>	
3.0 Materials and Methodology	28
3.1 Preliminary process was studied and lab sample was produced according to previous process. Sample was tested for stability.	28
3.1.1 Chemicals	28
3.1.2 Check for the stability	29
3.1.3 Check for the Quality	29
3.2 Most appropriate, cost effective, stable formula was selected by changing the ingredients.	29
3.2.1 Selection of best emulsifier combination	29
3.2.1.1 Check for the stability	30
3.2.2 Selection of most stable emulsifier ratio	31
3.2.2.1 Check for the stability	31
3.2.3 Selection of most suitable oil/water ratio	31
3.3 Improvement of stability	32
3.4 Carry out bulk production	32
3.4.1 Check for the stability	33
3.5 Evaluation of baking performance	33
3.6 Microbiological evaluation	33
3.6.1 Enumeration of Yeast and moulds	33
3.6.2 Total plate count	34

<b>Chapter 4</b>	
4.0 Results and Discussion	36
4.1 Drawbacks of preliminary process	36
4.2 Identification of best emulsifier combination	36
4.3 Identification of best emulsifier ratio	38
4.4 Identification of cost effective, most stable water:oil ratio	39
4.5 Stability improvement	40
4.6 Bulk production	41
4.6.1 Evaluation of baking performance	41
4.6.2 Microbiological evaluation	42
4.6.2.1 Enumeration of Yeast and mould	42
4.6.2.2 Enumeration of Total plate count	42
4.7 Developed recipe for Pan grease	43
4.8 Costing	43
<b>Chapter 5</b>	
<b>5.0 Conclusion and Recommendation</b>	<b>45</b>
5.1 Conclusion	45
5.2 Recommendation	45
<b>REFERENCES</b>	<b>46</b>
<b>APPENDIX</b>	<b>48</b>
Appendix 1	48
Appendix 2	49
Appendix 3	50

## LIST OF TABLES

Table	Page No
Table 2.1 Properties of emulsions	8
Table 2.2: HLB values of surfactants	23
Table 2.3 Required HLB values of oils	26
Table 3.1 Sample No 01	30
Table 3.2 Sample No 02	30
Table 3.3 Sample No 03	30
Table 3.4 Selection of most stable emulsifier ratio	31
Table 3.5 Selection of most suitable oil/water ratio	32
Table 3.6 MgSO <sub>4</sub> concentrations	32
Table 4.1 Results of the emulsifier combination selection	37
Table 4.2 results of the emulsifier ratio selection	38
Table 4.3 Results of the water: oil ratio selection	39
Table 4.4 Results of the selection of suitable MgSO <sub>4</sub> concentration	40
Table 4.5 Bakery performance evaluation record	42
Table 4.6 Costing for old recipe	43
Table 4.7 Costing for new recipe	43

## LIST OF FIGURES

<b>Figure No</b>	<b>Page</b>
Figure 2.1 structure of tween 60	4
Figure 2.2 Structure of PGPR	5
Figure 2.3 Structure of water molecule	6
Figure 2.4 Structure of BHA	6
Figure 2.5 Structure of Sodium benzoate	7
Figure 2.6 Electrostatic Stabilization	14
Figure 2.7 Sterically stabilization	15
Figure 2.8 Structure of emulsifier	20
Figure 3.1 Manufacturing procedure of Raigam pan grease	28
Figure 4.1 Scatter plot of phase separation ratio vs emulsifier combinations	37
Figure 4.2 Scatter plot of phase separation ratio vs emulsifier ratios	38
Figure 4.3 Scatter plot of phase separation ratio vs water/oil ratios	39
Figure 4.4 Scatter plot of phase separation ratio vs MgSO <sub>4</sub> concentrations	40
Figure 4.5 Planetary mixer	41

## LIST OF ABBREVIATIONS

BHA	Butylated Hydroxy Anisole
°C	Celsius
CFU	Colony Falling Units
Conc.	Concentrate
ect.	and so on
et al.	and others
eg.	example
°F	Fahrenheit
g	gram
HLB	Hydrophile Lipophile Balance
Hrs.	Hours
i.e.	That is
Kg	Kilograms
L	Liters
Max.	Maximum
mg	milligram
ml	milliliter
min	minute
No.	Number
o/w	oil in water
PGPR	Polyglycerole Polyrecinolate
PDA	Potato Dextrose Agar
Vs.	versus
w/o	water in oil

# CHAPTER 1

## INTRODUCTION

### 1.0 Introduction

#### 1.1 Background

Raigam Marketing services (Pvt) ltd, is a leading company in Sri Lanka engaged in manufacturing of food products including Textured Soya Protein (Soya Meat), Raigam X, Gango, Raimite, varieties of biscuits, pan grease, Neeroga etc.

Pan release is the integral component of the baking process. It is applied in very small amounts as very thin layers on equipment to prevent the sticky nature of baked goods. In other word a thin, uniform, full-coverage spread of release agents provide a slick release surface that enables to slide off without sticking of product. The basic ingredients of pan grease are oil, water and emulsifiers. In addition to that preservatives are added to increase the shelf life and it exists as an emulsion.

An emulsion is a two-phase system consisting of two incompletely miscible liquids, the one being dispersed as finite globules in the other. The process of emulsification is used to disperse one material throughout another in separate droplets, for industry purposes, effecting a dispersion that will retain its physical characteristics for a period of 1 to 2 years at least (Sztukowski *et al.*, 2005). Emulsifier (also known as an emulgent or surfactant) is a substance which stabilizes an emulsion. These agents have both a hydrophilic and a lipophilic part in their chemical structure. All emulsifying agents concentrate at and are adsorbed onto the oil: water interface to provide a protective barrier around the dispersed droplets (Rajah, 2002). There are two types of emulsions, including water in oil and oil in water emulsions. The nature of the emulsion is determined by the emulsifier.

There are three types of emulsion instability: flocculation, where the particles form clumps; creaming, where the particles concentrate towards the surface of the mixture while staying separated; and breaking, where the particles coalesce and form a layer

of liquid (Bot *et al.*, 2007). The stability depends on particle size distribution, the nature, effectiveness and amount of emulsifiers used, the difference in density of the two phases and condition of storage etc. Emulsifiers have HLB values. It is determined by the ratio of the weight of the hydrophilic portion of the molecule to the lipophilic molecules.

Raigam Pan-grease is a mixture of water, palm oil, emulsifier Polyglycerol Polyricinoleate and preservatives like Butylated hydroxy Anisole (BHA) , Sodium Benzoate. The major drawback associated with product is instability of the emulsion before the expected shelf life. Therefore it is beneficial to identify the causes for the instability and make suggestions to improve stability with low cost method.

**1.2 Overall Objective:** Identification the Causes for instability of pan grease emulsion with the time and Development of Cost Effective Procedure to improve the stability of Pan Grease.

**1.3 Specific Objectives:**

- Determination the effect of blend of emulsifiers on stability of pan grease.
- Determination the appropriate emulsifier ratio.
- Determination the optimum water: oil ratio of product with increased stability

# CHAPTER 2

## LITERATURE REVIEW

### 2.0 Literature Review

#### 2.1 Pan releasing agent (Pan Grease)

Release agents are film-forming lubricating oils, solid lubricants, waxes, or fluids that prevent sticking or adhering of materials to an underlying surface. Unlike permanent non-stick coatings, release agents typically require replenishment and are non-curing. There are two basic types of mold release agents; non-permanent and semi-permanent.

It can be used with molds that are made of rubber, plastic, stone, fiberglass, or various metallic elements and alloys. Common mold manufacturing techniques include transfer molding, compression molding, injection molding and liquid injection molding.

##### 2.1.1 Ingredients used in preparation

###### 2.1.1.1 Coconut oil

Coconut oil is a fat, consist of 90% saturated fat. The oil contains predominantly medium chain triglycerides, mainly 92% saturated fatty acids, 6% monounsaturated fatty acids, and 2% polyunsaturated fatty acids. Out of the saturated fatty acids, coconut oil is containing 44.6% lauric acid, 16.8% myristic acid and 8.2% palmitic acid; although it contains seven different saturated fatty acids as whole Oleic is the only monounsaturated fatty acid and Lenoleic is the polyunsaturated fatty acid found in coconut oil.

Unrefined coconut oil is melt at 24-25°C (76°F) and smoked at 170°C (350°F), while refined coconut oil has a higher smoke point of 232°C (450°F)( William, 1925)

Coconut oil has a long shelf life compared to other oils, lasting up to two years due to its high saturated fat content; saturated fats resist rancidity. Coconut oil is best stored





PGPR is strongly lipophilic, soluble in fats and oils but insoluble in water and ethyl alcohol. In chocolates it is used as a viscosity reducing agent. It is virtually always paired with lecithin or another plastic viscosity reducing agent (Azcon *et al.*, 2005).

It can also be used as an emulsifier in spreads and in salad dressings

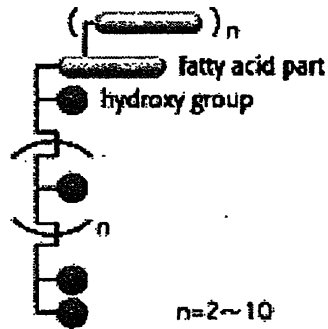
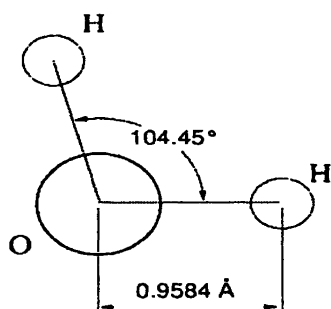


Figure 2.2 Structure of PGPR

Source: <http://www.rikenvitamin.jp/int/emulsifier/basic/kind.html>

#### 2.1.1.4 Water

Water is a common chemical substance that is essential to all known forms of life. In typical usage, water refers only to its liquid form or state, but the substance also has a solid state, ice, and a gaseous state, water vapor. Water has a partial negative charge ( $\sigma^-$ ) near the oxygen atom due to the unshared pairs of electrons, and partial positive charges ( $\sigma^+$ ) near the hydrogen atoms. In water, this happens because the oxygen atom is more electronegative than the hydrogen atoms — that is, it has a stronger "pulling power" on the molecule's electrons, drawing them closer (along with their negative charge) and making the area around the oxygen atom more negative than the area around both of the hydrogen atoms (Martin, 2007).



**Figure 2.3 Structure of water molecule**

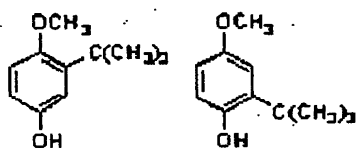
Source: <http://reddit.com/user/austinb/>

#### 2.1.1.5 Butylated hydroxyanisole (BHA)

Butylated hydroxyanisole (BHA) is a mixture of two isomeric organic compounds, 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole. It is prepared from 4-methoxyphenol and isobutylene. It is a waxy solid that exhibits antioxidant properties.

Like butylated hydroxytoluene (BHT), the conjugated aromatic ring of BHA is able to stabilize free radicals, sequestering them. By acting as free radical scavengers, further free radical reactions are prevented (George, 1997).

Evidence that BHA is a carcinogen has been obtained from animal trials. According to Codex standards usage level of BHA for pan grease is 175mg.



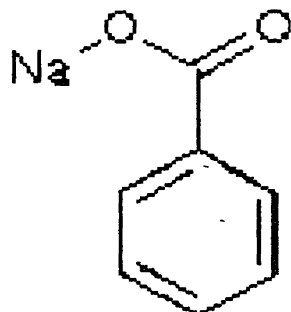
**Figure 2.4 Structure of BHA**

Source: <http://reddit.com/user/austinb/>

#### 2.1.1.6 Sodium Benzoate

Sodium benzoate is the chemical benzoate of soda ( $C_7H_5NaO_2$ ), produced by the neutralization of benzoic acid with sodium bicarbonate, sodium carbonate, or sodium

hydroxide. The salt is not found to occur naturally (George, 1997). According to Codex standards usage level of sodium benzoate for pan grease is 1000mg/kg.



**Figure 2.5 Structure of Sodium benzoate**

## 2.2 Emulsion

### 2.2.1 Definition

Emulsions are biphasic systems consisting of two immiscible liquids, one of which (the dispersed phase) is finely subdivided and uniformly dispersed as droplets throughout the other phase (the dispersion medium) (Sztukowski *et al.*, 2005).

The dispersed phase is also called as the internal phase and dispersion medium as external phase. These immiscible liquids are made miscible by adding a third substance known as emulsifying agent. They stabilize the system by forming a thin film around the globules of the dispersed phase.

### 2.2.2 Structure of emulsions

When water, oil and a surfactant are mixed, the surfactant rests at the water-oil interface. These systems depending on their stability are called emulsions or micro emulsions (thermodynamically stable). Although, the properties for an emulsion and a micro emulsion are different, both obey the same principle: they try to form enough interface for preventing the polar non-polar solvent contact (Adam *et al.*, 1994).

Micro emulsions are very interesting systems, because the oil-surfactant-water interface forms a wide variety of structures to avoid the direct oil/water contact. The

sizes of these structures are in the range of a few hundreds of nanometers, so the solutions are transparent (Fechner *et al.*, 2007). Micelles are the simplest structures: spherical or cylindrical objects formed by surfactant molecules, separating oil and water. Micelles are drops of oil in water and reverse micelles are drops of water in oil (Franck *et al.*, 2006)

Another micro emulsion structure is the lamellae: water and oil consecutive layers separated by surfactant layers conveniently oriented. The lamellae structure is similar to the smectic thermotropic phase; it presents birefringence and maintains the order even at diluted concentrations. This structure is related with the spherulite structure (onion structure). It is possible that spherulites are only out-of-equilibrium transient lamellar phases induced by mechanical work (yet to be proved) or by other stimulus. The thermodynamic stability of spherulites is still under study (Franck *et al.*, 2006).

### 2.3 Types of emulsions

- 1 Oil in water emulsions
- 2 Water in oil emulsions

**Table 2.1 Properties of emulsions**

oil in water emulsion	water in oil emulsion
Water is the dispersion medium and oil is the dispersed phase.	Oil is the dispersion medium and water is the dispersed phase.
They are non greasy and easily removable.	They are greasy and not water washable.
O/W emulsions give a positive conductivity test as water is the external phase which is a good conductor of electricity.	W/O emulsions go not give a positive conductivity test as oil is the external phase which is a poor conductor of electricity.

Source: <http://da.wikipedia.org/wiki/Emulsion>

## **2.4 Test used to identify emulsion type**

Since emulsion (O/W or W/O) looks the same in appearance with naked eyes, therefore certain tests have been developed to differentiate between them. At least two tests should be done to reach a conclusive decision to identity of the emulsion.

### **2.4.1 Dilution test**

In this test the emulsion is diluted either with oil or water. If the emulsion is o/w type and it is diluted with water, it will remain stable as water is the dispersion medium but if it is diluted with oil, the emulsion will break as oil and water are not miscible with each other. oil in water emulsion can easily be diluted with an aqueous solvent whereas water in oil emulsion can be diluted with a oily liquid (Swarbrick *et al.*, 1996).

### **2.4.2 Conductivity test**

This test is based on the basic principle that water is a good conductor of electricity. Therefore in case of o/w emulsion, this test will be positive as water is the external phase. In this test, an assembly consisting of a pair of electrodes connected to a lamp is dipped into an emulsion. If the emulsion is o/w type, the lamp glows (Swarbrick *et al.*, 1996).

## **2.5 Properties of emulsions**

The properties that are most important in a emulsion are appearance feel and effectiveness. For any given formula, these properties will depend on the components or ingredients and their properties, the type of emulsion and the ratio of the major phases. The appearance of a emulsion depends on a variety of more specific properties such as viscosity or consistency and stability or lack of stability.

The type of emulsion, whether the emulsion is oil-in-water or water-in-oil influences both the surface appearance and application qualities. Water-in-oil emulsions usually have a shiny or oily appearing surface versus a sheen or matte surface for oil-in-water. W/O emulsions usually are more oily or emollient in feel (Rajah, 2002).

### 2.5.1 Texture, plasticity and consistency

Physical properties of the emulsion typically being measurable by elasticity, viscoelasticity and work softening. Texture, plasticity and consistency are equally important because they encapsulate those aspects of product benefit sought by the consumer in food applications. For instance, texture is primarily used to describe the state of the emulsion structure, and can range from smooth to floury, grainy, granular, or sandy to coarse and lumpy (Thomasset *et al.*, 2002).

Very close to this are the aspects of consistency, a temperature-dependent property. It describes a smooth, even plastic, state varying from soft, medium, firm and tough to hard and brittle. Since it is possible to encounter smooth as well as grainy and coarse plastic products, plasticity is associated with both these terms (Becher, 1965).

Plasticity remains distinct from other two terms because it describes the ability of a fat or emulsion to retain its shape under slight pressure, such as that encountered during rolling, mixing or spreading (Zukerman *et al.*, 1998).

### 2.5.2 Spreadability

The term 'spreadability' is often used in connection with the solid state of table spreads. It is connected with consistency, texture and shear but is also influenced by the material on which the fat is spread.

### 2.5.3 Viscosity

The viscosity or consistency of an emulsion when the continuous phase is in excess is essentially the viscosity of the continuous phase. As the proportion of internal phase increases, the viscosity of the emulsion increases to a point where the emulsion is no longer fluid. When the volume of the internal phase exceeds the volume of the external phase, the emulsion particles become crowded and the apparent viscosity is partially "structural viscosity"(Kroy, 1999). Under these conditions, particle size, particle charges, and similar relationships assume greater importance in determining the apparent viscosity. Theoretically, the maximum volume that can be occupied by uniform spherical particles for the dispersed phase of an emulsion is 74% of the total volume. Emulsion have been prepared that contain as high as 99% internal phase, and

under these conditions considerable distortion from the usual spherical particle shape of the dispersed phase occurs (Sztukowski *et al.*, 2005).

The viscosity of an emulsion may be controlled as follows;

To lower viscosity

- Increase the proportion of continuous phase
- Decrease the viscosity of the continuous phase
- Add various types of surface-active agents

To raise viscosity

- Add thickeners (finely divided silica)
- Increase the proportion of internal phase
- Reduce the particle size of the emulsion or reduce clumping of existing particles

(William *et al.*, 1991)

#### 2.5.4 Dispersibility

Dispersibility of an emulsion is determined by the continuous phase, thus if continuous phase is water soluble, the emulsion may be diluted with water, conversely, if the continuous phase is oil soluble, the emulsion may be diluted with oil (Sherman, 1968).

#### 2.5.5 Appearance

The appearance of emulsion is a combination of the properties of the two phases, the particle size of the internal phase, and the colours and or pigmentation of the internal and the external phases (William *et al.*, 1991).

#### 2.5.6 Particle size

The particle size and particle size distribution of an emulsion are governed by the quantity and efficiency of the emulsifier, the order of mixing, and the agitation



employed. If the particle size of an emulsion is gradually reduced, the colour and appearance of the emulsion will change (Sherman, 1968).

#### 2.5.7 Electrical conductivity

The electrical conductivity of an emulsion depends on the conductivity of the continuous phase and is closely related to the type of emulsion, o/w or w/o (Vipulanadan, 1998).

#### 2.5.8 Ease of formation

The ease of formation of the emulsion is influenced most by the degree of reduction of interfacial tension, inherent properties of the two phases (density, viscosity, surface properties, etc.), and the amount and nature of the emulsifier (Dukkiml, 1992).

### 2.6 Methods of emulsion preparation

Commercially, emulsions are prepared in large volume mixing tanks and refined and stabilized by passage through a colloid mill or homogenizer. Extemporaneous production is more concerned with small scale methods. Several methods are generally available to the pharmacist. Each method requires that energy be put into the system in some form. The energy is supplied in a variety of ways: trituration, homogenization, agitation, and heat.

#### 2.6.1 English (Wet Gum) Method

It is discussed, the proportions of oil, water, and emulsifier are the same (4:2:1), but the order and techniques of mixing are different. The 1 part gum is triturated with 2 parts water to form mucilage; then the 4 parts oil is added slowly, in portions, while triturating. After all the oil is added, the mixture is triturated for several minutes to form the primary emulsion. Then other ingredients may be added as in the continental method. Generally speaking, the English method is more difficult to perform successfully, especially with more viscous oils, but may result in a more stable emulsion (Sotoyama *et al.*, 1999).

## 2.6.2 Beaker Method

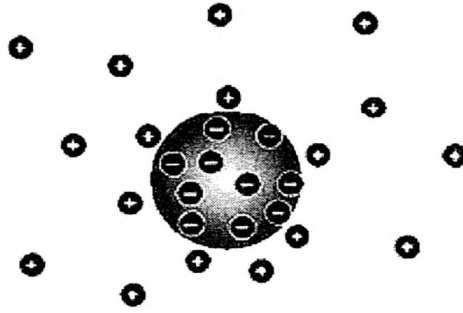
When synthetic or non-gum emulsifiers are used, the proportions given in the previous methods become meaningless. The most appropriate method for preparing emulsions from surfactants or other non-gum emulsifiers is to begin by dividing components into water soluble and oil soluble components. All oil soluble components are dissolved in the oily phase in one beaker and all water soluble components are dissolved in the water in a separate beaker. Oleaginous components are melted and both phases are heated to approximately 70°C over a water bath. The internal phase is then added to the external phase with stirring until the product reaches room temperature. The mixing of such emulsions can be carried out in a beaker, mortar or blender or in the case of creams and ointments, in the jar in which they will be dispensed (Sotoyama *et al.*, 1999).

## 2.7 Stability of emulsions

In order to improve emulsion stability, Understand of the mechanisms of stabilizing an emulsion is its step. Generally there are two mechanisms to achieve emulsion stability.

### 2.7.1 Electrostatic stabilization

This type of emulsion stability is provided by the adsorption of anionic surfactants around an oil droplet resulting in an electrically negative charged layer, which is balanced by positive counter ions. It is called a double layer. When two droplets come too close to each other, the electric double layers will repulse each other to prevent the two droplets from coalescing. If emulsifiers in a product are mainly anionic surfactants, the emulsion is stabilized mainly by electric forces (double layers). Therefore, water hardness, pH and ion concentration (electric conductivity) will greatly affect the emulsion stability (Zhao *et al.*, 2002).



**Figure 2.6 Electrostatic Stabilization**

Source : <http://www.fis.unical.it/~biophysics/lipids.htm>

### 2.7.2 Steric stabilization

If an emulsion contains significant levels of nonionic or polymeric emulsifiers, the system is stabilized mainly by steric effects. These steric effects generate Van der Waals repulsion forces when two particles are too close. The size and type of emulsifiers (or polymers), pH and temperature are important parameters in the stability of these emulsions.

In many oil in water (O/W) emulsions both electrostatic and steric mechanisms

play roles in the stability of emulsions. However, water in oil (W/O) emulsion, also called an invert emulsion will be mainly stabilized by the steric mechanism because of the low dielectric medium of the continuous oil phase. Because only one mechanism can be used, W/ O emulsions are more difficult to stabilize (Zhao *et al.*, 2002).



**Figure 2.7: sterically stabilization**

Source: <http://www.fis.unical.it/~biophysics/lipids.html>

## **2.8 Emulsion instability**

An emulsion is a thermodynamically unstable preparation, so care has to be taken because chemical as well as physical stability of the preparation remains intact throughout the shelf life. There should be not appreciable changed in the mean particle size or the size distribution of droplets of the dispersed phase and secondly droplets of the dispersed phase should be remained uniformly distributed throughout the system. Instabilities seen in emulsion can be grouped as;

- Creaming
- Cracking
- Phase inversion

### **2.8.1 Creaming**

An emulsion is said to cream when the oil or fat rises to the surface, but remains in the form of globules, which may be redistributed throughout the dispersion medium by shaking. An oil of low viscosity tends to cream more readily than one of high viscosity. Increasing the viscosity of the medium decreases the tendency to cream. Creaming is a reversible phenomenon which can be corrected by mild shaking (Sobisch *et al.*, 2002). The factors affecting creaming are best described by stroke's law.

$$V = \frac{2r^2(d_1 - d_2)g}{9\eta}$$

Where V = rate of creaming

r = radius of globules

d1 = density of dispersed phase

d2 = density of dispersion medium

g = gravitational constant

$\eta$  = viscosity of the dispersion medium

The following approaches can be used for decreasing Creaming

**Reduction of globule size:** According to Stoke's law, rate of creaming is directly proportional to the size of globules. Bigger is the size of the globules, more will be the creaming. Therefore in order to minimize creaming, globule size should be reduced by homogenization.

**Increasing the viscosity of the continuous phase:** Rate of creaming is inversely proportional to the viscosity of the continuous phase i.e. more the viscosity of the continuous phase, less will be the problem of creaming. Therefore to avoid creaming in emulsions, the viscosity of the continuous phase should be increased by adding suitable viscosity enhancers like gum acacia, tragacanth etc. (Lerche *et al.*, 2002).

### 2.8.2 Cracking

Occasionally, it happens that an emulsion cracks during preparation, i.e., the primary emulsion does not become white but acquires an oily translucent appearance. In such a case, it is impossible to dilute the emulsion nucleus with water and the oil separates out. Cracking of emulsion can be due to addition of an incompatible emulsifying agent, chemical or microbial decomposition of emulsifying agent, addition of electrolytes, exposure to increased or reduced temperature or change in pH (Lerche *et al.*, 2002).

### 2.8.3 Phase Inversion

In phase inversion o/w type emulsion changes into w/o type and vice versa. It is a physical instability. It may be brought about by the addition of an electrolyte or by changing the phase volume ratio or by temperature changes. Phase inversion can be minimized by using the proper emulsifying agent in adequate concentration, keeping the concentration of dispersed phase between 30 to 60 % and by storing the emulsion in a cool place (Lerche *et al.*, 2002).

## 2.9 Preservation of emulsions

Reduced shelf-life of emulsions can be caused by either microbiologically or by chemical processors.

### 2.9.1 Microbiological rancidity

Yeast, Bacteria or mold are the major causes for microbiological rancidity. Their activity lead to the hydrolytic decomposition of fats and some times the splitting of the proteins in the water phase, with either event resulting in unpleasant taste and odour. Hence prevention of microbiological activity is important. Since micro-organisms cannot grow in fat, it follows that it takes place in the water droplets and on the surface of yellow fat spreads. A number of additives and procedures can be used to address the problem.

#### 2.9.1.1 Preservatives

Benzoates or sorbates are added to the water phase, and benzoic acid or sorbic acid is added to the fat phase, to prevent the growth of micro-organisms. Usually, the decomposition of fats as a result of microbiological activity will result in an increase in it's acid value, from below 0.1% in a neutral oil to 0.3%-1.0%, which is considered too high. It should be noted that addition of sorbic acid or benzoic acid may cause the acid value to increase by 0.5%-1.0% without any decomposition of the fats having taken place (Rajah, 2002).

Dosage rate depend on the composition of the water phase and the degree of contamination. Typical rates are 0.1%-0.2%.

### 2.9.1.2 Salt

Sufficient addition of salt to the water phase should stop the growth of micro-organisms, but this is dependent on the type of micro-organisms. In normal w/o emulsions recipes, a 1% addition of salt will prevent the growth of many micro-organisms, but addition of 2% will stop most. However, equally, in some instances small quantities of salt, (i.e. 0.1-0.2%), may even support the growth of micro-organisms (Rajah, 2002).

### 2.9.1.3 pH in the water phase

A low pH, of 4.0-4.5, retards the growth the growth of micro-organisms.

### 2.9.1.4 Starch

Micro-organisms can thrive in the conditions created by the presences of starches but they can also be adversely affected.

### 2.9.1.5 Pasteurization

Pasteurization of the aqueous phase or the liquid emulsion will improve shelf-life stability.

### 2.9.1.6 Fat blend

Some fats are more prone to hydrolytic activity by micro-organisms than others (e.g. the lauric oils, mainly coconut oil and palm kernel oil, which develop a soapy taste).

### 2.9.1.7 Temperature

Low storage temperatures, of 5-10<sup>0</sup>C for water in oil emulsion is recommended. Most micro-organisms thrive at 20-30<sup>0</sup>C (Kuo *et al.*, 1985).

## 2.9.2 Preservation from oxidation:

Oxidative changes such as rancidity and spoilage mainly due to atmospheric oxygen and effects of enzymes produced by micro-organisms is seen in many emulsions

containing vegetables, mineral oils and animal fats. Antioxidants can be used to prevent the changes occurring due to atmospheric oxygen.

Antioxidants are agents having a high affinity for oxygen and compete for it with labile substances in the formulation. The ideal antioxidant should be nontoxic, nonirritant, and effective at low concentration under the expected conditions of storage and use, soluble in the medium and stable (Ramanathan *et al.*, 1993). Antioxidants for use in oral preparation should also be odorless and tasteless.

Some of the commonly used antioxidants for emulsified systems include alkyl gallate such as ethyl, propyl or dodecyl gallate, butylated hydroxyanisole (BHT), butylated hydroxytoluene (BHT).

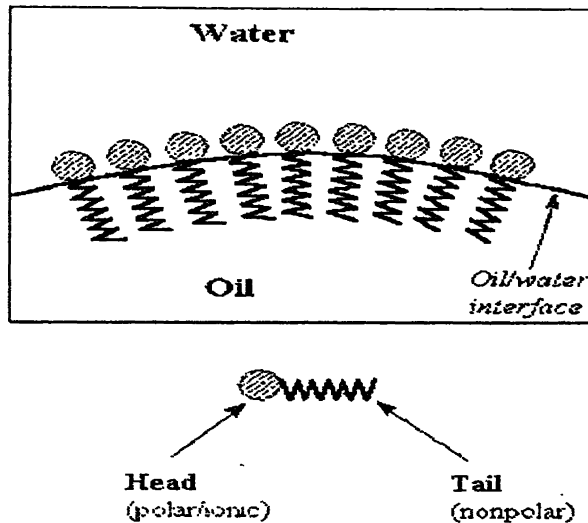
## **2.10 Emulsifiers**

### **2.10.1 Definition of Emulsifiers**

Emulsifying agents are amphiphilic compounds whose chemical structure has both hydrophilic and hydrophobic functions. This special chemical structure gives them their emulsifying properties since by locating themselves at the oil/water interface these molecules can help to stabilize a thermodynamically unstable system (Rajah, 2002).

However, this functional definition is restrictive as, in fact, the role of emulsifying agents goes far beyond their capacity to stabilize emulsions. The amphiphilic structure gives these molecules unique physical properties which allow them to form complexes with biopolymers (starch, protein etc.), and to control the crystallization of fats. These properties have important consequences for both the texture and preservation of foods and on the 'workability' of raw materials used in the composition of foods (Lerche *et al.*, 2002).





**Figure 2.8 structure of emulsifier**

Source: [http://en.wikipedia.org/wiki/Portal:Pharmacy\\_and\\_Pharmacology](http://en.wikipedia.org/wiki/Portal:Pharmacy_and_Pharmacology)

## 2.10.2 Major Types of Surfactants

### 2.10.2.1 Synthetic Emulsifying Agents

- Cationic, e.g., benzalkonium chloride, benzethonium chloride
- Anionic, e.g., alkali soaps (sodium or potassium oleate); amine soaps (triethanolamine stearate); detergents (sodium lauryl sulfate, sodium dioctyl sulfosuccinate, sodium docusate).
- Nonionic, e.g., sorbitan esters (Spans), polyoxyethylene derivatives of sorbitan esters (Tweens, or glyceryl esters) (Stauffer, 1987).

Cationic and anionic surfactants are generally limited to use in tropical, o/w emulsions. Cationic agents (quarternary ammonium salts) are incompatible with organic anions and infrequently used as emulsifiers. Soaps are subject to hydrolysis and may be less desirable than the more stable detergents.

### 2.10.2.2 Natural Emulsifying Agents

A variety of emulsifiers are natural products derived from plant or animal tissue. Most of the emulsifiers form hydrated lyophilic colloids (called hydrocolloids) that form multimolecular layers around emulsion droplets (Russ, 1950). Hydrocolloid type emulsifiers have little or no effect on interfacial tension, but exert a protective colloid effect, reducing the potential for coalescence, by:

- providing a protective sheath around the droplets
- imparting a charge to the dispersed droplets (so that they repel each other)
- swelling to increase the viscosity of the system (so that droplets are less likely to merge)

Hydrocolloid emulsifiers may be classified as:

- vegetable derivatives, e.g., acacia, tragacanth, agar, pectin, carrageenan, lecithin
- animal derivatives, e.g., gelatin, lanolin, cholesterol
- Semi-synthetic agents, e.g., methylcellulose, carboxymethylcellulose
- Synthetic agents, e.g., Carbopols

(Fischer, 1944)

Naturally occurring plant hydrocolloids have the advantages of being inexpensive, easy to handle, and nontoxic. Their disadvantages are that they require relatively large quantities to be effective as emulsifiers, and they are subject to microbial growth and thus their formulations require a preservative. Vegetable derivatives are generally limited to use as o/w emulsifiers (Griffin, 1954).

The animal derivatives general form w/o emulsions. Lecithin and cholesterol form a monomolecular layer around the emulsion droplet instead of the typically multimolecular layers. Cholesterol is a major constituent of wool alcohols and it gives lanolin the capacity to absorb water and form a w/o emulsion. Lecithin (a phospholipid derived from egg yolk) produces o/w emulsions because of its strong hydrophilic character. Animal derivatives are more likely to cause allergic reactions

and are subject to microbial growth and rancidity. Their advantage is in their ability to support formation of w/o emulsions (Griffin, 1954).

### 2.10.3 Properties of Emulsifiers

#### 2.10.3.1 Hydrophile-lipophile Balance (HLB)

The HLB of an emulsifier is an expression of the relative simultaneous attraction of an emulsifier for water and for oil (or two phases of a system to be emulsified) (Robert, 2004).

An emulsifier that is lipophilic in character is assigned a low HLB number, and an emulsifier that is hydrophilic in character is assigned a high number. The mid point is approximately 10, and the assigned values have ranged from 1 – 40 (Michaelides, 2007).

When two or more emulsifiers combine or blended, the HLB values are additive in behavior.

The HLB value is useful because it allows a prediction of the control action or behavior that may be expected from a surfactant, e.g,

A low value, about 4 - water in oil emulsifier

A high value, about 16 – solubilizer (Rajah, 2002)

According to the HLB number, surfactants may be utilized for different purposes:

**Table 2.2: HLB values of surfactants**

Function	HLB Range
Antifoaming agent	1 to 3
Emulsifier, water-in-oil	3 to 6
Wetting agent	7 to 9
Emulsifier, oil-in-water	8 to 18
Detergent	13 to 15
Solubilizer	15 to 20

Source: <http://www.womeninmining.org/pdfs/Surfactants.pdf>

The control also depends upon the “required HLB” of the oil being emulsified. “Required HLB” is a numerical classification of ingredients in accordance with their emulsification requirements. This is the control or type of behavior that can be expected from the emulsifier, rather than the efficiency with which emulsification will be accomplished (Rajah, 2002).

The HLB values may be calculated for surfactants as follows;

$$HLB = 20 \left[ 1 - \frac{S}{A} \right]$$

Where S = saponification number

A = Acid number of fatty acids

### 2.10.3.2 Solubility

The solubility of an emulsifier is a greatest importance in the preparation of the emulsifiable concentrates. It is usually desirable that the concentrate remain homogeneous for an indefinite period and over a wide range of temperatures. The emulsifier must remain “dissolved” through all storage conditions. It is frequently possible to enhance the solubility of one emulsifier with a co emulsifier. HLB is not

the same as solubility, although there is an overall relationship. Thus materials having low values tend to be oil soluble and materials having high values tend to be water soluble. However, two emulsifiers may have the same HLB and exhibit different solubility characteristics.

It is recognized that there is a correlation between the behavior of surfactant and their solubility in water. For example, a water soluble surfactant or blend is normally used to make an o/w emulsion. Also a water soluble surfactant will be used for solubilization and almost completely water soluble surfactant as a detergent. All of the products of these application exhibit aqueous characteristics, i.e., they dilute readily with water and conduct electricity. Under no circumstances would an oil soluble surfactant be used for these purposes. However, to make a w/o emulsion, an oil soluble surfactant would be chosen.

#### 2.10.3.3 Ionization

Surface active agents may be classified as ionic or non-ionic. The ionic types are anionic or cationic, depending on the whether the characteristically surface-active portion of the compound lies in the anion or cation.

In the ionic surface active agents, the hydrophilic portion of the fatty moiety of the molecule is frequently weak. Its attraction to water may be increased by combination with an inorganic ion.

Non-ionic surface active agents depend mainly upon the hydroxyl groups and ether linkages to create the hydrophilic action. Since they do not ionize, they are comparatively insensitive to hard water and electrolytes.

#### 2.10.3.4 Particle charge

Ionic emulsifiers produce emulsions having a dispersed that exhibits particle charge. Emulsions produce with non-ionic agents and without added electrolytes generally exhibit little or no particle charge. In the presence of even small quantities of electrolytes, however, an appreciable charge is apparent.

### 2.10.3.5 Chemical reactivity

For most formulations, an emulsifier should have a low chemical reactivity. Exceptions to this are cases in which subsequent precipitation of the emulsion is desired (McKenna, 2005).

## 2.11 Selection of emulsifiers

With the overall composition approximated, the next step is selection of the emulsifier. An emulsifier has two actions that are distinctly different. The generally recognized action which can be designated as “efficiency” is that of promoting the formation of an emulsion, easier to prepare, producing a finer particle size, and aiding the stability of the emulsion. The second action which occurs along with the preparation of the emulsion, consists in controlling the type of emulsion that is to be prepared, o/w or w/o. It appears that the action of emulsifiers may be related to their structure roughly as follows;

Control, i.e., what the emulsifier will do (make an o/w or w/o emulsion), seems to depend on the “hydrophilic-lipophilic balance” of the emulsifier.

How efficiently the emulsifier will work seems to be related to overall chemical structure, that is, whether the emulsifier is a soap, a partial ester, a complete ester, whether the lipophilic group is saturated, etc (Karsa, 1999).

Emulsifiers are usually quite complex materials. It does indeed appear that the more complex they are, the more efficiently they function. This is recognized in formulating practice, and frequently combinations of two or more emulsifiers are used.

Some times the selection of the blend of emulsifiers can be accomplished by calculating the required HLB of the oil phase, followed by testing a series of emulsifiers of the proper HLB and chemical type to establish which provides the desired viscosity, application properties, stability, etc.

The “required” HLB of a mixture of ingredients to be emulsified may be estimated from the required HLB values of several individual ingredients.

**Table 2.3 Required HLB values of oils**

Class	Required HLB value
Vegetable oil family	6
Silicone oils	8-12
Petroleum oils	10
Typical ester emollients	12
Fatty acids and alcohols	14-15

Source: [http://lotioncrafter.com/pdf/The\\_HLB\\_System.pdf](http://lotioncrafter.com/pdf/The_HLB_System.pdf)

When two or more emulsifiers are combined, their HLB values are additive.

Example: A mixture of 30% Span 80 (HLB = 4.3) and 70% Tween 80 (HLB = 15) has an overall HLB value of:

$$\text{HLB} = (0.3 \times 4.3) + (0.7 \times 15) = 11.8$$

There is generalized equation to determine the ratios of emulsifiers to be used;

$$\text{Required HLB value} = (\text{HLB value of emulsifier A} \times \text{fraction A} + \text{HLB value of} \\ - \text{ emulsifier B} \times \text{fraction B})$$

## 2.12 Stability testing

Stability of emulsions is an important parameter for the formulator. Stability testing of emulsions involves determining stability at long term storage conditions, accelerated storage conditions, freezing and thawing conditions. Stress conditions are applied in order to speed up the stability testing (Rajah, 2002). The stress conditions used for speeding up instability of emulsions include:

Centrifugal force Agitational force Aging and temperature the following physical parameters are evaluated to assess the effect of any of the below stress conditions:

- Phase separation
- Viscosity
- Particle size and particle count

Particle size and size distribution, the freeze-thaw cycling technique used to assess emulsions for stress testing for stability testing result in increase of particle growth and may indicate future state after long storage (Zukerman *et al.*, 1998 ).



# CHAPTER 3

## MATERIALS AND METHODOLOGY

### 3.0 Materials and Methodology

3.1 Preliminary process was studied and lab sample was produced according to previous process. Sample was tested for stability.

#### 3.1.1 Chemicals

#### Equipments

Oil

Beakers

PGPR

Hand mixer ( Max. speed 4000 rpm, Type RQT-

BHA

124A, HP 1/8)

Sodium benzoate

Digital balance (Metler Tolido, China)

Water

Palm oil (32%) + PGPR(3%) + BHA(0.05%)

↓  
Mixing (10 min.)

↓  
Add Sodium Benzoate in to water (65%) and mix

↓  
Add water into oil mixture (within 20 min.)

↓  
Mix thoroughly

Figure 3.1 Manufacturing procedure of Raigam pan grease

325g of water, 160g of coconut oil, 15g of PolyGlycerole PolyRecenoleate (PGPR), 0.025g of BHA (Butylated Hydroxy Anisole) and 1g of Sodium Benzoate were measured accurately by using calibrated digital balance. PGPR was poured into oil with in 10 minutes. The speed of the hand mixer was adjusted to 180rpm while

mixing. Then 0.025g of BHA was added to the mixture and it was mixed thoroughly. Then after Sodium Benzoate was mixed with water and it was added to the oil while mixing using hand mixer. The Speed of the mixer was adjusted to 230rpm. When adding water should be completed with in 20 min, at the beginning very small quantities of water was added.

### 3.1.2 Check for the stability

After Pan grease was prepared, 3 samples was placed in room temperature, under 50°C and freezer. Several freeze-thaw cycles were followed.

### 3.1.3 Check for the Quality

Sample was observed under Light microscope.

## **3.2 Most appropriate, cost effective, stable formula was selected by changing the ingredients.**

### **Chemicals**

PGPR

Lecithin

Tween 60

Tween 20

Coconut oil

Water

BHA

Sodium benzoate

### **Equipments**

Hand Mixer ( Max. speed 4000 rpm, Type RQT-124A,  
HP 1/8)

Beakers

Caliberated Digital balance (Metler Tolido, China)

Measuring cylinder

### 3.2.1 Selection of best emulsifier combination

Required HLB (Hydrophile Lypophile Balance) value of the oil phase was calculated using reference values. Then 4 emulsifiers was selected and two emulsifiers were

mixed at once in according to required HLB value of the oil phase. As four emulsifiers PGPR, Lecithin, Polysorbate 60 and tween 20 were selected. In all mixtures PGPR was added definitely.

Table 3.1 Sample No 01

PGPR	Lecithin
23%	77%

Table 3.2 Sample No 02

PGPR	Polysorbate 60
67%	33%

Table 3.3 Sample No 03

PGPR	Tween 20
70%	30%

This was done by keeping other ingredients constant and by following same procedure.

#### 3.2.1.1 Check for the stability

Three samples were subjected to several freeze-thaw cycles and phase separation was calculated when watery phase become constant. Phase separation was measured by calculating the ratio of the total liquid separated relative to the entire sample volume. Most stable emulsifier combination was selected.

### 3.2.2 Selection of most stable emulsifier ratio

Selected emulsifiers (PGPR and Tween 60) were mixed in different ratios and samples were tested for stability. This was done by keeping other ingredients constant and by following same procedure.

Table 3.4 Selection of most stable emulsifier ratio

Sample No	% of PGPR	% of Tween 60
01	20%	80%
02	40%	60%
03	60%	40%
04	80%	20%
05	50%	50%

#### 3.2.2.1 Check for the stability

Five samples were subjected to several freeze-thaw cycles and phase separation was calculated when watery phase become constant. Phase separation was measured by calculating the ratio of the total liquid separated relative to the entire sample volume. Most stable emulsifier ratio was selected.

#### 3.2.3 Selection of most suitable oil/water ratio

Stability was measured by changing oil/water ratio. Cost effective ratio was identified with maximum stability. Four samples were subjected to several freeze-thaw cycles and phase separation was calculated when watery phase become constant. Phase separation was measured by calculating the ratio of the water/oil ratio was selected. Total liquid separated relative to the entire sample volume.

Table 3.5 Selection of most suitable oil/water ratio

Sample No.	% of oil	% of water
01	25%	75%
02	50%	50%
03	10%	90%
04	75%	25%

### 3.3 Improvement of stability

After selecting most appropriate recipe, several percentages of Magnesium sulphate was added and stability was measured.

Table 3.6 MgSO<sub>4</sub> concentrations

Sample No	1	2	3	4
% of MgSO <sub>4</sub>	0.2%	0.5%	0.7%	1%

### 3.4 Carry out bulk production

#### Chemicals

PGPR

Coconut oil

Tween 60

Water

BHA

Sodium benzoate

#### Equipments

Planetary Mixer

Beakers

Caliberated Digital balance (Metler Tolido, China)

Measuring cylinder

After selecting most appropriate recipe, bulk production was carried out. 1kg of coconut oil was mixed with 200g of PGPR and 50g of tween 20. Mixing speed was adjusted to 2<sup>nd</sup> level. Then 2.5g of BHA was added, 5g of Sodium benzoate and 25g

of MgSO<sub>4</sub> were added to 3.75kg of water. Then it was added to oil phase with in 20 minutes. The speed of the planetary mixer was adjusted to third level.

#### 3.4.1 Check for the stability

Samples of the bulk were subjected to several freeze-thaw cycles. Three samples were kept under 50<sup>0</sup>C and three were kept under room temperature.

### 3.5 Evaluation of baking performance

Baking performance of the 3 samples were evaluated using 7 bakeries. Samples were applied on bread pans and performances of the samples were evaluated using ballet paper.

The four samples were coded in three digits numbers as 385, 228, 337 and 245 seven bakeries were used to sense them for releasing property of bread, crust colour and overall acceptability. Acceptability of four samples was evaluated using 4-point scale subjectively (Appendix 03). Results were analyzed using the MINITAB statistical analysis package according to the Kruskal-wallis test at 5 % level of significance (Appendix 01 and Appendix 02)).

### 3.6 Microbiological evaluation

#### 3.6.1 Enumeration of Yeast and moulds.

##### **Materials:**

Potato Dextrose Agar

Tataric acid

Peptone water

1ml of 10<sup>-1</sup> diluted sample

##### **Equipments**

Petri dishes

Pipette

Incubator (oven)

Quebec colony counter

39g of PDA powder was dissolved in 1L distilled water and kept in the water bath to dissolved with frequent agitation just sufficient to dissolved the all the crystals. 50 ml quantities were distributed in test tubes, plugged with cotton wool, Covered with aluminum lids and autoclaved at 121 °C for 15 minutes.

Dilution of pan grease sample  $10^{-1}$  was plated using the pour plate technique. PDA used as culture media. Then Petri dishes were put in to the oven at 25 °C for 5 days for incubation. After 5 days Petri dishes were observed, under the colony counter. (Before use PDA was added small amount of tartaric acid).

### **3.6.2 Total plate count**

#### **Materials;**

Nutrient agar

Peptone water

1ml of  $10^{-3}$  diluted pan grease sample

#### **Equipments**

Petri dishes

Pipette

Incubator (oven)

Quebec colony counter

23g of Nutrient agar powder was dissolved in 1L distilled water and kept in the water bath and frequent agitation just sufficient to dissolve the all the crystals. 50 ml quantities were distributed in test tubes, plugged with cotton wool, Covered with aluminum lids and autoclave at 121 °C for 15 minutes.

Dilution of pan grease samples  $10^{-3}$  was plated using the pour plate technique. Nutrient agar was used as culture media. Then Petri dishes were put in to the

incubator at 32 °C for 48 hours. After two days shape of colony and viable count was observed.



# CHAPTER 4

## RESULTS AND DISCUSSION

### 4.0 Results and Discussion

#### 4.1 Drawbacks of preliminary process

The emulsifier percentage of the preliminary process is 3%. PGPR is only one emulsifier used in process. Each oily material requires a different strength of emulsifier to ensure stability. This is referred to as the HLB requirement for that oil. The required HLB value of the oil phase is 5.6. To being with, the HLB system is in place to help match the oils used with appropriate emulsifiers. So the HLB value of the emulsifier should match with Required HLB value of the oil. But the HLB value of PGPR is 1. So only PGPR is cannot stabilize the system. PGPR is the highly lipophilic in nature. The water in oil emulsion is stabilized by this type of emulsifier. And also the preliminary process contains 65% of water, 30% of oil and 3% of emulsifier. According to literature, I found that the percentage of emulsifier is not enough to stabilize the system. Many common emulsification systems obey the “20% rule”. This rule states that the total emulsifier content should be 20% of the oil weight (Rajah, 2002).

#### 4.2 Identification of best emulsifier combination

According to nature of the emulsifiers, PGPR, Lecithin, Tween 20 and Tween 60 are selected. By using the high HLB emulsifier, such as polysorbate as a part of the emulsification system will greatly enhance the ease of satisfying HLB requirement of the oil. The PGPR was combined with each one, calculating ratio by considering HLB values of the emulsifiers and required HLB of the oil phase. Oil phase only contains coconut oil. Ratio was calculated according to below equation.

HLB requirement – HLB B / ( HLB A – HLB B ) = amount of fraction A required

Eg ; (5.6 – 14.9) / ( 1 – 14.9) = amount of fraction A

$$= 0.67$$

The results of the stability test as follow;

Table 4.1 Results of the emulsifier combination selection

Sample no	Phase separation ratio(v/v)
01	0.5
02	0.1
03	0.4

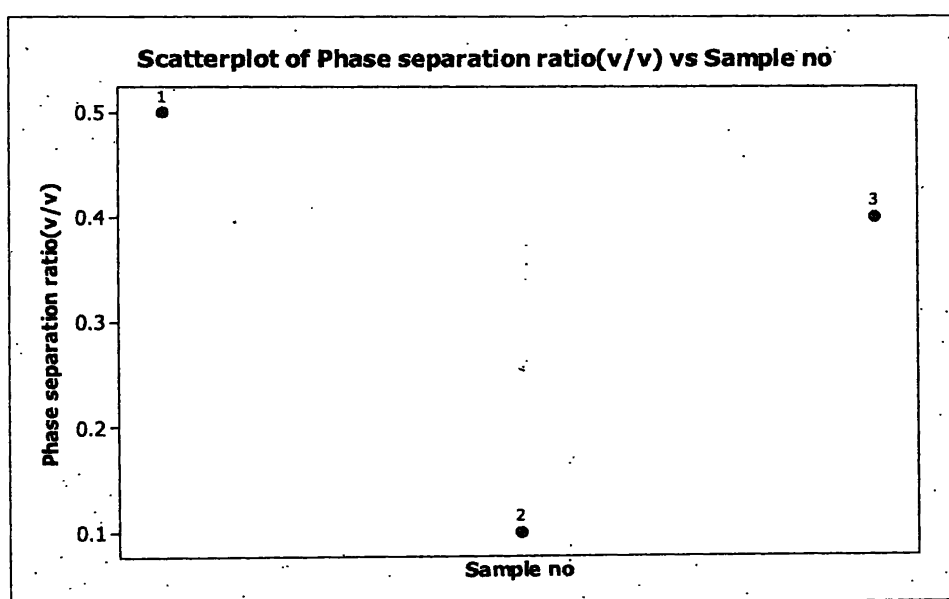


Figure 4.1 Scatter plots of phase separation ratio vs emulsifier combinations

According to above results sample No 02 has higher stability than other 2 combinations. The HLB match is not only consideration. Several other factors may influence the stability of an emulsion. The amount of change in emulsion stability caused by different chemical types of emulsifiers in relation to the amount of change caused by emulsifier HLB.

### 4.3 Identification of best emulsifier ratio

The results of the stability test as follow;

Table 4.2 results of the emulsifier ratio selection

Sample No	Phase separation ratio (V/V)
01	0.5
02	0.3
03	0.1
04	0.0
05	0.4

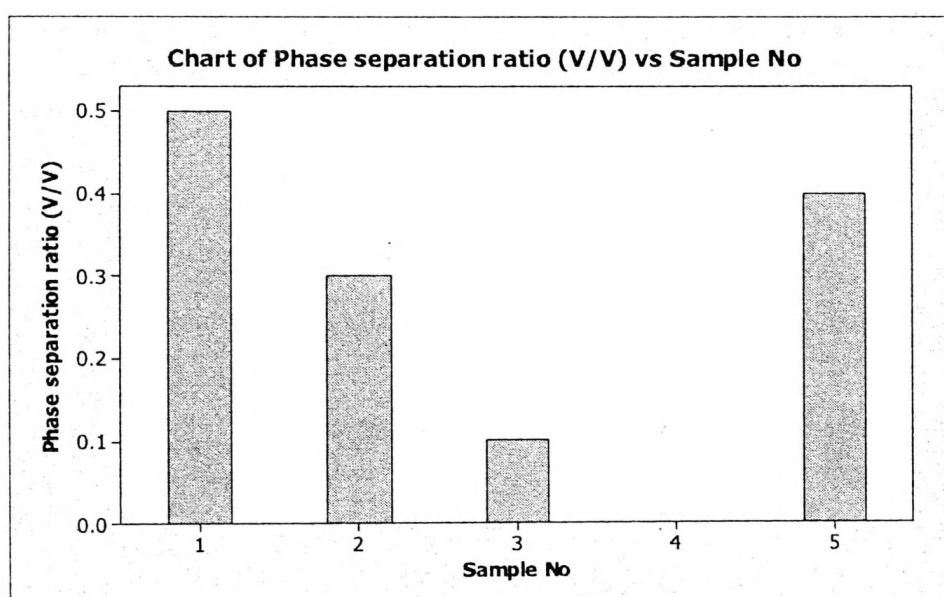


Figure 4.2 Scatter plot of phase separation ratio vs emulsifier ratios

According to above results sample No.4 is the most stable sample. It contains 80% PGPR and 20% Tween 60. The HLB value of the PGPR is 1 and Tween 60 is 14.9(Griffin, 2002). The required HLB of the coconut oil is 5.6 and oil phase only contain coconut oil. So HLB requirement of the oil phase is 5.6. Above two emulsifiers are fulfill this HLB requirement. PGPR is highly lipophilic in nature and Tween 60 is hydrophilic emulsifier. Stability of emulsifier combination is greater than stability of one emulsifier. So use of suitable ratio of the emulsifier combination is better.

#### 4.4 Identification of cost effective, most stable water: oil ratio

The results of the stability test as follows;

Table 4.3 Results of the water: oil ratio selection

Sample No.	Phase separation ratio(v/v)
01	0.0
02	0.0
03	0.6
04	0.4

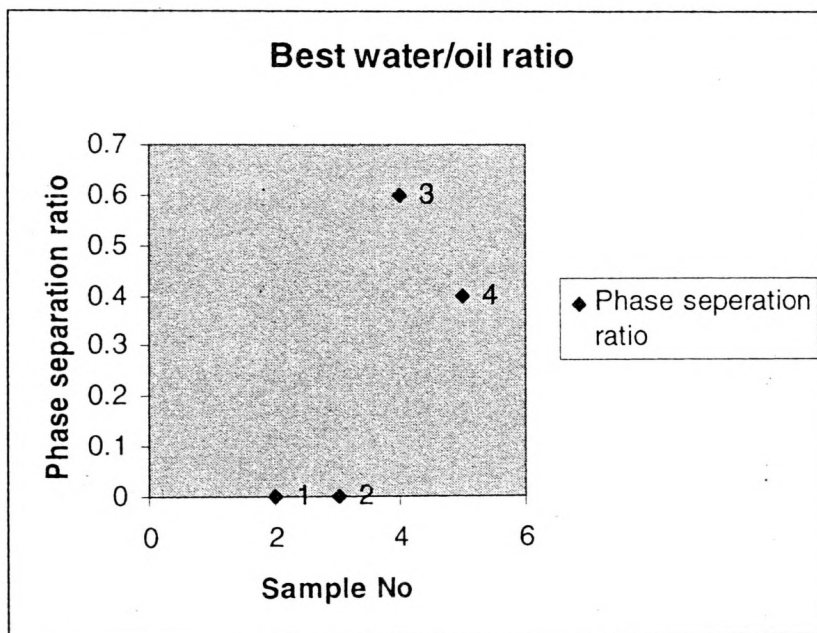


Figure 4.3 Scatter plot of phase separation ratio vs water/oil ratios

Sample No 01 and 02 are stable. Sample No 01 contains 80% of water and 20% of oil. Sample No 02 contains 50% of water and 50% of oil. Among these two samples sample No 01 is most cost effective. The cost of preliminary process is Rs.59.72. In this recipe percentage of emulsifier is higher than preliminary recipe. Cost of production can be reduce by only increasing the H<sub>2</sub>O percentage of the recipe. The viscosity or consistency of an emulsion when the continuous phase is in excess is essentially the viscosity of the continuous phase. As the proportion of the internal phase increases, the viscosity of the emulsion increases to a point where the emulsion

is no longer fluid. When the volume of the internal phase exceeds the volume of the external phase, the emulsion particles become crowded and viscosity increases. The maximum volume that can be occupied by uniform spherical particles for the dispersed phase of an emulsion is 74% of the total volume (Griffin, 2002).

#### 4.5 Stability improvement

Table 4.4 Results of the selection of suitable MgSO<sub>4</sub> concentration

Sample No	Phase separation ratio (v/v)
01	0.1
02	0.0
03	0.0
04	0.2

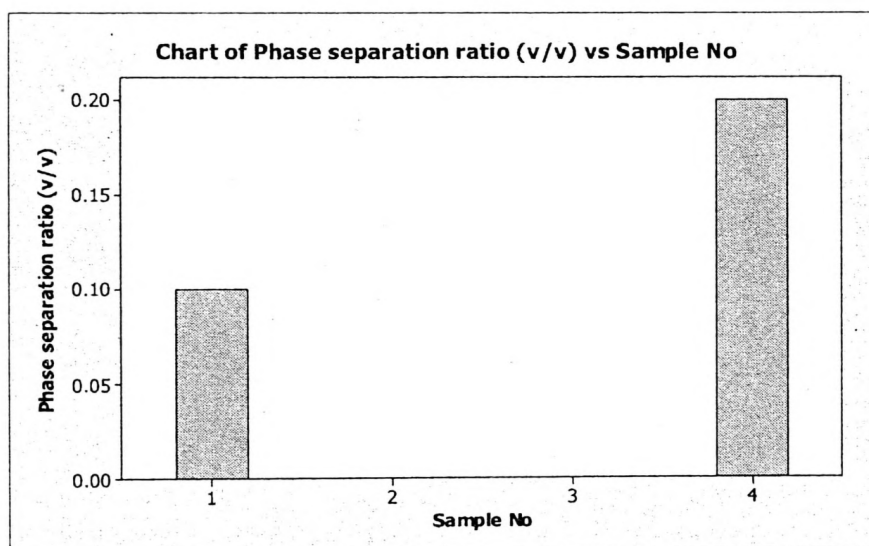


Figure 4.4 Scatter plot of phase separation ratio vs MgSO<sub>4</sub> concentrations

In this study the effect of added MgSO<sub>4</sub> on the properties of water-in-oil emulsions is investigated. Addition of MgSO<sub>4</sub> resulted in improved emulsion stability with respect to coalescence. The average droplet size of the emulsions increased with addition of MgSO<sub>4</sub>. However, the equilibrium oil-water interfacial tension was a decreasing function of MgSO<sub>4</sub>. It is proposed that MgSO<sub>4</sub> decreases the rate of surfactant adsorption at the oil-water interface, which results in increased non-equilibrium interfacial tension during emulsification and increased droplet size. This contrasts

with the interfacial tension measurements, which were made under conditions that favored the attainment of an equilibrium adsorbed surfactant concentrations. When the concentration of  $MgSO_4$  used during the emulsion preparation was greater than or equal to 1%, the tendency to flocculation upon standing increase. Behavior was described to greater sedimentation forces being operative for the emulsions containing a larger average droplet size. The relatively low energetic barrier to flocculation for W/O emulsions appears to make these emulsions more susceptible to differences in sedimentation forces (Kent *et al.*, 2001).

#### 4.6 Bulk production

Bulk production was carried out with 0.5% of  $MgSO_4$  and with out  $MgSO_4$  recipes. After preparation of 5 kg of Pan grease check for the stability. All two samples are stable yet. Planetary mixer is used to bulk production. It has only three speed levels.

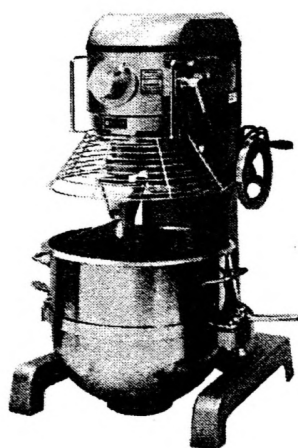


Figure 4.4 Planetary mixer

##### 4.6.1 Evaluation of baking performance

The evaluation was carried out using the seven bakeries for four samples and thus the data generated were dependent on each sample observation. Based on the above feature, Kruskal-Wallis test was selected to analyze the results of attributes. Once samples are seemed to have a statistically significant difference, mean ranks were calculated separately for those attribute, in order to determine the degree of difference and to select the best sample. After treating the data in such a manner outcomes could be able to summarize as appeared in the following table (table 4.5).

Table 4.5. Bakery performance evaluation record

Attribute	P-Value	Mean Rank				Median				Best Sample
		228	245	337	385	228	245	337	385	
Releasing property	0.000	228	245	337	385	228	245	337	385	245 and 337
		24.7	7.9	7.9	17.4	4	1	1	3	
Crust colour	0.001	25	9	12	12	4	1	2	2	245

The P-values obtained clearly emphasize that there is a statistically significant difference between samples coded as 228, 245, 337 and 385 in releasing property and crust colour. Based on corresponding rank means and medians, it is possible to say that sample code 245 and 337 are the best sample in releasing property and 245 is the best sample in crust colour.

#### 4.6.2 Microbiological evaluation

##### 4.6.2.1 Enumeration of Yeast and mould

According to yeast and mould count results, sample was safe. CFUs in yeast and mould count are zero. Standard value of yeast and mould count is less than 10/ml. Therefore, there is sufficient evidence to say that Sodium benzoate (anti microbial agent) is suitable for production of microbiologically safe product.

##### 4.6.2.2 Enumeration of Total plate count

According to Total plate count results, sample was safe. CFUs in Total plate count are 300. Standard value of Total plate count is less than 1000/ml. Therefore, there is sufficient evidence to say that Sodium benzoate (anti microbial agent) is suitable for production of microbiologically safe product.

#### 4.7 Developed recipe for Pan grease

Coconut oil	24.75%
PGPR	4%
Tween 60	1%
BHA	0.05%
Sodium benzoate	0.2%
Water	70%

#### 4.8 Costing

Table 4.6 Costing for old recipe

Item	Unit price(Rs)	Quantity(Kg)	Cost(Rs)
Coconut oil	133.00	0.32	42.56
PGPR	512.13	0.03	15.36
BHA	3030.15	0.0005	1.52
Sodium benzoate	140.00	0.002	0.28
water		0.65	
		1Kg	59.72

Table 4.7 Costing for new recipe

Item	Unit price(Rs)	Quantity(Kg)	Cost(Rs)
Coconut oil	133.00	0.2475	33.25
PGPR	512.13	0.04	20.56
Tween 60	350.00	0.01	3.50
BHA	3030.15	0.0005	1.52
Sodium benzoate	140.00	0.002	0.28
water		0.7	-
		1Kg	58.01



According to results of costing, by using new recipe can produce stability improved product with reducing cost.

## **CHAPTER 5**

### **CONCLUSIONS AND RECOMMENDATIONS**

#### **CONCLUSIONS**

- Mixtures of of emulsifiers can provide better stability than pure surfactants. PGPR with Tween 60 provide better stability.
- Optimal emulsifier ratio for Pan grease for (w/o emulsion) long – term stability is 20% Tween 60 and 80% of PGPR.
- Long- term stable cost effective water : oil ratio is 4 : 1.
- Crust colour of bread becomes dark brown in resulted sample with added  $MgSO_4$  and also there was ability to stick with trays.

#### **RECOMMENDATIONS**

- It is necessary to check the stability of developed Pan Grease mixture until phase separation. Because it is important to determine the shelf-life of the product
- Further research can be used to improve the stability of Pan Grease mixture
- It is better to replace the new machines to mixing purpose
- It is better to use new machines for filling purpose of Pan Grease

## REFERENCES

- Becher, P., and Berkmeier, R. ( 1964) The determination of HLB, first edition, 52-54pp
- Bot, A., Bouwman, G.W. and Duval, F.P., (2006) Effect of processing on droplet cluster structure in emulsion gels, International Journal of Food Science & Technology, Volume 6, 311-317pp
- Bot, A., Franck, P., and Bouwman, G. (2007) Effect of processing on droplet cluster structure in emulsion gels, Food Hydrocolloids, Volume 21, 844-854pp
- Decker, D. J., Donnelly, J.L., and McClements, D.J., (1998) Iron-Catalyzed Oxidation of Menhaden Oil as Affected by Emulsifiers, Journal of Food Science 63 (6), Volume 63, 176-215pp
- Dukkim, J., Geun Song, M., Ho Jho, S., and Yum Kim, J. (1982) Rapid evaluation of water-in-oil emulsion by turbidity ratio measurement, Journal of Food science, volume 47, 678-682pp
- Fechner, A., Knoth, A., Muschiolik, G., and Scherze, I. (2007) Stability and release properties of double-emulsions stabilised by caseinate-dextran conjugates, Food Hydrocolloids, Volume 21, 943-952pp
- Garti, N., and Remon, F., (1984) Relationship between nature of vegetable oil, emulsifier and the stability of w/o emulsion, International Journal of Food Science & Technology, Volume 19, 711-717pp
- George, J.B. (1987) New ingredients in food processing, First edition, CBS publishers and distributors, 164p
- Griffin, W.C. (1954) Calculation of HLB values of nonionic surfactants, published by JSCC, 249p
- Karsa D., (1999), Design and Selection of Performance Surfactants, Journal of Colloid and Interface Science, Vol.242, 203-205pp

- Li, F. and Vipulanadan, C., Conductivity of water in oil microemulsion system, (Accessed from: [http://gem1.cive.uh.edu/content/conf\\_exhib/02\\_poster/10.html](http://gem1.cive.uh.edu/content/conf_exhib/02_poster/10.html), 15<sup>th</sup> of may 2007)
- Matz, S.A. (1996) Bakery technology and Ingredients, Third edition, CBS publishers and distributors, 729p
- McKenna, B.M., (2005) Design and Selection of Performance Surfactants, Journal of Colloid and Interface Science, Volume 1, 146-208pp
- Rajah, K.K. (2002) Fats in food Technology, First edition, published by Sheffield Academic press ltd., 302p
- Stokoe, W. N., (1928) The rancidity of coconut oil produced by mould action, (accessed from:<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1252092>)
- Swarbrick, J., (1996) Emulsion preparation and stabilization, (accessed from: <http://pharmlabs.unc.edu/emulsions/text.html>, 03<sup>rd</sup> of June 2007)
- Sztukowski, D., Urrutia, P., and Yarranton, H.W. (2005) Effect of interfacial rheology on model emulsion coalescence, Journal of Colloid and Interface Science, Volume 310,246-252pp
- Whitehurst R.J., (2004), Emulsifiers in food technology, Bakers' Journal, Volume 34, 101-212pp

# APPENDIX

## Appendix 1

### Kruskal-Wallis Test: C2 versus C1

Kruskal-Wallis Test on C2

C1	N	Median	Ave Rank	Z
228	7	4.000	24.7	3.79
245	7	1.000	7.9	-2.44
337	7	1.000	7.9	-2.44
385	7	3.000	17.4	1.09
Overall	28		14.5	

H = 20.61 DF = 3 P = 0.000

H = 22.30 DF = 3 P = 0.000 (adjusted for ties)

## Appendix 2

### Kruskal-Wallis Test: C2 versus C1

Kruskal-Wallis Test on Result

sample	N	Median	Ave Rank	Z
228	7	4.000	25.0	3.90
245	7	1.000	9.0	-2.04
337	7	2.000	12.0	-0.93
385	7	2.000	12.0	-0.93
Overall	28		14.5	

H = 15.83 DF = 3 P = 0.001

H = 18.16 DF = 3 P = 0.000 (adjusted for ties)

### Appendix 3

Very good	1
Good	2
Not – bad	3
Bad	4

	228	245	337	385
Releasing property				
Crust colour				
Overall acceptability				

**National Digitization Project**

***National Science Foundation***

Institute : Sabaragamuwa University of Sri Lanka

1. Place of Scanning : Sabaragamuwa University of Sri Lanka, Belihuloya

2. Date Scanned : 2017-09-25

3. Name of Digitizing Company : Sanje (Private) Ltd, No 435/16, Kottawa Rd,  
Hokandara North, Arangala, Hokandara

**4. Scanning Officer**

Name : B.A.C. Badarayanan

Signature : 

**Certification of Scanning**

*I hereby certify that the scanning of this document was carried out under my supervision, according to the norms and standards of digital scanning accurately, also keeping with the originality of the original document to be accepted in a court of law.*

**Certifying Officer**

Designation : Librarian

Name : T. N. Neighsoorei

Signature : 

Date : 2017-09-25

**Mrs. T. N. NEIGHSOOREI**  
(MSSc, PhD, ASLA, BA)  
Librarian  
Sabaragamuwa University of Sri Lanka  
P.O. Box 02 Belihuloya, Sri Lanka  
Tele: 094 45 2280045  
Fax: 094 45 2280045

*“This document/publication was digitized under National Digitization Project of the National Science Foundation, Sri Lanka”*