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Development of a frozen yogurt fortified with a nano-emulsion containing purple rice bran oil

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**DEVELOPMENT OF A FROZEN YOGURT FORTIFIED WITH A NANO-EMULSION
CONTAINING PURPLE RICE BRAN OIL**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Food Science

by
Luis Alonso Alfaro Sanabria
B.S., Zamorano University, Honduras, 2007
December 2012

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ABSTRACT

The U.S. frozen dessert business, including retail and foodservice markets, is the largest in the world. There is a need for deserts that offer better nutritional attributes, such as antioxidants than traditional deserts. Purple rice bran oil (PRBO) is an excellent source of natural antioxidants including tocopherol, tocotrienol, and oryzanol. Adding nano-emulsions of purple rice bran oil (NPRBO) to many products including frozen yogurts (FY) will improve the nutritional profile of the product by substantially increasing the natural antioxidant content. This fortification may have the potential to broaden the FY market.

The objectives of this study were to develop and evaluate a frozen yogurt with NPRBO. A stable emulsion was prepared using PRBO (10%), sodium caseinate (5%) and water (85%). The emulsion was sonicated followed by ultra-shearing to produce the nano-emulsion. The nano-emulsion was mixed with the frozen yogurt ingredients to produce a FY-containing-NPRBO (FYNRO). Plain-frozen-yogurt (PFY) and frozen-yogurt-with-sodium caseinate (5%) (FYSC) were used as controls. The yogurts were analyzed for total aerobes (ACP), lactic acid bacteria (LAB), thiobarbituric-acid-reactive-substances (TBARS), peroxide value (PV), rheological properties, microstructure, and α -tocopherol, γ -tocotrienol, and γ -oryzanol content. Triplicate experiments were conducted and data was analyzed at $\alpha = 0.05$. All of the frozen yogurts had similar texture, melting rate, overrun, fat instability, pH, color and microbial counts. No coliforms were found, ACP counts were <10 CFU/g, and LAB counts were similar for all FY. PV's (mmol/kg of FY) were 3.55, 2.38 and 2.13 for FYNRO, FYSC, and PFY, respectively, while TBARS (μ moles malonaldehyde /kg of FY) values 5.2, 4.57, and 4.66 for FYNRO, FYSC, and PFY, respectively. FYNRO had 6.51 (μ g/g oil) α -tocopherol, 12.89 (μ g/g oil) γ -tocotrienol,

and 754.44 ($\mu\text{g/g}$ oil) γ -oryzanol. No significant physical, chemical, and or microbial properties changed in FYNRO during six weeks of storage at -22 °C. The study demonstrated that FY could be fortified with NPRBO to create a product with unique marketing potential.

CHAPTER 1. INTRODUCTION

Frozen yogurt is a frozen dessert characterized by having the texture properties of ice cream combined with the acidic taste of yogurt. Its process consists in mixing all ingredients to make natural stirred yogurt with stabilizers/emulsifiers and sugar, then freezing the mix in a conventional ice cream freezer (Tamine and Robinson 2007). Frozen yogurt popularity has increased and continues to grow; making it one of the most frequently consumed frozen desserts around the world.

Frozen yogurt's attractiveness to consumers include providing a low fat replacement for ice cream and the probiotic benefits of the live cultures present in the yogurt (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). According to Tamine and Robinson (2007), the official standard of identity for frozen yogurts has not been specified yet in most countries. However, some references specify that the final product should have a minimum of 0.15% titratable acidity (expressed as lactic acid), >3.25% milk-fat, a pH <5, and a minimum yogurt content >70% (Westerbeek, 1996; Marshall *et al.*, 2003).

Cholesterol levels in the US population are alarming. According to the Center for Diseases Control (CDC, 2012), approximately one in every six adults (16.3% of the U.S. adult population) has high cholesterol. In addition, the rate for deaths related with heart disease was 192.4 per each 100,000 population in 2008 (NVSS, 2011). As a result, a product that could help lower cholesterol levels could be both beneficial and highly marketable.

Rice bran oil (RBO) has been used for centuries in Asian countries and its popularity is increasing in the US due to its health benefits (Orthofer 2005). Rice bran oil is balanced in

saturated, monounsaturated, and polyunsaturated fatty acids and meets the American Heart Association (AHA) recommendations (AHA 2012). RBO which is rich in nutraceutical compounds such as tocopherols, tocotrienols and gamma oryzanol has been linked with health improvement by lowering serum cholesterol and having antioxidant functionality in humans (Most et al. 2005; El-Rhaman 2010; Kerckhoffs et al. 2002). Recently, a new rice variety harvested in the US called “purple rice” is gaining more attention due to the bioactive compounds found in the bran (Jang and Xu 2009).

Lately, the food industry has a strong interest in nano-emulsions due to its benefits such as stability and functional performance in food processing (Hu et al. 2004; Tadros et al. 2004; Anton et al. 2007). The objectives of this study were to produce a nano-emulsion containing purple rice bran oil (PRBO), and to determine the effect of addition of a nano-emulsion containing PRBO on the physicochemical characteristics of frozen yogurt. A nano-emulsion containing PRBO (NPRBO) was produced and a frozen yogurt containing NPRBO (10% w/v) was developed and the physical, chemical, and microbiological characteristics were evaluated during six weeks of frozen storage at -22 °C. Plain-frozen-yogurt (PFY) and yogurt-with-sodium caseinate (5%) (FYSC) were used as controls. Triplicate experiments were conducted and data was analyzed at $\alpha=0.05$.

CHAPTER 2. LITERATURE REVIEW

2.1 Frozen yogurt

2.1.1 Frozen yogurt definition and classification

The standard of identity of North Carolina State (02 NCAC 09K.0214, 2000) defines frozen yogurt as “the food prepared by freezing while stirring, a pasteurized mix consisting of the ingredients provided for ice cream which may contain other ingredients permitted under the Federal Food, Drug, and Cosmetic Act (21 USC 321 et seq.). All dairy ingredients are cultured after pasteurization by one or more strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Frozen yogurt, exclusive of any flavoring, should contain not less than 3.25 % milk fat, and not less than 8.25 % milk solids not fat. The finished frozen yogurt shall weigh not less than five pounds per gallon. The titratable acidity of frozen yogurt should be not less than 0.5 %, calculated as lactic acid, except if the frozen yogurt primary flavor is a non-fruit characterizing ingredient(s). This characteristic acidity is developed by the bacterial activity and no heat or bacteriostatic treatment, other than refrigeration, which may result in destruction or partial destruction of the organisms, shall be applied to the product after culturing. The product, when in package form, shall be labeled according to applicable Sections of 2 NCAC 9B .0016(f)(2) (21 CFR Part 101)”.

According to Tamine (2007), frozen yogurt can be classified into three categories: soft, hard, or mousse. Soft frozen yogurt consists of a mix of 80% yogurt base (cold) with 20% fruit syrup base and stabilizer/emulsifier; hard frozen yogurt is a mix containing 65% yogurt base with 35% fruit syrup plus stabilizer/emulsifier; and mousse frozen yogurt is a mix of the yogurt with hot mousse base mixture (skim milk, sugar and stabilizer/emulsifier).

The ice cream and frozen dairy products industry is increasing. According to the International Dairy Foods Association (IDFA), a total of 1.481 billion gallons of frozen yogurt mix were produced in 2010. In addition, frozen yogurt production increased by 8.1 % with 49.7 million gallons produced in 2010 compared with 2009 (NASS, 2012).

2.1.2 Frozen yogurt health benefits

Frozen yogurt is a dessert that combines the texture of ice cream with the nutritive and healthy properties of yogurt (Rezaei, *et al.*, 2011). Fermented products like yogurt possess probiotic properties. According to Havenaar *et. al* (1992), probiotics can be defined as “mono or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora”. Probiotics are recognized by generating an optimum balance in the microbial population in the digestive tract and this is associated with good nutrition and health (Rybka Kailasapathy, 1995). Bacteria related with these probiotic properties are mainly *lactobacilli* and *bifidobacteria* (Lourens-Hattingh and Viljoen, 2001). According to Holzapfel *et al.* (2001), a notable number of microorganisms have been considered as probiotics (Table 2.1).

Probiotic bacteria have increasingly been incorporated into foods as dietary adjuncts. The most popular bacteria used for fermented dairy products such as yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The survival of these lactic acid bacteria (LAB) in the human intestine have been studied (Del Campo, *et. al*, 2005; Johansson *et al.*, 1998; Kullen *et al*, 1997; Lick *et al*, 2001). The microflora, being composed mainly of *Lactobacillus* species, will occupy the surface area of the intestine. Therefore, there is a better absorption of nutrients and the secretion of lactic acid and other antimicrobial compounds would provide protection from

growth of pathogens. A summary of health promoting attributes from yogurt are reported in Table (2.2) (Tamime and Robinson, 2007).

Table 2.1 Microorganisms considered as probiotics

Lactobacillus species	Bifidobacterium species	Other lactic acid bacteria	Nonlactic acid bacteria
<i>L. acidophilus</i>	<i>B. adolescentis</i>	<i>Enterococcus faecalis</i>	<i>Bacillus cereus</i> var. <i>toyoi</i> <i>Escherichia coli</i> strain
<i>L. acidophilus</i>	<i>B. animalis</i>	<i>Enterococcus faecium</i>	<i>nissle</i> <i>Propionibacterium</i>
<i>L. amylovorus</i>	<i>B. bifidum</i>	<i>Lactococcus lactis</i> <i>Leuconostoc</i>	<i>freudenreichii</i>
<i>L. casei</i>	<i>B. breve</i>	<i>mesenteroides</i>	<i>Saccharomyces cerevisiae</i>
<i>L. crispatus</i>	<i>B. infantis</i>	<i>Pediococcus acidilactici</i>	<i>Saccharomyces boulardii</i>
<i>L. delbruekii</i> <i>subsp. Bulgaricus</i>	<i>B. lactis</i>	<i>Sporolactobacillus</i> <i>inulinus</i> <i>Streptococcus</i>	
<i>L. gallinarum</i>	<i>B. longum</i>	<i>thermophilus</i>	
<i>L. gasseri</i>			
<i>L. johnsonii</i>			
<i>L. paracasei</i>			
<i>L. plantarum</i>			
<i>L. reuteri</i>			
<i>L. rhamnosus</i>			

2.2 Nano-emulsions

2.2.1 Definition and properties of nano-emulsions

A food emulsion consists of two immiscible liquids such as oil and water, with one of the liquids dispersed as small spherical droplets in the other (McClements, 1999). Emulsions can be classified into two types, oil-in-water (o/w) and water-in-oil (w/o). Most food emulsions are of the o/w type (Fennema, 2008). According to Dickinson and Patino (1999), in most food emulsions the diameters of the droplets usually range between 0.1 and 100 µm. These types of emulsions are thermodynamically unstable and tend to breakdown quickly over time.

Table 2.2 Some health-promoting activities attributed to dairy starter cultures and an indication of their likely validity for humans

Action/effect	Alleged health benefit	Established in humans ^{a,b}
In digestive tract	Active against <i>Helicobacter pylori</i>	
	Enhanced lactose digestion	✓
	Stimulation of intestinal immunity	
	Stabilization of Crohn's disease	
	Stimulation of intestinal peristalsis	
On intestinal microflora	Improves balance between microbial populations	Increase in faecal bifidobacteria
	Decrease in faecal enzyme activity	✓
	Colonization of intestinal tract	✓
	Reduced carrier time for <i>Salmonella</i> spp.	
On diarrhea	Prevention/treatment of acute diarrhea	✓
	Prevention/treatment of rotavirus diarrhea	✓
	Prevention of antibiotic-induced diarrhea	✓
Other effects	Improved immunity to disease	
	Suppression of some cancers	
	Reduction in serum cholesterol	
	Reduction in hypertension	

^aMore than one publication and no conflicting evidence. ^bA tick indicates confirmed in humans. Source: Tamime and Robinson (2007)

Nano-emulsions are nanometric-sized emulsions with droplet sizes in the range of 20-300 nm (Anton *et al.*, 2007; Solans *et al.*, 2005, Anton *et al.*, 2008, Jafari, Yinge and Bhandari, 2006). The food industry is highly interested in nano-emulsions because of certain inherent advantageous of nano-emulsions. The very small droplet size results in low gravity forces such as that Brownian motion may be sufficient to prevent creaming or sedimentation occurrence during storage. Weak flocculation is prevented and this enables the system to remain dispersed with no separation. The significant film thickness prevents any thinning or disruption of the liquid film between the droplets (Tadros *et al.*, 2004). According to Qian & McClements (2011), a key advantage of nano-emulsions is they can be made to be optically transparent. Thus, nano-

emulsions can be used to incorporate lipophilic functional components into transparent aqueous beverage products.

Some studies have also advised that the bioavailability of encapsulated non-polar components is higher in nano-emulsions than conventional emulsions due to the small particle size and high surface-to-volume ratio (Acosta, 2009; Huang, Yu & Ru, 2010). McClements and Xiao (2012) have proposed a potential biological fate of ingested nano-emulsions. They state that absorption of nano-emulsions by cells could be done through a number of different mechanisms: *paracellular mechanism*, in which particles that are small enough can pass between the narrow gaps of epithelial cells. A second mechanism, which is the *transcellular mechanism*, particles that are sufficiently small are absorbed directly through epithelial cell membranes by either passive or active transport mechanism (Fig 2.1).

Troncoso *et al.* (2012) studied the *in vitro* digestibility of nano-emulsions. O/W nano-emulsions stabilized by Tween 20 were evaluated by measuring the rate of lipid hydrolysis under simulated intestinal fluid conditions using a pH-Stat method. It was found that the lipid digestion profile depended strongly on the particle size of the nano-emulsions. As the lipid droplet size was decreased, the lag time was decreased, the digestion rate was increased, and the total amount of free fatty acids released was increased.

2.2.2 Stability of nano-emulsions

Although stable in regards to sedimentation or creaming (Solans *et al.*, 2005), nano-emulsions may be susceptible to Ostwald ripening, which results from differences in solubility between small and large droplets (Tadros *et al.* 2004). Ostwald ripening leads to condensation of droplets

into a single drop (Fennema, 2008). This phenomenon can be modeled for two droplets of radii r_1 and r_2 ($r_1 < r_2$) by the following equation:

$$\left(\frac{RT}{V_m}\right) \ln \left[\frac{c(r_1)}{c(r_2)}\right] = 2\gamma \left(\frac{1}{r_1} - \frac{1}{r_2}\right) \quad (2.1)$$

Where (RT/V_m) is called the characteristic length, $c(r)$ is the solubility surrounding a particle of radius r , and γ is the interfacial tension at the disperse phase-continuous phase interface.

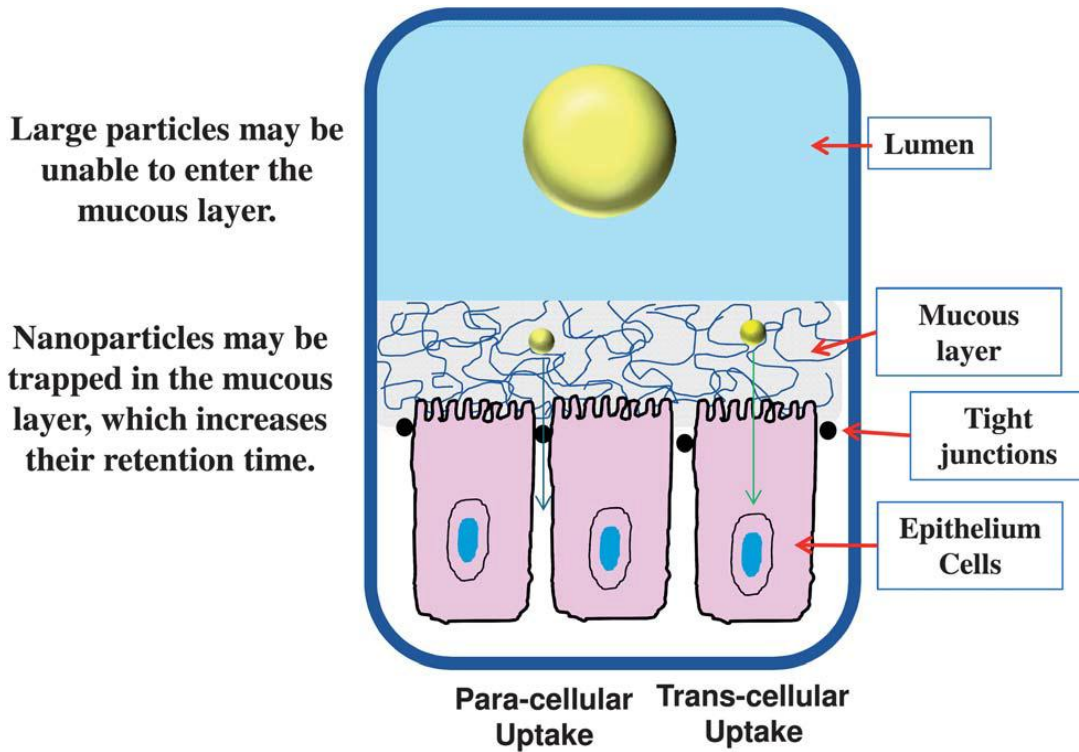


Figure 2.1 Schematic diagram of the potential absorption mechanisms of particles by cells in the GI tract. Source: McClements and Xiao (2012).

2.3 Ultrasonication

2.3.1 Definition of ultrasonication

Ultrasound is defined as sonic waves with frequencies >20 kHz which are above the range audible to humans (Feng, Barbosa-Canovas, and Weiss, 2011). Ultrasound has been divided into two main categories: low intensity (“non-destructive”) and high intensity (also called “power ultrasound”). Low-intensity ultrasound uses low energy, with power intensities below 1 W/cm^2 and high frequencies between 1-10 MHz. High-intensity ultrasound uses high energy with power intensities above 10 W/cm^2 and low frequencies in the range of 10-100 kHz (Povey and Mason, 1998). The main phenomena occurring during ultrasonication is the formation of microbubbles in a liquid media, this bubble formation is known as cavitation. Cavitation is explained as the cavitation or the Blake threshold (P_B) (Eq. 2.2), which is a function of the solution vapor pressure (P_v), the surface tension (σ), the initial nanobubble radius (R_0), and the system pressure (P_0) (Leighton, 1994).

$$P_B = P_0 - P_v + \frac{4}{3} \sigma \sqrt{\frac{2\sigma}{3(P_0 + 2\frac{\sigma}{R_0} - P_v)R_0^3}} \quad (2.2)$$

2.3.2 Use of ultrasonication to produce nano-emulsions

Ultrasonication is a well-known method to produce nano-emulsions and nanoparticles. According to Anton *et al.* (2008), the cavitation phenomena is responsible for the formation of nano-emulsions using ultrasonication. The high energy required to produce a nano-emulsion can be described by the Laplace pressure (p), which is the difference in pressure between inside and outside the droplets (Tadros *et al.*, 2004):

$$p = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad (2.3)$$

Where R_1 and R_2 are the principal radii of curvature of a drop, thus for a spherical drop, $R_1 = R_2 = R$, therefore this equation can be described as follows:

$$p = \frac{2\gamma}{R} \quad (2.4)$$

Jafari *et al.* (2006) evaluated the use of sonication to produce nano-emulsions. Oil (20% w/w) in water nano-emulsion using whey protein concentrate (WPC)/maltodextrin as emulsifying agents was produced using a 24 KHz sonicator (Dr Hielscher series, Model UP 400S) at full power (100 % amplitude). It was found that it was possible to produce an emulsion with 243 nm size after 20 seconds sonication. Jafari *et al.* (2006) states that the powerful shock waves produced by cavitation radiate throughout the solution, breaking the dispersed liquid into very small drops. In addition, Canselier *et al.* (2002) assert that a two-step mechanism has been discussed for ultrasound emulsification: a first step, in which a combination of interfacial waves and instability leads to the eruption of dispersed phase droplets into the continuous phase; and a second step that consists of reducing size of droplets through cavitation near the interface. The disruption and mixing of shock waves and cavitation explain the very small droplet size obtained.

2.4 Rice bran oil (RBO)

2.4.1 Rice bran oil production

Rice is one of the most produced grains in the world for human consumption. According to the USDA (2011), the world rough and milled rice production for the year 2010 was 659.2 and 440.6 million metric tons respectively. Rice bran (RB) is the hard outer layer of the rice grain and together with the germ consists of about 10% of the grain composition. The structure of the rice grain is given in Figure 2.2.

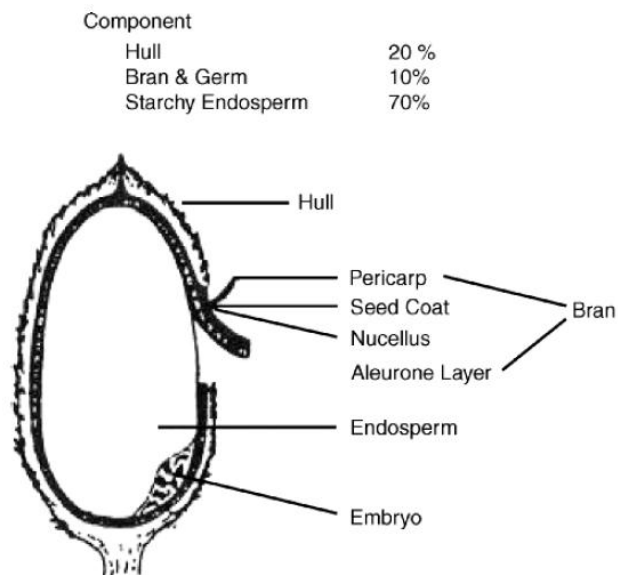


Figure 2.2 Composition of rice grain. Source: (Orthoefer, 2005)

Depending on the variety, rice bran contains 12-25% oil. Rice bran oil has been widely used in Asian countries such as Japan, Korea, China, Taiwan, Thailand, and Pakistan (Cheruvanky *et al.*, 2003), and it is preferred for its flavor and odor. Rice bran oil world production is estimated at 722.2 thousand metric tons, with India, China, and Japan as leading producers. According to Orthoefer (2005), more than half of rice is processed in small rice mills, leaving approximately 20-25 million metric tons of bran available for oil production.

2.4.2 Rice bran oil composition and health benefits

Rice bran oil (RBO) is mainly composed by triglycerides, which consist about 80% of the oil. Minor constituents of RBO are phospholipids, glycolipids, sterols, waxes, and tocols. The composition of crude RBO is given in Table 2.3.

RBO is the most balanced and versatile oil closest to the American Heart Association (AHA) recommendations (AHA, 2011). RBO has a good balance in its fatty acid composition, and it is

composed mainly of oleic (37-41%), linoleic acid (37-41%) (essential fatty acid), and palmitic acid (22-25%). Fatty acid composition of crude RBO is presented in Table 2.4. Studies have demonstrated that a diet enriched in unsaturated fatty acids (UFA) lowers LDL cholesterol when replacing saturated fat (Shepherd *et al.*, 1980; Schaefer *et al.*, 1981).

Table 2.3 Composition of crude rice bran oil

Triglycerides	80%
Phospholipids	2%
Glycolipids	1%
Sterols	5%
Waxes	2-5%

Source: Marshall and Wadsworth (1994).

2.4.3 Natural antioxidants in RBO

Rice bran oil has natural antioxidants such as tocopherols, tocotrienols, and gamma oryzanol (Table 2.5). Tanabe *et al.* (1981) reported 19-46 mg of α -tocopherol, 1-3 mg of β -tocopherol, 1-10 mg of γ -tocopherol, and 0.4-0.9 mg of δ -tocopherol per 100 g of oil. Gamma oryzanol has been reported to be at 6.42 mg/g and 5.17 mg/g in long grain rice and medium grain rice respectively (Lloyd *et al.*, 2000).

Table 2.4 Major lipid classes of crude rice bran oil and their fatty acid composition

Lipid class ^a	Wt.%	14:0	16:0	18:0	18:1	18:2	18:3	20:0	Saturated	Unsaturated
TL	20.1	0.4	22.21	2.21	38.85	34.58	1.14	0.61	25.43	74.57
NL	89.2	0.43	23.41	1.88	37.24	35.29	1.07	0.68	26.4	73.6
GL	6.8	0.09	27.34	0.28	36.45	35.76	0.18		27.61	72.39
PL	4	0.11	22.13	0.16	38.11	39.32	0.17		22.4	77.6

^aTL = total lipids; NL = neutral lipids (nonpolar lipid and free fatty acids); GL = glycolipids; PL = phospholipids. Source: Shin and Godber (1996).

Table 2.5 Tocopherol (T) and tocotrienol (T3) concentrations (mg/100g) in raw rice bran and commercially available refined oil

Source	α -T	β -T	γ -T	δ -T	α -T3	γ -T3	δ -T3
Rice bran	6.3	0.9	3.2	0.2	3.8	12	0.7
Brown rice ^a	0.63	0.09	0.32	0.02	0.38	1.2	0.07
Crude oil ^a	31.5	4.5	16	1	19	60	3.5
Refined oil	8.2		12.8	1.3	2.1	42.9	3.5

^aCalculated values. Source: Shin and Godber (1996).

It has been demonstrated that rice bran oil lowers cholesterol in humans (Most *et al.* 2005; Raghuram *et al.*, 1989; Lichtenstein *et al.*, 1994; Sugano and Tsuji, 1997; Orthoefer, 1996). Most *et al.* (2005) determined the effects of defatted rice bran and rice bran oil in an average American diet on blood lipids in moderately hypercholesterolemic persons. They found that total cholesterol was not significantly changed by the rice bran diet. On the other hand, it was found that the diet containing rice bran oil had a significant effect by lowering total cholesterol, LDL cholesterol, and lipoprotein B on the subjects. Investigators in this study concluded that the cholesterol-lowering capacity of RBO is due to components present in the oil since there were no substantial differences in the fatty acid composition between the diets.

Rice bran oil is one of the food sources with the highest phytosterols content such as γ -oryzanol, which contains 10 different compounds (esters of transferulic acid and phytosterols). Among these compounds, the major components of γ -oryzanol are cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesterol ferulate (Xu and Godber, 1999). The health properties of γ -oryzanol have been studied before (Patel and Naik, 2004; El-Rahman, 2010). According to Yoshino *et al.* (1989) and Rogers *et al.* (1993), γ -oryzanol health properties include a decrease of plasma cholesterol, decrease of platelet aggregation, decreased hepatic cholesterol biosynthesis, increased fecal bile acid excretion, decreased cholesterol absorption, and also γ -

oryzanol has been used to treat nerve imbalance and disorders of menopause. El-Rahman (2010) investigated the effect of γ -oryzanol extracted from RBO on rats fed a high-cholesterol diet. γ -oryzanol was administrated at different doses of 250, 500 and 750 mg/100 g diet for 50 days. In this study it was found that serum total cholesterol concentration decreased significantly by using 500 mg and 750 mg of γ -oryzanol compared with the control. Therefore, according to these results, it is recommended to use γ -oryzanol as a natural source for lowering cholesterol. The chemical structure of γ -oryzanol is given in Fig. 2.3.

Besides γ -oryzanol, tocotrienols are present in high quantities in RBO, with the major components as β -tocotrienol and γ -tocotrienol. According to Kerckhoffs *et al.* (2002), tocotrienols have been suggested to lower cholesterol. This is possible due to an inhibition of the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in endogenous cholesterol synthesis. Kerckhoffs *et al.* (2002) also states that the mechanism of this inhibitory action by tocotrienols may involve a reduced HMG-CoA reductase protein synthesis rate and an increased degradation rate as found in human hepatoma HepG2 cells. The chemical structures of tocotrienols are given in Fig. 2.4.

2.5 Purple rice bran oil (PRBO)

Studies have reported that pigments that are responsible for the red and purple color of colored rice have many health functions (Canter and Ernst, 2004; Youdim *et al.*, 2002; Tsuda *et al.*, 2003). Purple rice is a new rice variety being harvested in the US, thus, information on tococls, γ -oryzanol, and other components in this rice is limited. Jang and Xu (2009) determined the antioxidant content in purple rice bran from rice harvested in the Southern US. In this study they reported a total phenolic content of 6.0 μg of catechin equiv/g of lipophilic extract (purple rice bran oil) from the outer layer fraction of the bran. In addition, they found an average total tococls

(α -tocopherol, γ -tocopherol, α -tocotrienol, and γ -tocotrienol) content of 411.8 $\mu\text{g/g}$ in the purple bran oil fraction, and γ -oryzanol was reported with a value of 3.4 mg/g of rice bran oil.

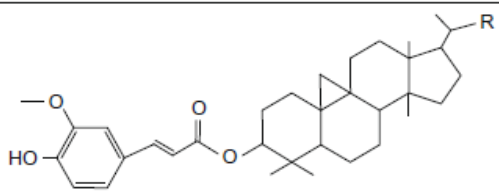


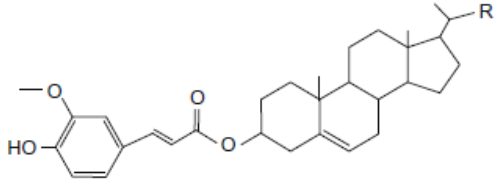


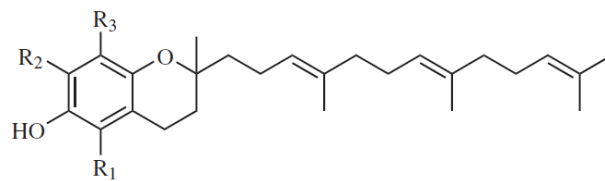
Molecular structure	R	Compound
		24-Methylen-cyclo-artanylferulate
		Cycloartenylferulate
		β -Sitosteranylferulate
		Campesteranylferulate

Figure 2.3 γ -Oryzanol and its four chemical structures. Source: Lerma-Garcia *et al.* (2009)



Tocotrienols (T3)

Figure 2.4 Chemical structure of tocotrienol. Source: Orthoefer (1995)

CHAPTER 3. MATERIALS AND METHODS

3.1 Rice processing, rice bran collection and stabilization

Raw purple rice (*Blanca Isabel* variety) was purchased from a local producer (Rush Rice Products, L.L.C, Louisiana, USA). Rice was milled using a pilot scale mill (Satake Engineering Co., Tokyo, Japan) which operated in a continuous process. The rice mill consisted of a husker (Model GPS300A), a mill (Model VAF10AM), a wet polisher (Model BA3AW), and a color sorter (Model GS3AA). Rough rice (approximately 500 lb. rice was used in this study) was processed using the following pilot scale operational procedure: (1) Husking procedure: (a) the power switch was turned on to run the motor of the husker; (b) the paddy (rough rice) reservoir hopper was filled with paddy; (c) roll handle was turned to the right in order to have a gap of “3” (from 1-10); (d) the main lever was positioned at number 1 in order to open the compartment and circulate for husking for approximately 2 min per batch of 0.5 lb. paddy; (e) the main lever was positioned to number 2 to open the compartment and discharge husked rice for the whitening step. (2) Milling operation: (a) the switch of the rice bran collector motor was turned on; (b) the mill machine motor switch was turned and the feeding valve was opened; (c) the rice polishing chamber became full with rice rains (3) Rice whitening operation: (a) the rice whitener machine was turned on; (b) the valve was opened in order to set the flow to “6” (from 1-10). (4) Color sorter operation: (a) the compressor was turned on and the stop valve was opened to supply compressed air to the grain sorter; (b) the knob of the filter regulator was turned and adjusted so the pointer of pressure of the gauge read 2.5 kg/cm²; (c) the color sorter switch was turned on (d) the feed switch was turned on and adjusted to a feeding rate number “8” (from 1-10); (d) the sensitivity for primary and secondary sorting were set to number “6” (from 0-10).

Rice bran was collected in a barrel lined with a black plastic bag (Hefty Cinchsak®, 13 gallon size, Reynolds Consumer Products, Inc., Lake Forest, IL) and rapidly cooled (from ~40 °C to room temperature) by placing the bag in an ice bath. Rice bran was then sieved using a mesh with 1.4 mm opening (Fisher Scientific Co., Pittsburg, PA) under a cooler at 4 °C and frozen (-22 °C) until stabilization of lipases to prevent hydrolytic rancidity of lipids. Rice bran was stabilized according to Ramezanzadeh *et al.* (1999). One hundred and fifty grams per batch of rice bran were heated in a microwave oven (Model R-8320, Sharp Electronics Corp., Paramus, NJ) at 650 W and 2450 MHz. The moisture content of the rice bran was adjusted from 12.1 to 21% (w/w) by adding water. Rice bran samples (300 g) were placed in plastic zipper-top microwavable bags (Ziploc Double Zipper, gallon size, Johnson and Son, Inc., Racine, WI). Samples were spread evenly to a thickness of 0.5 cm and bags were sealed. The microwave chamber was preheated and samples were heated at 100% power for 3 min. Then, samples were allowed to cool to room temperature (~25 °C) and stored under freezing conditions (-22 °C) until oil extraction.

3.2 Purple rice bran oil (PRBO) extraction

Stabilized purple rice bran samples were mixed with hexane at 1:3 w/v (300 g rice bran: 900 mL hexane) in a 2000 mL beaker. Purple rice bran oil (PRBO) was then extracted by stirring the mixture using an impeller (IKA RW 20 digital, IKA Works Inc., NC) at 350 rpm for 2 h followed by centrifugation at 3840 g for 15 min. The solvent was evaporated using a vacuum rotary evaporator (Rotavapor RE121, BUCHI Labortechnik AG, Flawil, Switzerland) set at 40 °C for 30 min and any residual solvent was removed by nitrogen flushing using an evaporator (Zantec Analytical Evaporator, Glas-Col, LLC, Terre Haute, IN) for 2 hr.

3.3 Free fatty acids, peroxide value, color, moisture, FAMES, and antioxidant contents of PRBO

After extraction, PRBO was characterized for free fatty acids (FFA%), peroxide value (PV), moisture, color, fatty acid methyl esters (FAMES), and α -tocopherol, α -tocotrienol, γ -tocopherol, γ -tocotrienol, and oryzanol contents. The FFA and PV of PRBO were determined following American Oil Chemists Society (AOCS) Official Methods (1997). FFA content (%) was determined using a method described by the AOCS Ca 5a-40 Official Method (1997) with some modification in the weight of sample used (2 g instead of 5 g sample). Two grams of oil sample were mixed with 10 mL of an ethanol solution (prepared by mixing 120 mL of ethanol with 2 mL of phenolphthalein, titrated with 0.01 N NaOH solution until faint pink color appeared followed by immersion in water bath set at 60 °C for 5 min) and 0.4 mL of phenolphthalein. Then, solution was titrated with 0.1 N NaOH solution until it retained a pink color. The % FFA expressed as oleic acid was calculated by Eq. 3.1:

$$FFA (\%) = \frac{\text{Normality of NaOH} \times \text{volume of titrant used (mL)} \times 28.2}{\text{Weight of sample (g)}} \quad (3.1)$$

The PV (meq/Kg) of the PRBO was measured by the acetic acid-chloroform method (AOCS Cd 8-53, 1997) to determine primary lipid oxidation products. Two grams of oil were weighed into a 125 mL Erlenmeyer flask and 12 mL of a 3:2 acetic acid: chloroform solution (Fisher Scientific, Fair Lawn, NJ) were added. The flask was swirled (manually) and 0.2 mL of a saturated potassium iodide (Mallinkrodt Baker Inc., Phillipsburg, NJ) solution were added followed by 12 mL of distilled deionized water and 4 drops of starch solution (Sigma Aldrich, Co., St. Louis, MO). The resulting purple solution was titrated with 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$ (Sodium thiosulfate) (Mallinkrodt Baker Inc., Phillipsburg, NJ) under constant shaking until purple color disappeared.

A blank solution was prepared following the same procedure. The PV was calculated using equation Eq. 3.2:

$$PV = \frac{(S-B) \times M \times 1000}{W} \quad (3.2)$$

S is the volume (mL) of Na₂S₂O₃ (Sodium thiosulfate) solution used to titrate the sample, *B* is the volume (mL) of Na₂S₂O₃ solution used to titrate the blank, *M* is the molar concentration of the Na₂S₂O₃ solution (N), and *W* is the weight of the sample in grams.

Color of the PRBO was measured using a Lab Scan XE spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA). The spectrophotometer was standardized using white and black tiles. Oil samples (10 g) were placed in polystyrene hexagonal weigh boats (Fisherbrand®, Fisher Scientific, Pittsburg, PA) for measurement. Results were reported in L* (lightness), a* (greenness - redness), and b* (blueness - yellowness) values.

Moisture content of PRBO was measured following Karl Fischer titration method using a Mitsubishi Karl Fisher Moisturemeter (Mitsubishi Chemical Analytech Co., Ltd., Japan). Oil samples of 0.3-0.4 g were aspirated with a syringe and a silicon rubber was placed on the needle tip. Then syringe was weighed in an analytical balance (Mettler Toledo AG245, Mettler-Toledo Inc., Switzerland) and the weight of syringe with sample was recorded. Sample was injected into the titration cell and empty syringe was weighed to get the final sample size. Sample weight was inputted into equipment's software. Moisture was reported in ppm (parts per million) of water per gram of oil after titration.

Fatty acid composition expressed as fatty acid methyl esters (FAMES) of the PRBO was determined at the USDA-ARS Laboratory, University of Alaska Fairbanks, AK. FAMES were prepared using a modified method of Maxwell and Marmer (1983). A 20 mg sample of PRBO

was dissolved in 4.5 mL isooctane and 500 μ L of internal standard (10 mg methyl tricosanoate (23:0)/mL isooctane and 500 μ L 2 N KOH (1.12 g/10mL MeOH) was added to the mixture. The mixture was vortexed for 1 min and centrifuged to separate the upper layer. The separated upper layer was mixed with 1 mL of saturated ammonium acetate solution and the aqueous layer was removed and discarded. The mixture was centrifuged and the upper layer of the mixture was separated. Then 1 mL of distilled water was added to the separated upper layer and centrifuged, then 2-3 g anhydrous sodium sulfate was added, vortexed, and held for 20-30 min. The mixture was centrifuged and the liquid containing methyl ester was separated. Half milliliter of isooctane containing methyl ester and 0.5 mL of isooctane were added to the amber GC vial. The gas chromatographic (GC) analysis used a GC model 7890A (Agilent) fitted with a FAMEWAX™ (30m, 0.32mm x 0.25 μ m, Restek, Bellefonte, PA) column was used for fatty acid analysis. Data was collected and analyzed using the GC ChemStation program (ver E.02.00.493 Agilent Technologies, Inc.). Helium was used as the carrier gas at an average velocity of 64cm/sec. Injector and detector temperatures were held at 250 °C and 280 °C, respectively. A split injection (50:1 split ratio) was used and the oven programming was 195 °C to 240 °C at a rate of 5 °C/min and held 2 min at 240 °C for a total run time of 11 min. An autosampler performed the GC injection of standards and samples. The injection volume was 1 μ L. Samples were identified by comparing retention times to standards. Data expressed were as percent of total integrated area.

Tocopherol, tocotrienol, and oryzanol contents for PRBO were determined using the method described by Jang and Xu (2009). For HPLC analysis, a sample (0.2 g) of oil was dissolved in 2 mL of hexane in a glass test tube and vortexed. The mixture was transferred to HPLC vials and 25 μ L were injected into the HPLC system for analysis. The HPLC system consisted of Waters

(Milford, MA) 510 pumps, a 715 Ultra WISP injector, and fluorescence detectors. Chromatograms were recorded and processed using Waters Millennium chromatography software. Samples were injected into a 25 cm X 4.6 mm diameter 5- μ m Supelcosil LC-Si (Supelco, Bellefonte, PA) column. The column was preceded by a 5 cm X 4.6 mm i.d. guard column packed with 40- μ m pellicular silica. The mobile phase consisted of 0.5% (v/v) ethyl acetate and 0.5% (v/v) acetic acid in hexane at a flow rate of 1.5 mL/ min. Concentrations were expressed as μ g/g of PRBO.

3.4 Preparation of nano-emulsion containing purple rice bran oil (NPRBO)

An emulsion was prepared following the formulation of Wan *et al.* (2011) with some modifications in the ingredient percentages (Table 3.1). The following materials were used to produce the emulsion: water, purple rice bran oil, and sodium caseinate provided by American Casein Company (Burlington, NJ). Sodium caseinate was used since is a natural emulsifier (flexible protein) which has been used to produce nano-emulsions (Qian and McClements, 2011). Addition rate (% w/w) of the ingredients was modified to emulsify the highest percent of PRBO and produce the lowest droplet size (nm) without affecting the emulsion stability. Sodium caseinate was dissolved in water and stirred using a RCT Basic S1 magnetic stirrer (IKA Labortechnik, Janke & Kunkel GmbH & Co., Staufen, Germany) at 700 rpm for 1 hr. at room temperature. Purple rice bran oil was added gradually into the sodium caseinate solution and mixed by stirring for 5 min. A CPX-500 ultrasonic processor (Cole Parmer Instruments, Vernon Hills, IL) set for 10 min at 80% amplitude was used to produce a stable micro-emulsion to process each batch (200 mL/batch) of solution in a 250 mL glass beaker. The emulsion was constantly cooled to maintain a temperature of approximately 20 °C by means of immersion in an ice bath during the sonication process. The emulsion was then processed by an ultrashearing

device (OMNI Ultrashear M, Omni International, Kennesaw, GA) to produce a nano-emulsion. Emulsion batches (100 mL/batch) in a 200 mL beaker were sheared at 26,000 rpm for 10 min to reduce droplet size to nano-scale. The beaker was set in an ice bath to maintain a temperature of approximately 20 °C during ultrashearing. An emulsion produced only by ultrasonication was prepared as a control. The nano-emulsion and the control emulsion were kept refrigerated at 5 °C.

Table 3.1 Emulsion formulation

Ingredient	Amount (%)*
PRBO	10
Sodium caseinate	5
Water	85
Total	100

PRBO = Purple rice bran oil

*Mass basis

3.5 Physicochemical properties of NPRBO emulsion and control emulsion

Particle size distribution and mean droplet diameter of NPRBO and the control emulsion (only sonicated) was measured by a Dynamic Light Scattering (DLS) device (Zetasizer Nano-ZS, Malvern Instruments Ltd, Worcestershire, UK). Samples were diluted by a factor of 1:100 prior to particle size measurements. Samples of approximately 0.5 mL were placed in spectrophotometer cuvettes for measurement. Mean particle diameter was reported as “Z-average” diameter.

The microstructure of NPRBO and the control emulsion was determined using a light microscope (Leica DM RXAZ, Leica Microsystems, Germany). The purple rice bran oil was stained with 0.030% (w/w) of Oil Red O dye powder (Sigma-Aldrich Co., St. Louis, MO) before making the measurements. A drop of the emulsion was observed at an objective magnification of

80. Images of the emulsion's structure were acquired using image processing software with a CCD camera (Cooke Sensi Cam QE, Cooke Corporation, Germany). The analysis was conducted in the Louisiana State University Socolofsky Microscopy Center.

Peroxide value (PV) was determined to measure primary lipid oxidation products in the NPRBO and control emulsion during 0, 24, 48, 96, and 168 hr. of storage at 4 and 40 °C. PV of the NPRBO and control emulsion was determined following the IDF method (74A, 1991). Emulsion samples (10 mL) were mixed with 200 mL chloroform/methanol (7:3, 140 mL/ 60 mL) in a separation funnel. The mixture was allowed to rest for 10 min and then the solvent layer was separated from the mixture. Two more extractions were performed in the layer remaining in the separation funnel. The extracts were collected and the solvent was evaporated in a vacuum rotary evaporator (Rotavapor RE121, BUCHI Labortechnik AG, Flawil, Switzerland). Oil samples between 0.01-0.30 g were weighed in a test tube, 9.8 mL of chloroform/methanol were added and the mixture was vortexed for 4 seconds. Then 50 µL of 3.94 M ammonium thiocyanate were added and vortexed for 4 seconds. The addition of 50 µL of acidified iron (II) chloride solution was followed by vortexing for 4 seconds and incubating for 5 min. The absorbance of the incubated solution was read at 500 nm against a blank that contained all of the reagents except the sample in a spectrophotometer (Genesys 20, Thermo Fisher Scientific, Fair Lawn, NJ). The concentration of PV was determined by a standard curve constructed with known concentrations of Fe III (1-40 µg Fe III).

Thiobarbituric acid-reactive substances (TBARS) were analyzed to determine final oxidation products from the lipids in the NPRBO and the control emulsion at 0, 24, 48, 96, and 168 hr. of storage at 4 and 40 °C. An emulsion made by ultrasonication (10 min, 80% amplitude) was used as the control. TBARS of emulsions were measured according to Mei *et al.* (1998) with slight

modifications. A thiobarbituric acid (TBA) solution was prepared by mixing 15 g of trichloroacetic acid, 0.375 g of TBA reagent, 1.76 mL of 12 N HCL, and 82.86 mL of H₂O in a 100 mL volumetric flask. TBA solution (100 mL) was mixed with 3 mL of 2% butylated hydroxytoluene in ethanol, and 2 mL of this solution were mixed with 1 mL of the emulsion sample. The mixture was vortexed for 10 sec and heated in a boiling water bath for 15 min. The mixture was rapidly cooled to room temperature with running water and centrifuged at 3400 \times g for 25 min. The absorbance of the supernatant was measured at 530 nm and concentrations of TBARS (mmol/L emulsion) were determined from a standard curve prepared using known concentrations of 1, 1, 3, 3-tetraethoxypropane (0-0.05 mmol/L).

Emulsion stabilities of NPRBO and the control emulsion were determined following the method of Min *et al.* (2003) with little modifications. Emulsion samples of 5 mL were placed into 15 mL centrifugal tubes and stored at -20 °C for 2 days. Then the samples were allowed to thaw at room temperature for 4 hr. The thawed samples were centrifuged at 15,000 \times g for 40 min at -2 °C and the amount of oil separated was measured. Oil recovery % was calculated as (mL of oil recovered / 5 mL of emulsion sample) \times 100.

3.6 Fatty acid methyl esters (FAMES) of NPRBO and control emulsion

FAMES content for lipids extracted from NPRBO and the control emulsion were measured by gas chromatography. Lipids were extracted from emulsions using Folch (1957) extraction method with some modifications. A 70 g sample of an emulsion was dissolved in 1 L of 2:1 chloroform: methanol solution and filtered through Whatman No. 4 filter paper. The filtrate was mixed with 200 mL of a 0.66% sodium chloride (Mallinckrodt Baker Inc., Phillipsburg, NJ) solution and subsequently centrifuged at 649.6 \times g for 15 min. The chloroform layer containing

the lipids was evaporated under vacuum using a rotary evaporator at 40 °C. FAMEs were prepared using the same method used for PRBO.

3.7 Rheological properties of NPRBO and control emulsion

Viscosity, flow behavior and viscoelastic properties of NPRBO and the control emulsion were determined using an AR 2000 ex Rheometer with fitted plate geometry using plates of 40 mm in diameter and Universal Analysis (TA Instrument, New Castle, DE, USA) software. A 200 µm gap between the plates was used to determine the flow behavior of the emulsion samples (2-3 g). The shear stress was measured at shear rates from 0.00185 to 116 s⁻¹. The flow behavior was modeled using the Power Law model (Eq. 3.3) since samples did not present a yield stress:

$$\sigma = K (\dot{\gamma})^n \quad (3.3)$$

Where σ is shear stress (Pa), K is the consistency index (Pa.sⁿ), $\dot{\gamma}$ is shear rate (s⁻¹) and n is the flow behavior index. Logarithms were taken for σ and $\dot{\gamma}$ and a plot of $\log \sigma$ versus $\log \dot{\gamma}$ were constructed. The resulting line yielded the magnitude of $\log K$ (i.e., intercept) and n (i.e., slope). The viscosity at a shear rate of 116 s⁻¹ was obtained. Frequency sweep tests were conducted in angular frequencies between 0.1 and 15 Hz. A 200 µm gap between plates was used. The elastic modulus (G') and viscous modulus (G'') of the emulsion samples were obtained using the Universal Analysis software.

3.8 Production of frozen yogurt fortified with NPRBO

Frozen yogurt containing nano-emulsion of purple rice bran oil (FYNRO) and frozen yogurt containing emulsion materials without purple rice bran oil (FYSC) and plain frozen yogurt (PFY) (both yogurts were used as controls) were produced following the formulations presented in Table 3.2. All equipment, implements, and containers were sanitized with a 200 ppm sodium

hypochlorite (Eurochem International Corporation, Atlanta, GA) solution before use. Pasteurized whole milk (Great Value®, Bentonville, AR) was obtained from a local store. Grade A Non-fat dry milk (NFDM) was obtained from Dairy America, Fresno, CA. Extra fine granulated sugar was obtained from Domino Foods, Inc., Yonkers; NY. Maltodextrin (M040) and stabilizer (PGX) were obtained from Danisco, Inc., Madison, WI. Starter cultures of *L. bulgaricus* (LB-12) and *S. thermophilus* (ST-M5) were obtained from Chr. Hansen, Milwaukee, WI.

Table 3.2 Frozen yogurt formulations

Ingredient	FYNRO^{a*}	FYSC^b (Control)	PFY^c (Control)
Whole milk (Kg)	7.56	7.56	7.56
NPRBO ^d (g)	620.00	-	-
Sodium caseinate solution (g)	-	620.00	-
Sucrose (g)	1362.00	1362.00	1362.00
M040 Maltodextrin (g)	363.20	363.20	363.20
Stabilizer (g)	20.00	20.00	20.00
NFDM (g)	399.52	399.52	399.52
<i>Lactobacillus bulgaricus</i> (mL)	2.00	2.00	2.00
<i>Streptococcus thermophilus</i> (mL)	2.00	2.00	2.00

^aFYNRO= Frozen yogurt containing nano-emulsion of purple rice bran oil, ^bFYSC = Frozen yogurt containing emulsion material without purple rice bran oil, ^cPFY = Plain frozen yogurt, ^dNPRBO = Nano-emulsion containing purple rice bran oil. *Both the FYNRO emulsion and the FYSC solution contain 5% sodium caseinate.

Yogurt mixes were prepared in 7.56 Kg (2 gal.) batches. Whole milk was placed in 8 L stainless steel containers. Dry ingredients such as NFDM, sucrose, maltodextrin, and stabilizer were added to milk with concurrently with constant manual stirring. NPRBO and sodium caseinate solution were added to the yogurt mix in order to form FYNRO and FYSC respectively. Three

hundred ten grams of NPRBO were added to each gallon of yogurt mix in order to provide approximately 7.05% of the RDI (required daily value) of vitamin E in every cup (120 g portion size) of FYNRO. This amount was calculated as follows:

310 g emulsion/ gall

$310 \text{ g} * 10\% \text{ PRBO} = 31 \text{ g PRBO}$

Total vitamin E content in PRBO = 1.075 mg/g

$1.075 \text{ mg/g} * 31 \text{ g} = 33.325 \text{ mg vitamin E in 310 g emulsion.}$

If 3780 g (1 gall) yogurt contain 33.325 mg vitamin E;

120 g (cup size) frozen yogurt = 1.0579 mg vitamin E

RDI for vitamin E = 15 mg

$1.058 \text{ mg} * x/100 (15)$

X= 7.05% of RDI for vitamin E in every cup (120 g) of frozen yogurt

All mixes were heated to 60 °C for 1 min to increase the incorporation of the ingredients before homogenization. Yogurt mixes were then homogenized in a two stage Gauling 300 DJF 4 2PS homogenizer (APV Gaulin, Wilmington, MA) at 2,000 PSI for 15 s and then pasteurized at 72.78 ± 1 °C for 30 min. After pasteurization, mixes were rapidly cooled in a water bath to 40.55 ± 1 °C for inoculation of starter cultures. Incubation was done in an incubator Model 815 (Thermo Scientific, Two Rivers, WI) at 43 ± 1 °C until the pH dropped to approximately 4.6. Yogurt mixes were quickly cooled in an ice bath and stored overnight at 4 °C in a cooler. Yogurts were manually stirred prior to freezing using a stainless steel perforated milk stirrer (Nelson Jameson, Marshfield, WI) in order to breakdown the gels formed during incubation. Stirred yogurt mixes with a semi-liquid consistency were frozen in an ice cream freezing machine (Taylor 430-12, Taylor Co. Rockton, IL) in 2 gallon batches. The frozen yogurt batches were packed into and tightly sealed in ½ gallon containers (Reynolds Plastics, Richmond, VA). Containers were labeled and then placed in a freezer at -22 °C for hardening and storage (Fig. 3.1).

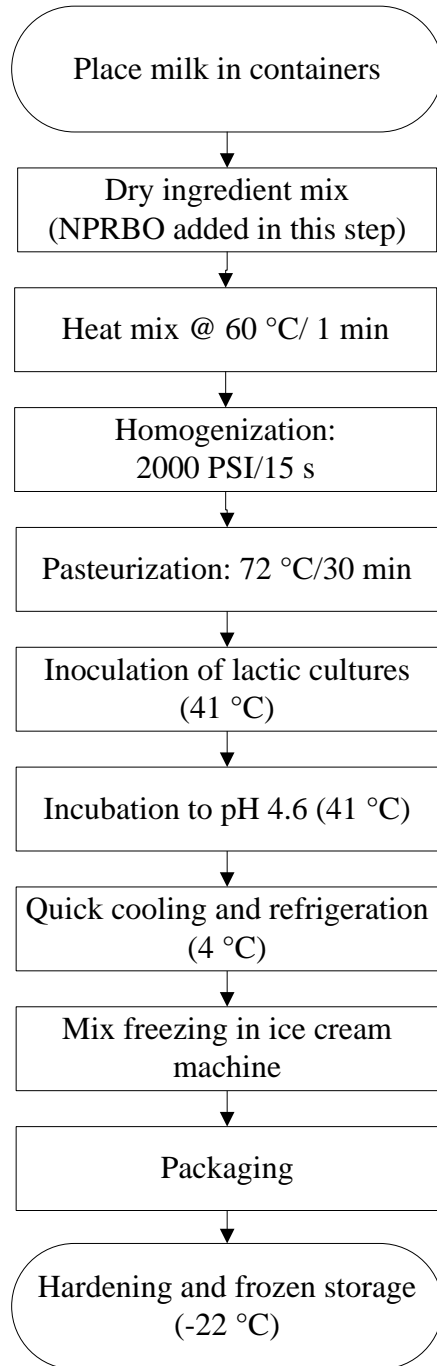


Fig. 3.1 Frozen yogurt flow process

3.9 Rheological properties of yogurt mixes before freezing

Rheological properties of yogurt mixes were determined before freezing. This was done as a quality control analysis since mixes may have a semi-liquid consistency in order to flow in the ice cream machine. Viscosity, flow behavior and viscoelastic properties of yogurt mixes at 5 °C (Isik *et al*, 2011) were determined following same procedure of NPRBO (detailed above).

3.10 Texture analysis of FYNRO, FYSC and PFY

Texture (hardness) of the frozen yogurts was determined according to Soukoulis and Tzia (2008). Before determination of hardness, samples were transferred from -22 °C to -15 °C and held for 24 h. A texture analyzer (Instron 5544, Canton, MA) coupled with a 500 N static load cell was used. Measurements were carried out using a 6 mm stainless steel cylindrical probe. The penetration depth at the geometrical center of the samples (placed in cups of 65 x 30 mm diameter and height respectively) was 10 mm and the penetration speed was set at 2.0 mm/s. Hardness (N) of the samples was determined as the peak compression force during penetration.

3.11 Melting percent (%) of FYNRO, FYSC and PFY

The melting percent (weight basis) of the frozen yogurts was determined according to Isik *et al*. (2011). A 2.5-mm iron wire mesh screen was used. Samples were prepared by weighing a cubic cut of frozen yogurt (of approximately 40 g) in a balance (Mettler Toledo AG245, Mettler-Toledo Inc., Switzerland). Samples were placed on the mesh screen, which was mounted on a beaker. The weight of the collected samples in the beaker was recorded at 15, 30, and 45 min of melting. The melting percent was calculated as the ratio of these values to the initial weight of the samples.

3.12 Overrun of FYNRO, FYSC and PFY

Overrun was done to determine the percent of aeration of the frozen yogurts after freezing, (Goh *et al.*, 2008). The % (w/w) overrun was obtained by measuring the weight of a known volume of yogurt mix and frozen yogurt (both 70 mL) in a 200 mL beaker (200 mL) and calculated using following equation (Eq. 3.4):

$$\text{Overrun (\%)} = \frac{\text{Wt. of yogurt mix} - \text{Wt. of the same volume of frozen yogurt}}{\text{Wt. of yogurt mix}} \times 100 \quad (3.4)$$

3.13 Color of FYNRO, FYSC and PFY

Changes in color of frozen yogurts were measured during 6 weeks storage at -22 °C. A Lab Scan XE spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA) was used. The spectrophotometer was standardized using white and black tiles and results were reported in L* (lightness), a* (greenness- redness), and b* (blueness- yellowness) values. Total color differences (ΔE^*) during 6 weeks storage of frozen yogurts were calculated using the following equation (Eq. 3.5):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3.5)$$

3.14 Coliforms, aerobic count plate (ACP), and lactic acid bacteria (LAB) counts of FYNRO, FYSC and PFY

Coliforms, Aerobic Count Plate (ACP), and lactic acid bacteria (LAB) counts were conducted the same day of freezing and packaging and during 6 weeks of frozen storage at -22 °C. ACP and Coliform counts were determined by plating 1 ml of the diluted (10^{-1}) frozen yogurt samples on 3M TM Petrifilm TM aerobic count plates and coliform count plates (3M Microbiology, St. Paul

MN) in duplicate. Petrifilms were labeled and incubated in a gravity convection incubator (Thermo Fisher Scientific, Fair Lawn, NJ) at 35 °C during 48 h and at 35 °C for 24 h for ACP and coliforms respectively. For LAB counts, the two cultures used for yogurt; *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were counted separately. For *Streptococcus thermophilus* a ST agar was made. Ten grams of Tryptone, 10 g of Sucrose, 5 g of Yeast extract and 2 g of Di potassium phosphate (K_2HPO_4) were dissolved in 1 L distilled water. The pH of the solution was adjusted to 6.8 ± 0.1 using 1 M HCl, then 6 ml of 0.5% (v/v) bromocresol purple and 12 g of agar were added to the medium. The media was boiled and sterilized at 121°C for 45 min. After cooling, the medium was aseptically poured into Petri dishes containing the diluted samples. Dilutions of frozen yogurt were made with 99 ml of sterilized peptone (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7}). Plates were incubated aerobically at 37 °C for 24 hours.

A pH modified MRS agar was prepared for *Lactobacillus bulgaricus* counts with 55 g of MRS broth powder weighed and suspended into 1 L of distilled water. The pH of the media was adjusted to 5.2 with 1 M HCl and 15 g of agar were added. The appropriate dilutions of frozen yogurt were made with 99 ml of sterilized peptone (10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7}). The media was heated to boiling with agitation to completely dissolve the powder before autoclaving at 121°C for 45 min. After cooling, media was aseptically poured into Petri dishes containing the diluted samples. Petri dishes were placed in BBL GasPaks and incubated anaerobically at 43 °C for 72 h (Dave and Shah., 1996).

3.15 Oxidative stability of FYNRO, FYSC and PFY

The oxidative stability of the frozen yogurts was determined by measuring the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). Both analyses were conducted the same day of freezing and packaging and after 6 weeks of frozen storage at -22 °C. PV was

determined to measure primary oxidation products (hydroperoxides) from the lipid components of the frozen yogurts. PV was determined according to Boon *et al.* (2008). Frozen yogurt samples (~0.3 g) were mixed with 1.5 mL iso-octane/2-propanol (3:1, v/v) and vortexed 10 s, three times. The mixture was centrifuged at $3400 \times g$ for 2 min (Beckman J2-HC, Fullerton, CA, USA). A 100 μL volume of the resulting organic solvent phase was added to 2.8 mL of methanol/1-butanol (2:1 v/v). Fifteen μL of 3.94 M ammonium thiocyanate solution followed by 15 μL of a ferrous iron solution (prepared by mixing equal volumes of 0.144 M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ with 0.132 M BaCl_2 , centrifuging, and separating the clear solution) were added to the methanol/1-butanol mixture, vortexed, and incubated at room temperature for 20 min. After incubation, sample absorbances were read at 510 nm using a spectrophotometer (Genesys 20, Thermo Scientific, Madison, WI). The hydroperoxide content of the samples (mmol/Kg frozen yogurt) was determined by a standard curve developed using known concentrations of cumene hydroperoxide (0-2 mmol/L).

TBARS value is an analysis in which final oxidation products of the lipid soluble components of the frozen yogurt react with Thiobarbituric Acid (TBA) forming condensation products. Absorbance of those compounds is measured at 530 nm. The analysis was conducted as described by Hekmat and McHamon (1997). One gram of frozen yogurt was weighed into a glass screw top test tube, 9 ml of a 15% (w/v) trichloroacetic acid (Sigma Aldrich, Co., St. Louis, MO) and 0.375% (w/v) of TBA reagent (Sigma Aldrich, Co., St. Louis, MO) in hydrochloric (0.25N) acid (Sigma Aldrich, Co., St. Louis, MO) solution were added, mixed well and heated in a boiling water bath (VWR Instruments Inc., Radnor, PA) for 15 min. Samples were quickly cooled to room temperature with water and centrifuged at $6,311 \times g$ for 15 min at 20 °C. Absorbance was measured at 530 nm and concentrations of TBARS ($\mu\text{mol/Kg}$ frozen

yogurt) were determined from a standard curve prepared using known concentrations of 1, 1, 3, 3-tetraethoxypropane (0-0.05 mmol/L).

3.16 Confocal Scanning Laser Microscopy of FYNRO, FYSC and PFY Structure

Confocal scanning laser microscopy (CSLM) was used to observe the gel structure of melted yogurt samples. A Leica TCS SP2 spectral confocal microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany) and Leica LCS software was used. Each frozen yogurt sample was placed over a micro slide (Gold Seal® Products, Portsmouth, NH) and covered with a No. 1½ cover glass (Corning®, Lowell, MA) for analysis. Optical sectioning of the frozen yogurt samples was initiated 5 µm beneath the cover glass using the reflectance mode and images at various sections of the slide were digitally captured. The analysis was conducted in the Louisiana State University Socolofsky Microscopy Center.

3.17 Fatty acid methyl esters (FAMES) composition of FYNRO, FYSC and PFY

FAMES content for lipids extracted from the frozen yogurts was measured by gas chromatography during 6 weeks of frozen storage at -22 °C. Lipids were extracted from frozen yogurts following same procedure of NPRBO. FAMES were prepared using the method described by Maxwell and Marmer (1983) detailed above.

3.18 Tocopherols, tocotrienols, and oryzanol contents of FYNRO

Tocopherol, tocotrienol, and oryzanol contents from lipids extracted from the frozen yogurt during 6 weeks of frozen storage at -22 °C were determined using the method described by Jang and Xu (2009). Lipids were extracted from frozen yogurt using the method described above.

For HPLC analysis, a sample (0.2 g) of oil was dissolved in 2 mL of hexane in a glass test tube and vortexed. The mixture was transferred to HPLC vials and 25 µL were injected into the HPLC

system for analysis. The HPLC system consisted of Waters (Milford, MA) 510 pumps, a 715 Ultra WISP injector, and fluorescence detectors. Chromatograms were recorded and processed using Waters Millennium chromatography software. Samples were injected into a 25 cm X 4.6 mm diameter 5- μ m Supelcosil LC-Si (Supelco, Bellefonte, PA) column. The column was preceded by a 5 cm X 4.6 mm i.d. guard column packed with 40- μ m pellicular silica. The mobile phase consisted of 0.5% (v/v) ethyl acetate and 0.5% (v/v) acetic acid in hexane at a flow rate of 1.5 mL/ min. Concentrations were expressed as μ g/g of frozen yogurt.

3.19 Freezing behavior of FYNRO, FYSC and PFY

Freezing behavior of the FYNRO, FYSC, and PFY was analyzed according to Alvarez *et al.* (2005) using a differential scanning calorimeter (DSC Q100, TA Instruments Inc., New Castle, DE). Frozen yogurt samples of 10 to 15 mg were weighed into large volume o-ring sealed stainless steel sample pans (TA Instruments, Inc., New Castle, DE) and sealed with a press. An empty large volume pan was used as a reference. The differential scanning calorimeter experimental program consisted of cooling to -50 °C, holding isothermally for 5 min, and heating at a rate of 5 °C per minute until 50 °C.

3.20 Statistics analysis

Mean values from three separate replications were reported with standard deviations. The statistical significance of differences observed among treatment means was evaluated by analysis of variance (ANOVA) (SAS Version 9.2, SAS Institute Inc., Cary, NC, U.S.A), followed by post hoc Tukey's studentized range test. Furthermore, the statistical significance of differences observed among treatment means during storage time was determined by ANOVA followed by post mixed procedure to analyze the time*treatment effect.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Physicochemical characteristics of purple rice bran oil (PRBO)

Physicochemical characteristics of the crude PRBO were determined after extraction (Table 4.1). Free fatty acids (FFA %) was 2.77 ± 0.08 % which is less than 3% and therefore acceptable for edible oils (Gracey *et al.*, 1999). Initial FFA% in the PRBO is produced by a lipase enzyme present in the bran; therefore the rice bran should be stabilized right after collection (Saunders, 1990). Peroxide value (PV) for PRBO was 2.67 ± 0.58 meq/kg, which was in concordance with the standard for quality characteristics for vegetable oils established by the Codex Alimentarius (1999) (up to 15 miliequivalents of active oxygen/kg oil). Moisture content was 300.80 ± 4.38 ppm. L*, a*, and b* values were 2.38 ± 0.11 , 5.01 ± 0.10 , and 3.11 ± 0.11 respectively, which indicated the oil was very dark and green. Antioxidant compounds such as α -tocopherol, γ -tocotrienol and γ -oryzanol were present in higher amounts in the PRBO than were α -tocotrienol or γ -tocopherol. Similar results were found by Jang and Xu (2009), in which it was found that γ -oryzanol (3400 $\mu\text{g/g}$), α -tocopherol (122.15 $\mu\text{g/g}$), and γ -tocotrienol (177.65 $\mu\text{g/g}$) were the highest lipophilic antioxidant components found in lipids extracted from purple bran. Moreover, most antioxidants in PRBO were present in greater concentrations than were reported by Zigoneau *et al.* (2008) for brown rice bran oil extracted by conventional hexane extraction (α -tocopherol = 270.83 $\mu\text{g/g}$, α -tocotrienol = 70.66 $\mu\text{g/g}$, γ -tocopherol = 49.5 $\mu\text{g/g}$, and γ -tocotrienol = 337.83).

Fatty acid methyl ester composition of PRBO is presented in Table 4.2. Only major fatty acids are reported. Oleic (C18:1) acid (42.2 ± 0.04 %) was the predominant fatty acid detected in PRBO, followed by palmitic (C16:0) and linoleic (C18:2) acid with values of 25.4 ± 0.02 % and 21.1 ± 0.02 %, respectively. It could be noted that the predominant fatty acid composition of

PRBO was somewhat similar to the brown rice bran oil fatty acid composition reported by Shin and Godber (1996), of oleic (38.85 %), linoleic (34.58 %), and palmitic (22.21 %) acid. The fatty acid composition means that purple rice bran oil has a good balance in monounsaturated, polyunsaturated and saturated fatty acids.

Table 4.1 Physicochemical characteristics of PRBO

Physicochemical characteristics	Values
FFA (%)	2.77 ± 0.08
PV (meq/Kg)	2.67 ± 0.58
Moisture (ppm)	300.80 ± 4.38
L* value	2.38 ± 0.11
a* value	5.01 ± 0.10
b* value	3.11 ± 0.11
Chroma	6.71 ± 0.14
Hue angle	30.70 ± 0.75
α-Tocopherol (µg/g)	414.00 ± 0.05
α-Tocotrienol (µg/g)	248.00 ± 0.12
γ-Tocopherol (µg/g)	102.00 ± 0.13
γ-Tocotrienol (µg/g)	311.00 ± 0.06
γ-Oryzanol (µg/g)	3477.00 ± 0.11

Table 4.2 FAMES* composition (%) for PRBO

FAMES	%
Myristic acid (C14:0)	0.7 ± 0.01
Palmitic acid (C16:0)	25.4 ± 0.02
Heptadecenoic acid (C17:1)	1.8 ± 0.02
Oleic acid (C18:1)	42.2 ± 0.04
Linoleic acid (C18:2)	21.1 ± 0.02
Arachidic acid (C20:0)	0.5 ± 0.01
Erucic acid (C22:1)	6.7 ± 0.06

FAMES = Fatty acid methyl esters, PRBO = purple rice bran oil. *Only major fatty acids were reported.

4.2 Stability, size and microstructure of nano-emulsion containing purple rice bran oil (NPRBO)

NPRBO was significantly ($p < 0.05$) more stable than control emulsion since had less oil recovery % (Table 4.3). Hence, our results are in agreement with the findings in previous studies, which indicate that nano-emulsions provide stability against sedimentation or creaming (Solans *et. al*, 2005; Tadros *et. al*, 2004; McClements and Xiao, 2012).

The droplet size and size distribution of the nano-emulsion containing purple rice bran oil (NPRBO) and a control emulsion (prepared by ultrasonication only) are presented in Table 4.4 and Fig. 4.1 and 4.2 respectively. From the results it can be observed that NPRBO had significantly ($p < 0.05$) lower size compared with the control, which indicated that the 10 min of shearing effectively decreased the emulsion size. The polydispersity index (PdI) is a dimensionless number that describes the heterogeneity of the sample, and it can range from 0 (monodisperse) to 1 (very polydisperse) (Cao, Ziener, and Landfester, 2010). PdI value was significantly lower for NPRBO when compared with the control, which indicates that NPRBO was more monodisperse than the control (Fig. 4.1 and 4.2). Qian and McClements (2011) reported an average diameter size of 179 nm for a nano-emulsion with sodium caseinate prepared by high-pressure homogenization, which is similar to the size found in this study.

Table 4.3 Stability of emulsions

Sample	Oil recovery (%)
Control emulsion	8.67 ± 1.15^a
NPRBO	4.67 ± 1.15^b

^{ab}Different superscripts between rows indicate significant ($p < 0.05$) differences between samples.

Table 4.4 Average size diameter for emulsion droplets

Sample	Z-Ave d. (nm)	PdI
Control emulsion	419.6 ± 8.75 ^a	0.44 ± 0.01 ^a
NPRBO	199.2 ± 1.04 ^b	0.10 ± 0.02 ^b

Z-Ave d. = Average size diameter (nanometers), PdI = Polydispersity index, NPRBO = Nano-emulsion containing purple rice bran oil. ^{ab}Different superscripts between rows indicate significant ($p < 0.05$) differences between samples.

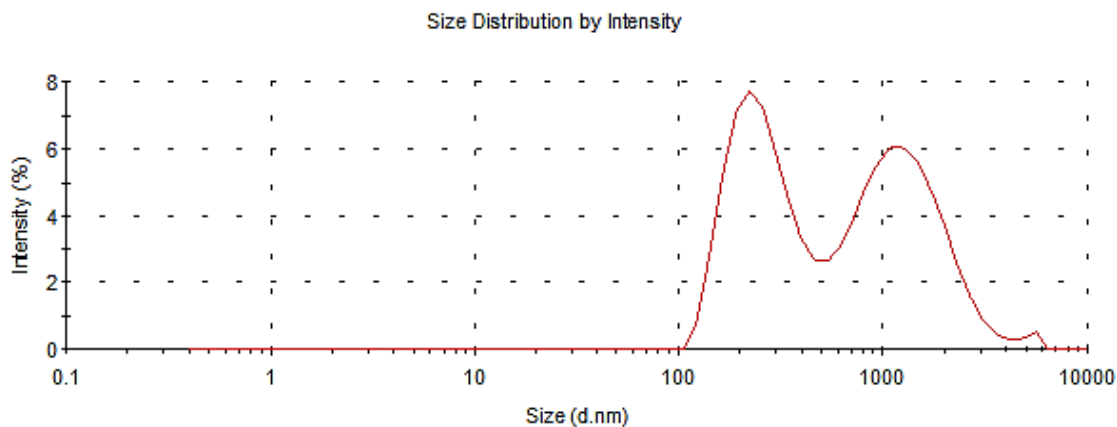


Figure 4.1 Size distribution of emulsion done with ultrasonication (control)

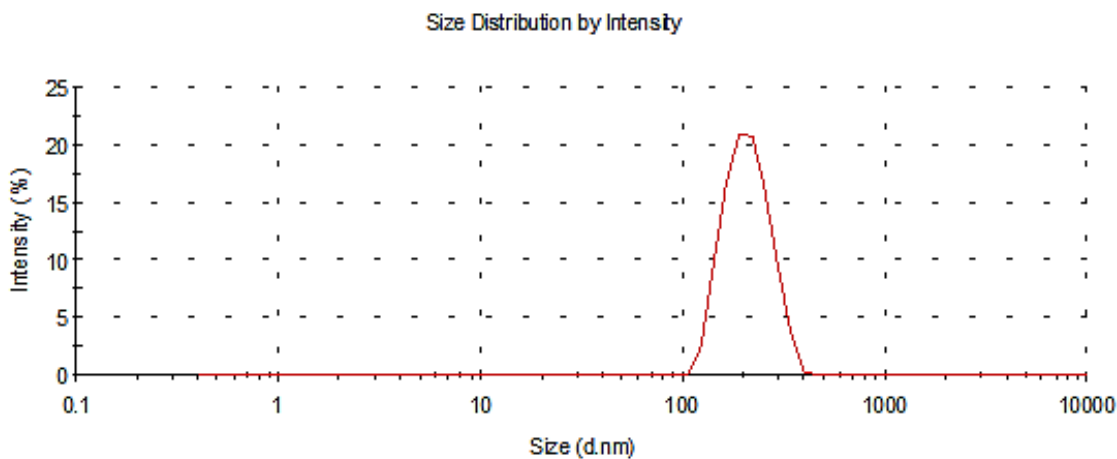


Figure 4.2 Size distribution of nano-emulsion containing purple rice bran oil (NPRBO)

The microstructures of the NPRBO and the emulsion produced by ultrasonication are shown in Fig. 4.3 and 4.4 respectively. The purple rice bran oil was stained with 0.030% (w/v) of Oil Red O dye powder in order to differentiate the oil droplets from air bubbles in the emulsions. This step is needed since nano-emulsions are difficult to observe due to their very small droplet sizes. From the pictures it can be observed that the nano-emulsion has lots of very small droplets (red points) with the sizes presented in the graphs above. On the other side, the picture for the control emulsion shows less droplets with bigger sizes. Therefore, there was a difference in the microstructure of the NPRBO and the control emulsion, which corresponds with the differences in size found by the DLS size measurements.

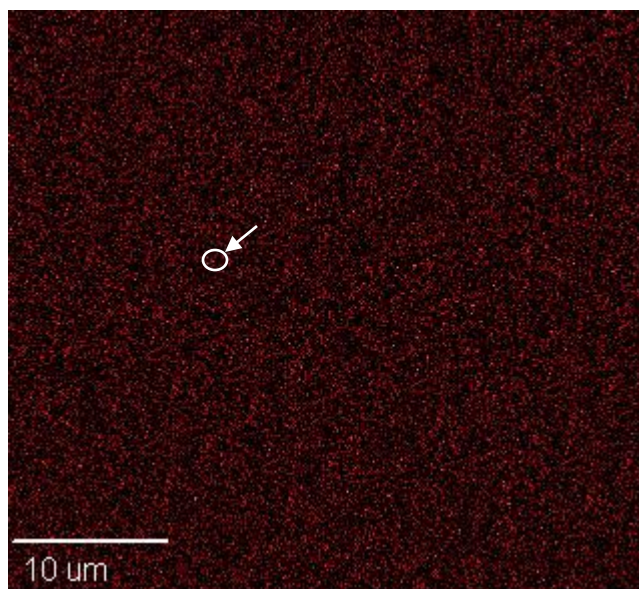


Figure 4.3 Optical light microscopy of NPRBO

4.3 Rheology of emulsions

The rheological properties of NPRBO and control (sonicated emulsion) are shown in Table 4.5. NPRBO and control emulsion had low consistency index (K) with non-significant differences

($p > 0.05$), which indicate the fluids have a low viscosity consistency. Both NPRBO and control emulsion presented flow behavior index (n) values below 1, thus emulsions had pseudoplastic shear thinning characteristics.

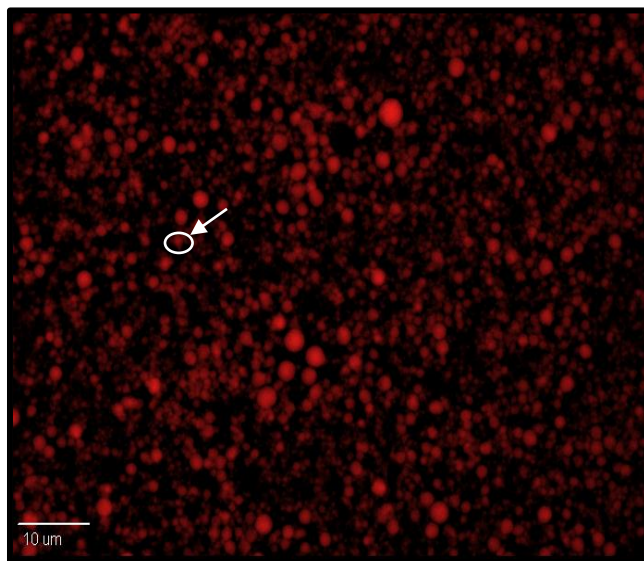


Figure 4.4 Optical light microscopy of control emulsion

Similarly to K value, non-significant differences were found for n index between emulsions. Apparent viscosity of NPRBO was significantly ($p < 0.05$) lower than the control, which means that the small droplet size of the nano-emulsion (199 nm) reduced the viscosity of the emulsion. Our results are in agreement with the ones reported by Weiss and McClements (2000), in which nano-emulsions with radius (r) droplet sizes between 80-90 nm did not exhibit a yield stress and behaved as fluids. They stressed that these dramatic changes in emulsion rheology were due to changes in microstructure. The viscosity of emulsions can vary depending to the purposes or applications needed by the industry. For example, emulsions with high viscosities may be desirable for producing fat products that need to be highly viscous or gel like, whereas nano-

emulsions with very low viscosities may be required for mixing purposes such as in beverages (McClements, 2011).

Table 4.5 Flow behavior properties of NPRBO and control

Sample	K (Pa.s ⁿ)	η	Apparent Viscosity (Pa.s)
NPRBO	0.06 ± 0.01^a	0.69 ± 0.06^a	0.01 ± 0.001^b
Control (sonicated emulsion)	0.27 ± 0.30^a	0.64 ± 0.13^a	0.02 ± 0.001^a

^{ab}Means with different letters across columns are significantly different ($P < 0.05$). NPRBO = nano-emulsion containing purple rice bran oil, η = flow behavior index, K = consistency index.

Fig. 4.5 shows the viscoelastic properties of NPRBO and control (sonicated emulsion). Elastic or storage modulus (G') indicate solid like properties of a fluid, while the viscous or loss modulus (G'') indicate the liquid like properties. Results indicate that emulsions had more liquid properties since both had higher loss modulus (G'') values. Non-significant differences were found for G' and G'' modulus values between NPRBO and control at most frequencies. Similar results were reported by Junyaprasert *et al.* (2009), who studied the rheology of nano-emulsions containing coenzyme Q₁₀. They observed that nano-emulsions showed a weak structure after applying an oscillation test, with higher loss modulus (G'') in comparison to the storage modulus (G'). Therefore, indicating liquid properties.

4.4 Fatty acid methyl esters (FAMES) of emulsions

FAMES for lipids extracted from NPRBO and sonicated emulsion are presented in Table 4.6. The purpose of this analysis was to determine if the processing steps to produce the NPRBO and sonicated emulsion (control) could generate significant changes in the FAMES of the purple rice bran oil.

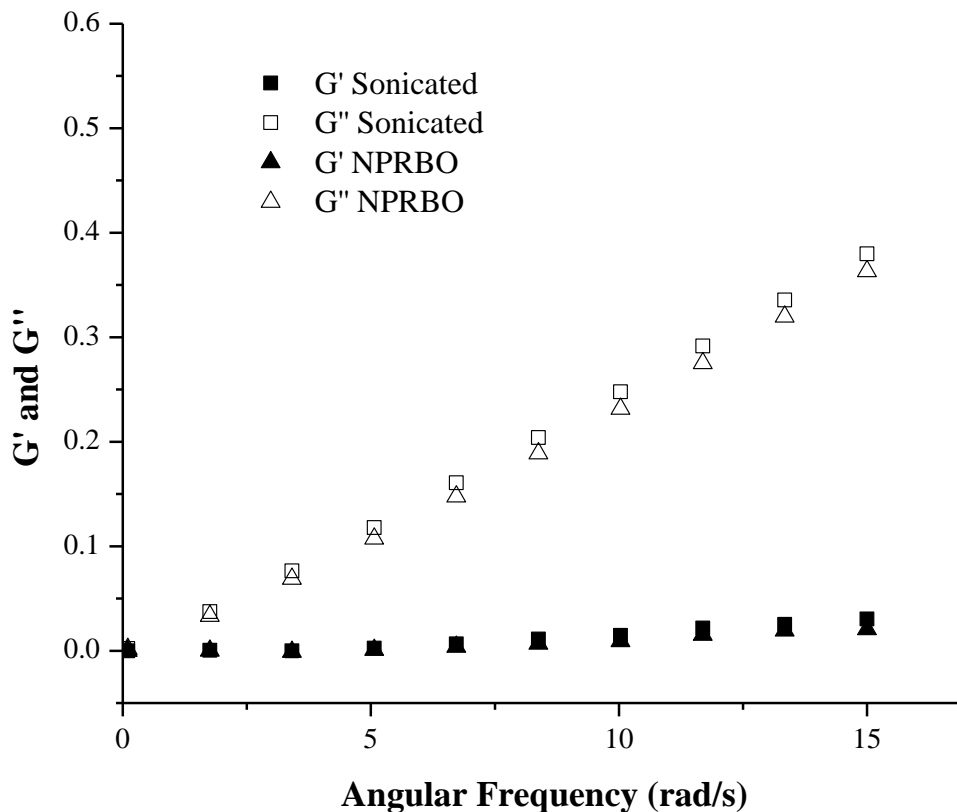


Figure 4.5 Frequency Sweeps Test of emulsions: Elastic Modulus (G') and Viscous Modulus (G'') vs. Angular Frequency

There was a slight decrease in most FAMES of NPRBO and sonicated emulsion, except for Heptadecenoic acid (C17:1) and Erucic acid (C22:1) which increased as PRBO>Sonicated emulsion>NPRBO. However, the results indicated non-significant ($p>0.05$) differences in the FAMES for NPRBO, control emulsion, and PRBO. Therefore, the processing steps did not produce significant changes in the FAMES of the rice bran oil.

4.5 Oxidative stability of emulsions

The peroxide value (PV) was measured to determine primary lipid oxidation products of the NPRBO and sonicated emulsion (control) during storage at 4 and 40 °C (Table 4.7).

Table 4.6 FAMES* composition (%) of NPRBO and sonicated emulsion

FAMES	PRBO (%)	Sonicated Emulsion (%)	NPRBO (%)
Myristic acid (C14:0)	0.7 ± 0.001 ^a	0.45 ± 0.004 ^a	0.52 ± 0.004 ^a
Palmitic acid (C16:0)	25.4 ± 0.020 ^a	24.54 ± 0.025 ^a	22.36 ± 0.056 ^a
Heptadecenoic Acid (C17:1)	1.8 ± 0.018 ^a	2.81 ± 0.016 ^a	2.87 ± 0.030 ^a
Oleic acid (C18:1)	42.2 ± 0.042 ^a	40.98 ± 0.064 ^a	40.30 ± 0.074 ^a
Linoleic acid (C18:2)	21.1 ± 0.021 ^a	20.79 ± 0.033 ^a	20.35 ± 0.045 ^a
Arachidic acid (C20:0)	0.5 ± 0.008 ^a	0.45 ± 0.004 ^a	1.39 ± 0.019 ^a
Erucic acid (C22:1)	6.7 ± 0.059 ^a	9.64 ± 0.097 ^a	11.07 ± 0.123 ^a

^{ab}Means with same letters across rows are not significantly different (P>0.05). FAMES = Fatty acid methyl esters, PRBO = purple rice bran oil, NPRBO = nano-emulsion containing purple rice bran oil. *Only major fatty acids were reported.

There was a slight increase in PV of emulsions stored at 4 °C, however, results indicate that there were not significant differences (p>0.05) in PV between NPRBO and sonicated emulsion during all storage times. The emulsions stored at 40 °C had similar PV during the first 48 hours storage. After 48 hr., NPRBO had significantly (p<0.05) higher PV than sonicated emulsion. This difference in PV may be due to the oil droplets in the nano-emulsion having a bigger exposed surface area because the high amount of very small droplets. Thus, this indicated that NPRBO is more susceptible to oxidation by heat compared with the control emulsion.

Table 4.7 PV (Meq/Kg oil) of NPRBO and control emulsions stored at 4 and 40 °C

Time, h	*Sonicated 4 °C	NPRBO 4 °C	*Sonicated 40 °C	NPRBO 40 °C
0	1.55 ± 0.36 ^a	1.71 ± 0.19 ^a	1.65 ± 0.18 ^a	1.88 ± 0.17 ^a
24	1.60 ± 0.24 ^a	2.06 ± 0.56 ^a	6.25 ± 1.24 ^a	8.42 ± 0.60 ^a
48	2.46 ± 0.57 ^a	2.31 ± 0.41 ^a	11.60 ± 1.71 ^a	14.03 ± 2.06 ^a
96	2.67 ± 0.68 ^a	2.92 ± 0.60 ^a	24.58 ± 3.02 ^b	39.76 ± 39.76 ^a
168	2.83 ± 0.26 ^a	2.97 ± 0.30 ^a	41.13 ± 6.97 ^b	49.60 ± 8.24 ^a

^{ab}Means with same letters across rows are not significantly different (P>0.05). PV = Peroxide Value, NPRBO = nano-emulsion containing purple rice bran oil. *Control.

The formation of secondary oxidation products is determined by measuring the thiobarbituric acid-reactive substances (TBARS). TBARS of NPRBO and sonicated emulsion stored at 4 and 40 °C are shown in Table 4.8. Similarly with the results for PV, the TBARS value of both emulsions stored at 4 °C had a slight increase during storage and non-significant ($p>0.05$) differences were found between emulsions in all storage times. In the case of the emulsions stored at 40 °C, TBARS increased significantly during the time of storage. Results are in agreement with those found by Tippetts and Martini (2010), in which study it was found that TBARS value had a maximum increase after 72 hr storage, when significant differences in off-odors were detected. Emulsions had similar TBARS values during the first 24 hr. After that time, NPRBO showed significantly higher ($p<0.05$) TBARS values than sonicated emulsion at 48 hr. and at the end of storage time (168 hr.). This also demonstrated that the nano-emulsion was more prone to oxidative reactions induced by heat. Badings (1970) states that hydroperoxides are very unstable and heat will accelerate their decomposition to secondary products.

Table 4.8 TBARS (mmol/kg oil) of NPRBO and control emulsions stored at 4 and 40 °C

Time, h	Sonicated 4 °C	NPRBO 4 °C	Sonicated 40 °C	NPRBO 40 °C
0	0.07 ± 0.03 ^a	0.09 ± 0.03 ^a	0.06 ± 0.01 ^a	0.11 ± 0.03 ^a
24	0.09 ± 0.05 ^a	0.12 ± 0.03 ^a	3.12 ± 1.91 ^a	6.12 ± 2.71 ^a
48	0.13 ± 0.03 ^a	0.17 ± 0.02 ^a	10.79 ± 3.00 ^b	27.23 ± 2.49 ^a
96	0.15 ± 0.03 ^a	0.21 ± 0.04 ^a	43.45 ± 4.71 ^a	38.56 ± 4.12 ^b
168	0.19 ± 0.04 ^a	0.34 ± 0.06 ^a	62.99 ± 6.68 ^b	85.06 ± 8.67 ^a

^{ab}Means with same letters across rows are not significantly different ($P>0.05$). TBARS = Thiobarbituric acid-reactive substances, NPRBO = nano-emulsion containing purple rice bran oil.

It is important to mention that there could be differences between oxidation in emulsions compared with bulk oils. Schwarz *et al.* (2000) studied the activity of antioxidants in oil in water

emulsions compared with bulk corn oil. They found that emulsions presented greater oxidation (as hexanal compounds) than bulk oil, indicating that oxidation in emulsions was caused mainly by the interaction of emulsifiers and minor compounds with water. In contrast with the emulsions, antioxidants in bulk oil showed higher activity and no prooxidant effects.

Oxidation of the PRBO also could be related with higher amounts of antioxidants, thus having a prooxidant activity. Previous investigations by Satue *et al.* (1995) studied the effect of different concentrations of α -tocopherol in the oxidative stability of olive oil. They found that α -tocopherol showed prooxidant activity at 500 ppm after 11 and 15 days of oxidation. In addition, Jung and Min (1992) found that hydroperoxides levels of purified soybean oil increased as concentrations of α -tocopherols increased from 0, 100, 250, 500 and 1000 ppm.

4.6 Rheological properties of yogurt mixes

Table 4.9 presents the rheological properties of the yogurt mixes. The viscosity of the frozen yogurt containing the nano-emulsion of purple rice bran oil (FYNRO) was not significantly different from the plain frozen yogurt (PFY) sample ($p > 0.05$). Hence the addition of NPRBO did not considerably change the viscosity of the yogurt mix compared to the plain yogurt mix. A low viscosity is required to have a good flow during freezing of yogurt mixes in the ice cream machine. The highest value for viscosity belonged to the FYSC sample (0.23 ± 0.01 Pa.s), which was significantly higher than FYNRO and PFY. This increase in viscosity is related to increased protein-protein interactions and protein bonds that increase the elastic character of the gel matrix of the yogurt (Damin *et al.* 2009). In addition, Marafon *et al.* (2011) reported an increase in viscosity (22.40 Pa.s) in yogurt prepared with sodium caseinate compared with the control (17.74 Pa.s). The addition of NPRBO also did not significantly affect the consistency index (K) of the FYNRO compared to the controls. All yogurt mixes had low K values, which is consistent with

the low viscosity of the yogurts. According to Batista et al. (2006), higher K values indicate high viscous consistency. Non-significant differences ($p < 0.05$) in flow behavior index (η) were observed for all yogurt mixes since all had $\eta < 1$, indicating that FYNRO, FYSC, and PFY mixes were pseudoplastic shear thinning fluids.

Table 4.9 Flow behavior properties of yogurt mixes

Sample	K^5 (Pa.s ⁿ)	η^4	Apparent Viscosity (Pa.s)
FYNRO ¹	2.35 ± 0.43 ^a	0.54 ± 0.06 ^a	0.19 ± 0.01 ^b
FYSC ²	3.52 ± 1.30 ^a	0.50 ± 0.07 ^a	0.23 ± 0.01 ^a
PFY ³	2.93 ± 0.40 ^a	0.46 ± 0.05 ^a	0.17 ± 0.01 ^b

^{ab}Means with different letters within columns are significantly different ($P < 0.05$). ¹FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, ²FYSC = Frozen yogurt with sodium caseinate, ³PFY = plain frozen yogurt, ⁴ η = flow behavior index, ⁵ K = consistency index.

The viscoelastic properties of the FYNRO, FYSC, and PFY yogurt mixes are shown in Fig. 4.6. The results indicate that mixes had some gel formation since G' values were higher than G'' values. However, G' and G'' values were not high, which indicate the mixes had running characteristics compared with higher values that correspond to stable gels. Estrada (2011), found that stirred plain yogurts formed stable gels with G' values higher than G'' with ranges of 500 to 900 and from 100 to 200 for G' and G'' respectively. The incorporation of NPRBO produced significantly ($p < 0.05$) lower values in both modules (G' and G'') compared with the PFY. Estrada (2011) reported different results, in which the addition of microencapsulated fish oils did not produced significant increase or decrease in either module for stirred yogurts when compared with a plain yogurt. Furthermore, Different results were found by Sanz et al. (2008), in which the incorporation of different types of fibre into yogurt produced an increase in the value of both modules in comparison with the control (white yogurt). The results found in our study can be

explained as the addition of NPRBO into the FYNRO caused a dilution effect, decreasing the structural compactness of the yogurt mix.

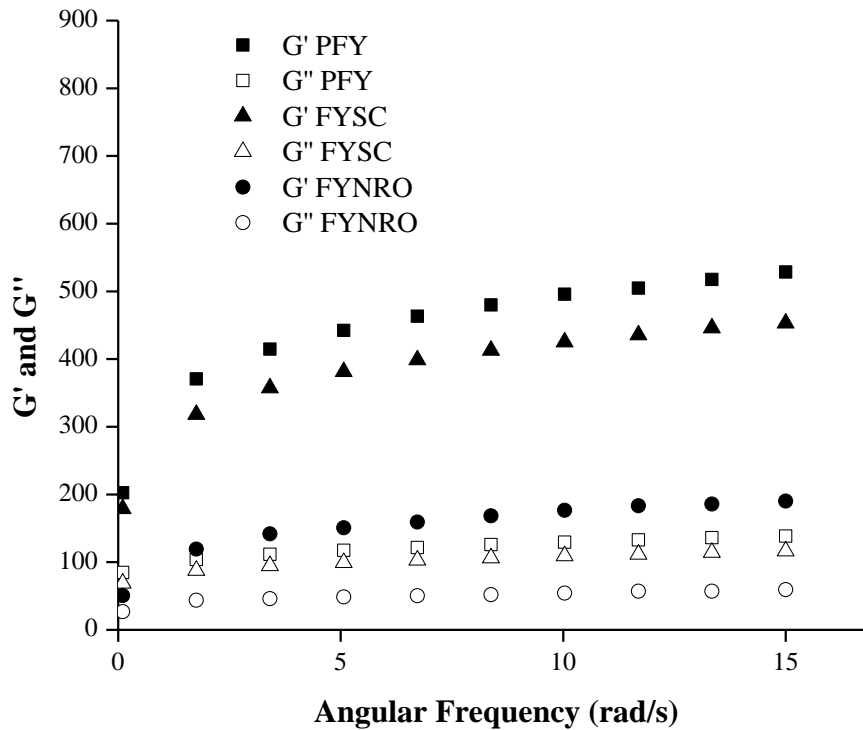


Figure 4.6 Frequency Sweeps Test of yogurt mixes: Elastic Modulus (G') and Viscous Modulus (G'') vs. Angular Frequency. FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, FYSC = frozen yogurt with sodium caseinate, PFY = plain frozen yogurt

4.7 Melting percent of FYNRO, FYSC, and PFY

The melting percent for frozen yogurts are presented in Fig. 4.7. No frozen yogurts had a significant melting at 15 min. FYNRO and FYSC presented the lowest melting % (w/w) at min 30, in comparison PFY had a significantly ($p < 0.05$) higher melting %. At min 45 FYNRO presented the lowest melting % (39.81 ± 2.81 %) and it was significantly different from FYSC and PFY (53.31 ± 4.80 and 56.39 ± 15.08 respectively). This indicated that the addition of NPRBO helped in the cohesion of the components in the frozen yogurt. According to Muse and

Hartel (2004), the melting percent can also be influenced by factors such as fat destabilization, ice crystal size formation, and the consistency coefficient of the mix.

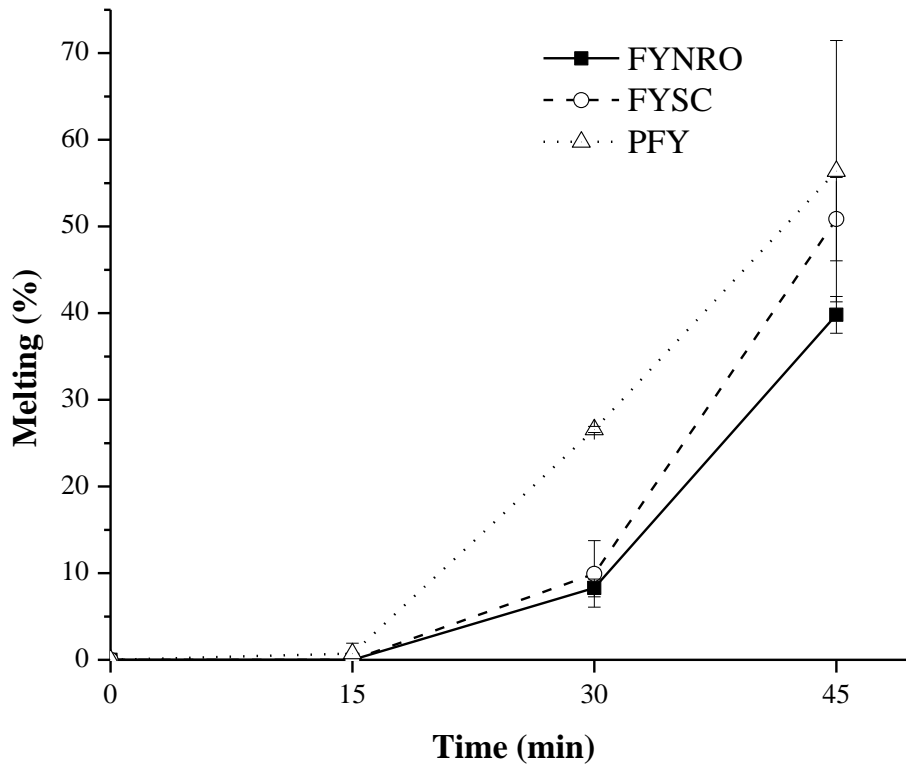


Figure 4.7 Melting (% w/w) of FYNRO, FYSC, and PFY. FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, FYSC = frozen yogurt with sodium caseinate, PFY = plain frozen yogurt

4.8 Overrun % of FYNRO, FYSC, and PFY

Overrun is one of the most important quality parameters of frozen desserts since it affects the texture and consequently the price of the products (Marshall *et al.*, 2003). All frozen yogurt samples had overruns % (w/w) between 19-22% (Table 4.10), which is in concordance with the standard of identity for frozen yogurt (02 NCAC 09K.0214, 2000). No significant differences

were observed among the samples ($p < 0.05$), therefore, it was observed that the addition of the NPRBO did not affect the overrun in the frozen yogurt.

Table 4.10 Overrun (%) of FYNRO, FYSC, and PFY

Sample	Overrun (%)
FYNRO ¹	20.46 ± 1.11 ^a
FYSC ²	21.96 ± 3.39 ^a
PFY ³	19.81 ± 2.71 ^a

Values are means ± standard deviations of triplicate determinations. ^aMeans with the same superscript are not significantly ($p > 0.05$) different. ¹FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, ²FYSC = Frozen yogurt with sodium caseinate, ³PFY = plain frozen yogurt.

4.9 Instrumental hardness of FYNRO, FYSC, and PFY

Hardness (N) of the frozen yogurts is reported in Table 4.11. No significant ($p < 0.05$) differences in hardness were found between frozen yogurts. Therefore, the addition of NPRBO did not affect the texture of the frozen yogurts. According to Hartel (2001), the texture is dependent on the composition (mainly protein and sugar content) and the effectiveness of the freezing process (e.g. overrun % and freezing time) of frozen desserts. Consequently, the high instrumental hardness of the frozen yogurts can be attributed to their lower overruns (%). Wilbey et al. (1997) found an inverse correlation between overrun and hardness of ice creams. Similarly, Sofjan and Hartel (2004) found that ice creams made with 80% and 100% overrun had the same hardness and were significantly harder than ice creams made with 120% overrun.

4.10 Lactic acid bacteria counts of frozen yogurts

Survival of cultures in frozen yogurt has great importance for the healthy properties of the product (Tamine and Robinson, 1999). The addition of a nano-emulsion containing purple rice

bran oil (NPRBO) did not affect the counts (Log CFU/ g frozen yogurt) of lactic acid bacteria (LAB) compared to LAB counts of the controls during 6 weeks of frozen storage at -22 °C.

Table 4.11 Instrumental hardness of FYNRO, FYSC, and PFY

Sample	Maximum load (N)
FYNRO ¹	155.76 ± 1.80 ^a
FYSC ²	155.82 ± 2.02 ^a
PFY ³	154.70 ± 1.19 ^a

Values are means ± standard deviations of triplicate determinations. ^aMeans with the same superscript are not significantly ($p > 0.05$) different. ¹FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, ²FYSC = Frozen yogurt with sodium caseinate, ³PFY = plain frozen yogurt.

Initial counts for *Streptococcus thermophilus* were 10.21 ± 0.18 , 10.12 ± 0.05 , and 10.31 ± 0.04 Log CFU/g frozen yogurt for FYNRO, FYSC, and PFY respectively (Fig 4.8). *Lactobacillus bulgaricus* counts were 10.17 ± 0.04 , 10.19 ± 0.05 , and 10.11 ± 0.1 Log CFU/g frozen yogurt for FYNRO, FYSC, and PFY respectively. *Lactobacillus bulgaricus* counts were 10.17 ± 0.04 , 10.19 ± 0.05 , and 10.11 ± 0.1 Log CFU/g frozen yogurt for FYNRO, FYSC, and PFY respectively. *Streptococcus thermophilus* counts remained similar at the end of the six weeks storage, with viable counts of 10.14 ± 0.09 , 10.03 ± 0.05 , and 10.17 ± 0.08 Log CFU/g frozen yogurt for FYNRO, FYSC, and PFY respectively. In addition, no significant differences were found between FYNRO and the controls. Similar results were observed for *Lactobacillus bulgaricus*, with viable counts of 10.02 ± 0.04 , 10.03 ± 0.20 , and 9.86 ± 0.20 Log CFU/g frozen yogurt for FYNRO, FYSC, and PFY respectively. In general, results indicate that starter cultures had a good stability during shelf life of the frozen yogurts. Results are in agreement with those obtained by Estrada et al. (2011); who found that the addition of microencapsulated menhaden oil (MMO) and microencapsulated salmon oil (MSO) had no effect on LAB counts of yogurts

during 4 weeks of storage. Lopez et al. (1998) studied the survival of LAB (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) in commercial frozen yogurt stored during 60 weeks at -23 °C. They reported initial counts of 7.57-7.58 and 4.29-6.79 log CFU/g for *Streptococcus thermophilus* and *Lactobacillus bulgaricus* respectively and found that LAB remained stable during storage and concluded that the shelf life period could be prolonged beyond 60 or 67 weeks.

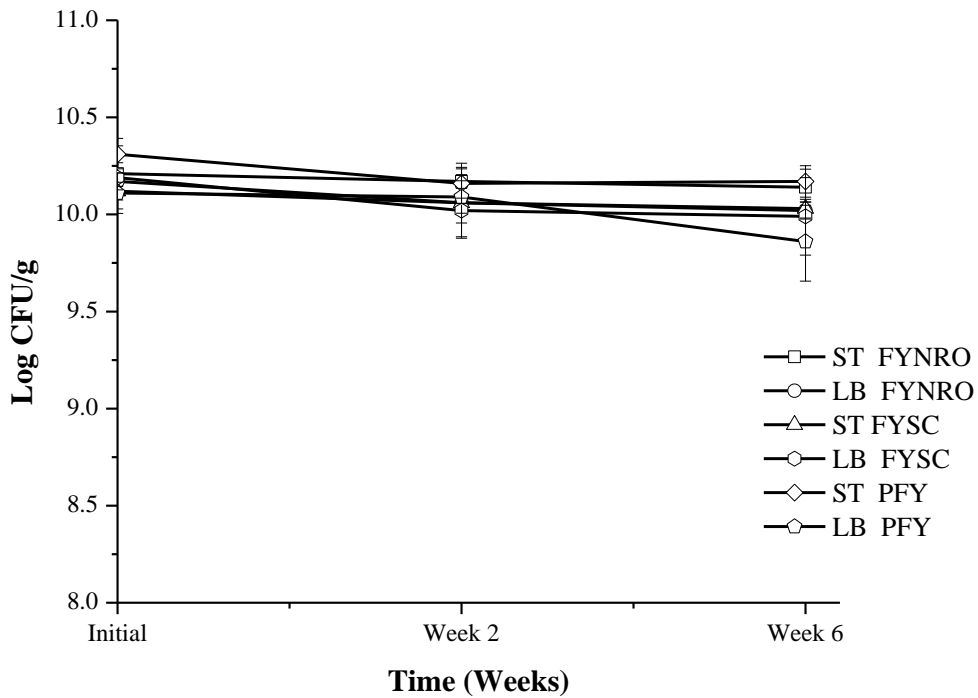


Figure 4.8 Lactic acid bacteria counts of frozen yogurts during 6 weeks storage. FYNRO=frozen yogurt with a nano-emulsion containing purple rice bran oil, FYSC=frozen yogurt with sodium caseinate, PFY=plain frozen yogurt, ST= *Streptococcus thermophilus*, LB = *Lactobacillus bulgaricus*

4.11 Peroxide value and TBARS of frozen yogurts

Peroxide value (PV) was measured to determine primary lipid oxidation products of the frozen yogurts during storage. Initial PV were 0.48 ± 0.15 , 0.27 ± 0.03 , and 0.17 ± 0.06 mmol/Kg

frozen yogurt for FYNRO, FYSC, and PFY respectively (Fig 4.11). The PV of FYNRO was significantly ($p < 0.05$) higher than the controls at the 6 week storage with PV of 3.55 ± 0.35 , 2.38 ± 0.75 , and 2.13 mmol/Kg frozen yogurt for FYNRO, FYSC and PFY respectively. This indicated that primary oxidation was initiated in FYNRO as an effect of the heat treatments during processing of the yogurt.

Final oxidation products during storage of the frozen yogurts were measured by thiobarbituric acid reactive substances (TBARS). No significant differences ($p < 0.05$) were found in TBARS values between all frozen yogurts during the 6 weeks of frozen storage (Fig. 4.12). Initial TBARS values for FYNRO, FYSC, and PFY were 3.88 ± 0.16 , 3.38 ± 0.23 , and 3.39 ± 0.39 μ moles MDA/Kg frozen yogurt respectively, and values remained similar at the 6 week time point. The effect of heat treatment on lipid oxidation of edible oils and dairy products has been studied previously (Al-Rowaily 2008; Albi et al. 1997; Downey 1969; Estrada 2011). Albi et al. (1997) reported that high oleic sunflower oil and virgin olive oil had an increase of peroxide value from 6.5 to 9.0 and from 16.1 to 19.8 mEq of O_2 /Kg respectively after a 120 min microwave oven heating treatment at half-power. Gonzales et al. (2003) reported a significant peroxide value increase from 2.91 to 7.77 and from 3.25 to 8.09 mEq of peroxide/kg oil during 2 months frozen (-20 °C) storage of ice creams made with modified milk containing high-oleic and high-linoleic fatty acids respectively.

4.12 Vitamin E vitamers and γ -oryzanol contents of FYNRO

Concentration of α -tocopherol, α -tocotrienol, γ -tocopherol, and γ -tocotrienol (vitamin E vitamers), during 6 weeks of storage of FYNRO are shown in Fig. 4.13. Initial contents were 6.98 ± 0.43 , 4.14 ± 1.28 , 11.39 ± 2.38 , and 16.80 ± 2.67 μ g/g lipids from FY for α -tocopherol, α -tocotrienol, γ -tocopherol, and γ -tocotrienol, respectively.

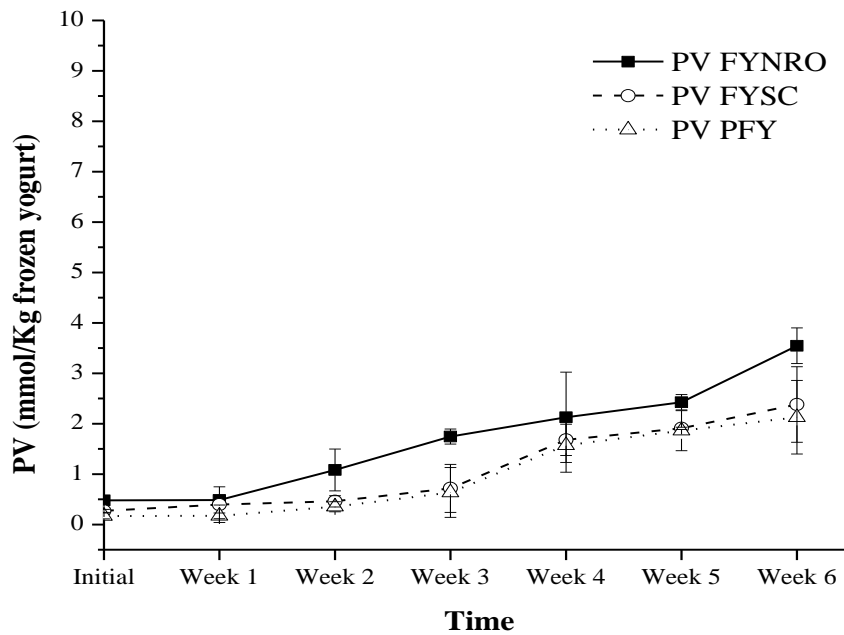


Figure 4.11 PV of frozen yogurts during 6 weeks storage. FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, FYSC = frozen yogurt with sodium caseinate, PFY = plain frozen yogurt

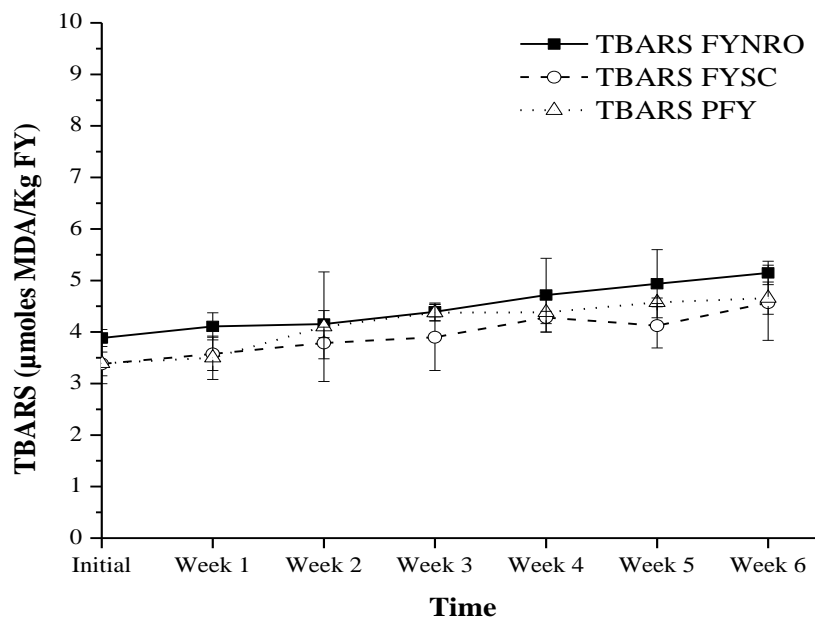


Figure 4.12 TBARS of frozen yogurts during 6 weeks storage. FY = frozen yogurt. FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, FYSC = frozen yogurt with sodium caseinate, PFY = plain frozen yogurt

These results are different from the values reported in the crude purple rice bran oil (PRBO), which indicate that there were losses in these compounds influenced by heating and/or lipid oxidation during processing of the nano-emulsions and frozen yogurts. According to Bramley et al. (2000), a number of factors such as oxygen, light, heat, alkali, trace minerals, and hydroperoxides can cause decomposition of vitamin E vitamers. Allouche et al. (2007) studied the effect of heat on the quality index and chemical composition of extra virgin olive oil and found a considerable loss of α -tocopherol (main component of vitamin E in olive oil) of 64% at the end of the heat treatment. Sabliov et al. (2009) also determined the effect of heat on degradation of α -tocopherol in both free and dissolved forms. They analyzed the degradation of α -tocopherol at temperatures of 40, 60, 120 and 180 °C under atmospheric pressure. It was found that for α -tocopherols, a first order degradation was observed in which the higher the temperature, the greater degradation. The decrease in vitamin E vitamers content could also be influenced by oxygen dissolved in the frozen yogurt from the aeration process during the yogurt mix freezing in the ice cream machine. In addition, the presence of hydroperoxides produced by primary oxidation reacts with these antioxidants, decreasing their content in the product (Diplock 1985). No significant changes ($p>0.05$) in the vitamin E vitamers were observed after 6 weeks of storage, with contents of 6.51 ± 0.89 , 2.98 ± 0.67 , 8.25 ± 0.44 , and 12.89 ± 2.58 $\mu\text{g/g}$ lipids from FY for α -tocopherol, α -tocotrienol, γ -tocopherol, and γ -tocotrienol respectively. This is related with the freezing temperatures of storage (-22 °C).

γ -Oryzanol concentration during 6 weeks storage of the FYNRO are reported in Fig. 4.14. The initial γ -oryzanol content from lipids extracted from the FYNRO was 858.05 ± 47.11 $\mu\text{g/g}$ lipids from FY. Similarly with results for vitamin E vitamers, this value was different from the value reported in PRBO, which indicated the heating processes and/or lipid oxidation caused

a decrease in this compound. No significant changes ($p>0.05$) in the γ -oryzanol content of FYNRO were observed at the end of 6 weeks of storage.

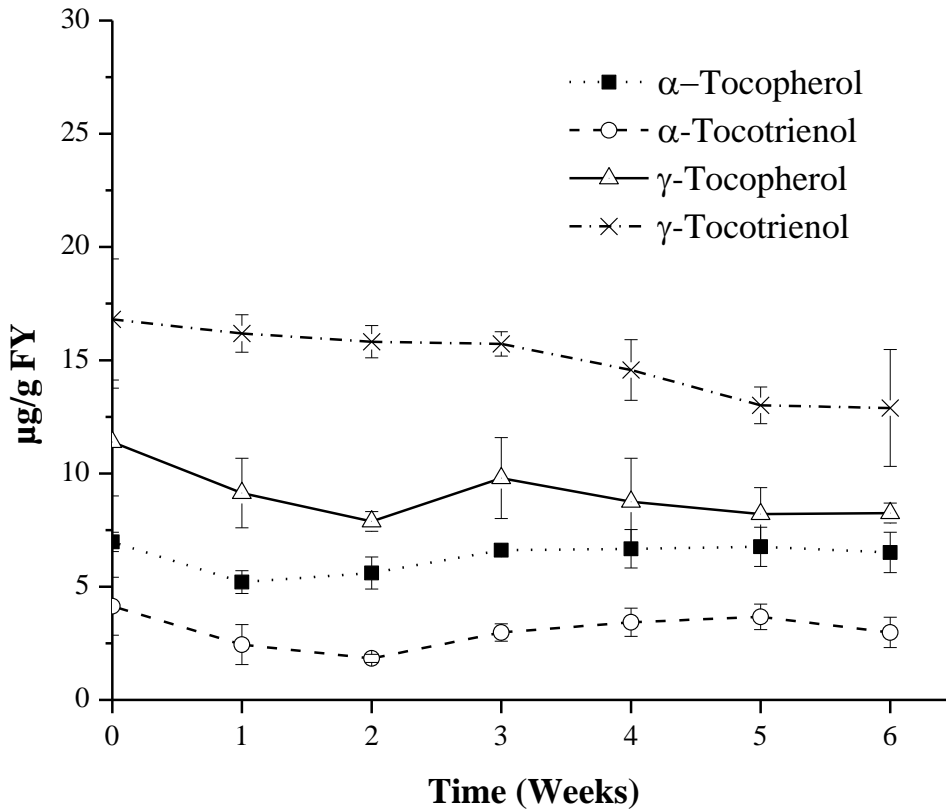


Figure 4.13 Changes of Vitamin E vitamers of FYNRO during 6 weeks storage. FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, FY = frozen yogurt

4.13 Color of frozen yogurts

Color is a very important attribute in food because it is usually the first property the consumer observes (Saenz and others, 1993). Changes in physical, chemical or microbiological parameters in yogurt affect shelf life and may cause color deterioration (Coggins *et al.*, 2010). L^* value changes for FYNRO, FYSC, and PFY during 6 weeks storage are reported in Fig. 4.15.

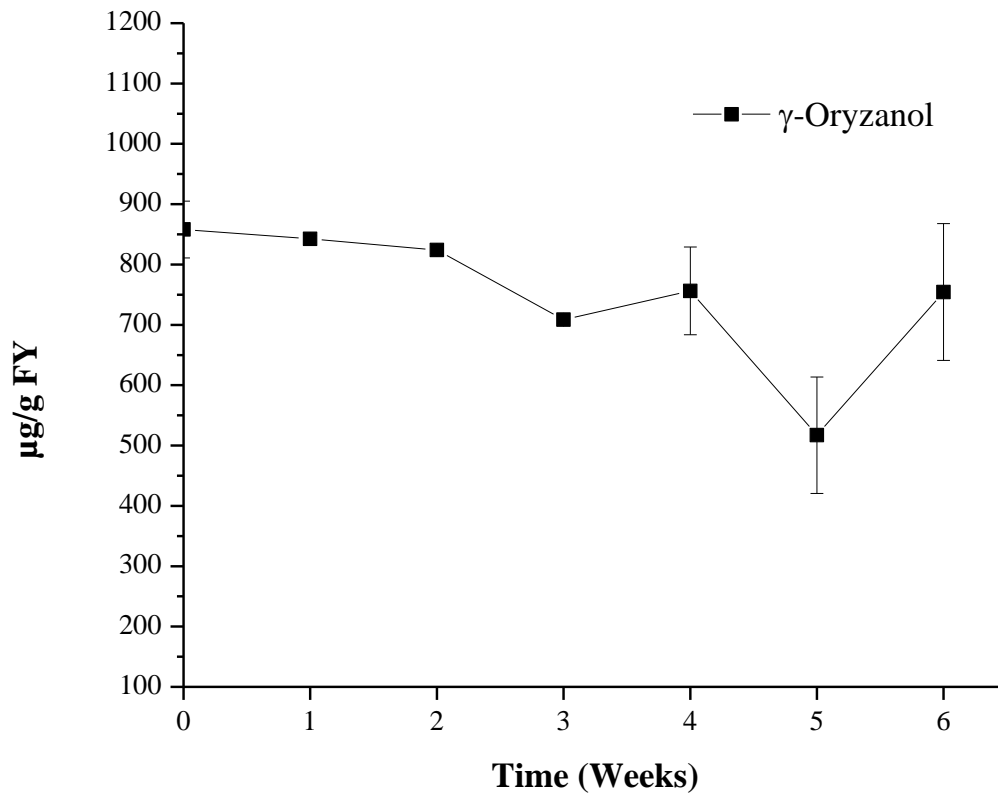


Figure 4.14 Changes of γ -Oryzanol content of FYNRO during 6 weeks storage. FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, FY = frozen yogurt

The L^* values at each sampling time were not significantly ($p > 0.05$) different among the different samples of frozen yogurt during the 6 weeks of storage, except at week 5 in which all frozen yogurts had different lightness. All frozen yogurts had negative a^* values (Fig. 4.16), which indicated that samples had a slight green color. A significantly ($p < 0.05$) lower greenness was observed between FYNRO and PFY from week 4 to week 6 storage and between FYNRO and FYSC at weeks 5 and 6. Not significant differences ($p < 0.05$) in yellowness between all frozen yogurts were observed for most of the storage time (Fig. 4.17). However, FYNRO showed a significantly higher yellowness (b^* value) compared with FYSC and PFY at week 6 of

storage. This could be related with a release of whey over time which had an effect on the yellowness of the FYNRO at the end of storage. The L^* , a^* and b^* values were converted to total color difference (ΔE^*) values using the initial (Day 1) values of each sample as reference for the calculations (Table 4.12). The ΔE^* values increased as storage time increased. FYSC and PFY had ΔE^* values of 0.23 to 2.70 and 0.46 to 1.80, respectively at the beginning and end of storage, which is acceptable since it is reported that ΔE^* values less than 3.0 cannot easily be detected by the naked human eye (Caner, 2005). FYNRO had significant changes with ΔE^* values of 0.37 to 3.41 at the beginning and end of the 6 week storage. Since L^* values for all frozen yogurts remained unchanged during the 6 weeks of storage, the differences in ΔE^* values between the FYNRO and FYSC and PFY are related with the decrease in a^* value and the increase in b^* value for FYNRO at the end of storage.

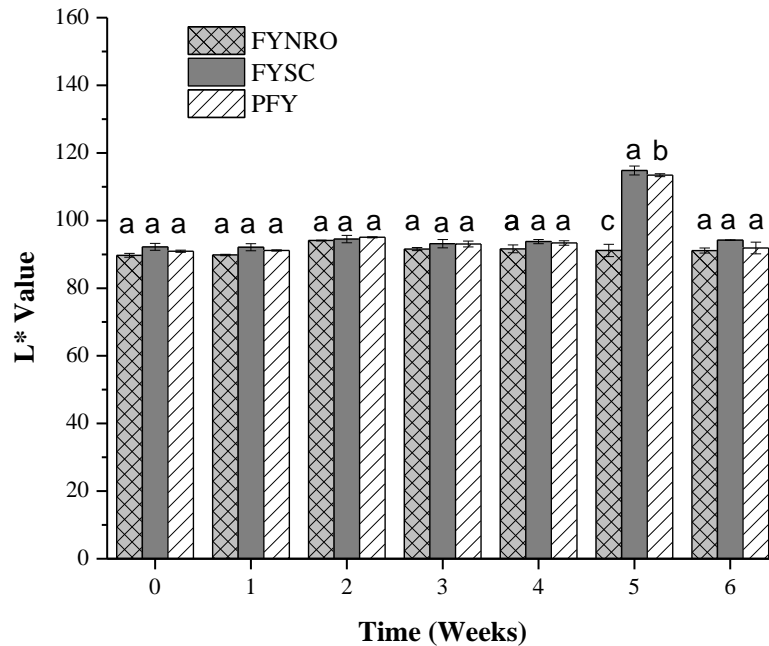


Figure 4.15 Color lightness values (L^*) of frozen yogurts during storage. ^{abc}Different superscripts indicate significant ($p < 0.05$) differences between samples at the same storage time.

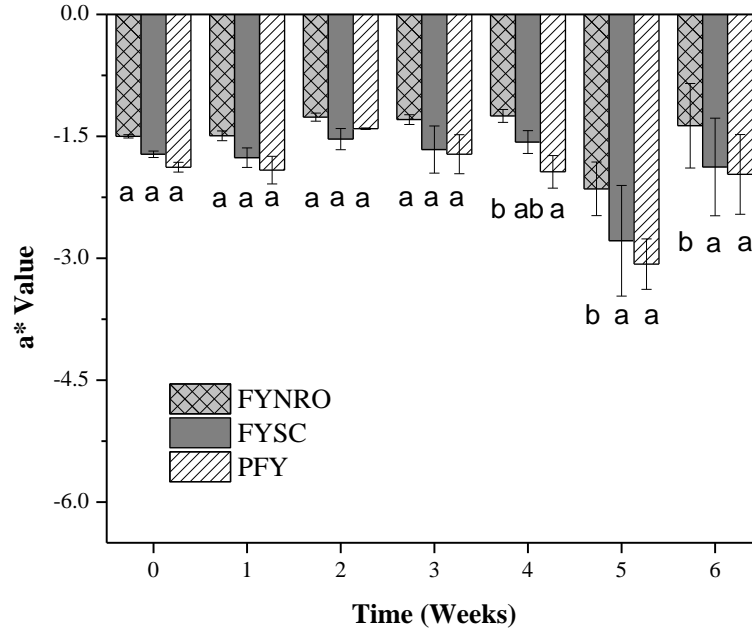


Figure 4.16 Color green (negative values)/ red (positive values) (a*) of frozen yogurts. ^{abc}Different superscripts indicate significant (p<0.05) differences between samples at the same storage time.

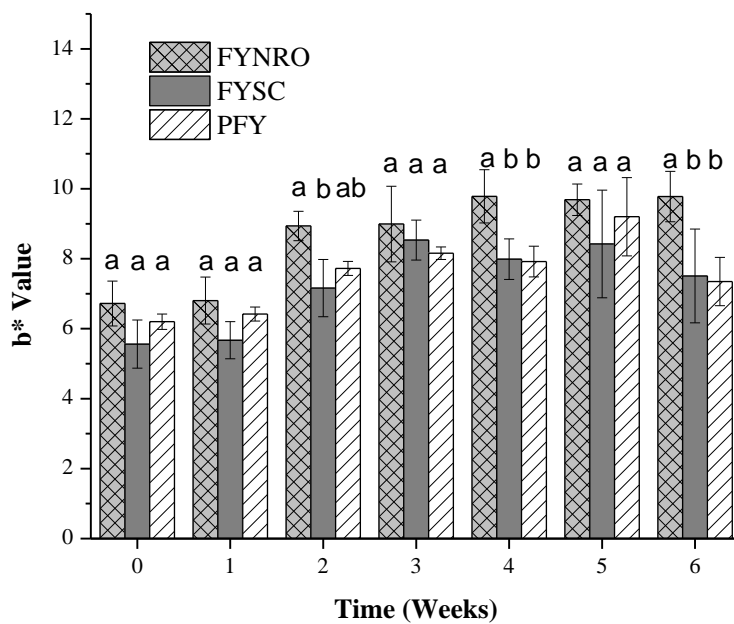


Figure 4.17 Color blue (negative values)/ yellow (positive values) (b*) of frozen yogurts. ^{ab}Different superscripts indicate significant (p<0.05) differences between samples at the same storage time.

Table 4.12 Total color differences of frozen yogurts during 6 weeks of storage

Time interval	FYNRO¹	FYSC²	PFY³
Day 1 - Week 1	0.37 ± 0.05 ^c	0.23 ± 0.20 ^b	0.46 ± 0.18 ^c
Day 1 - Week 2	4.97 ± 0.48 ^a	3.05 ± 1.39 ^b	4.44 ± 0.32 ^b
Day 1 - Week 3	2.84 ± 0.54 ^b	3.29 ± 1.61 ^b	3.44 ± 1.08 ^{bc}
Day 1 - Week 4	3.62 ± 0.95 ^{ab}	2.99 ± 1.74 ^b	3.60 ± 1.54 ^b
Day 1 - Week 5	3.75 ± 0.78 ^{ab}	17.68 ± 9.44 ^a	23.45 ± 1.51 ^a
Day 1 - Week 6	3.41 ± 0.29 ^{ab}	2.70 ± 0.37 ^b	1.80 ± 1.31 ^{bc}

Values are means ± standard deviations of triplicate determinations. ^{abc}Means within the same column with the same superscript are not significantly ($p>0.05$) different. ¹FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, ²FYSC = Frozen yogurt with sodium caseinate, ³PFY = plain frozen yogurt.

4.14 Fatty acid methyl esters (FAMES) analysis of FYNRO, FYSC, and PFY

FYNRO, FYSC and PFY FAMES compositions are shown in Table 4.13. . FYNRO had contents of palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acid of 39.92 ± 0.07 , 26.93 ± 0.04 , and 4.67 ± 0.01 % respectively. All were significantly ($p<0.05$) higher compared to FYSC and PFY. Therefore, it could be observed that the addition of NPRBO (22.36 % palmitic acid, 40.30 % oleic acid, and 20.35 % linoleic acid) significantly increased the content of these fatty acids in the FYNRO. Linoleic acid was found in very low amounts in the control frozen yogurts (PFY and FYSC). Estrada et al. (2011) reported significant decrease between 4.57-25.64 % in polyunsaturated fatty acids (PUFA) after processing and 4 weeks storage of stirred yogurts, which was attributed to lipid oxidation occurrence.

4.15 Freezing behavior of FYNRO, FYSC, and PFY

Overall enthalpy (ΔH), onset point, and endothermic peak temperature of FYNRO, FYSC, and PFY are reported in Table 4.14. ΔH is the heat energy required for melting the frozen yogurts. All frozen yogurts had similar overall enthalpy values with no significant differences ($p>0.05$) between treatments.

Table 4.13 FAMES composition (%) of FYNRO, FYSC and PFY*

Compound Name	FYNRO ¹ (%)	FYSC ² (%)	PFY ³ (%)
Myristic acid (C14:0)	11.28 ± 1.52 ^a	0.00 ± 0.00 ^b	4.87 ± 1.72 ^{ab}
Palmitic acid (C16:0)	39.92 ± 6.61 ^a	11.14 ± 3.63 ^b	19.14 ± 7.88 ^b
Heptadecenoic acid (C17:1)	1.63 ± 2.31 ^b	9.60 ± 2.39 ^{ab}	10.28 ± 5.85 ^a
Oleic acid (C18:1)	26.94 ± 4.16 ^a	6.73 ± 2.15 ^b	12.68 ± 4.55 ^b
Linoleic acid (C18:2)	4.67 ± 0.88 ^a	0.00 ± 0.00 ^b	1.82 ± 0.69 ^b
Arachidic acid (C20:0)	0.22 ± 0.31 ^b	7.06 ± 9.98 ^a	1.20 ± 1.69 ^b
Eicosadienoic acid (C20:2)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	1.95 ± 2.75 ^a
Erucic acid (C22:1)	8.21 ± 11.60 ^c	62.70 ± 0.41 ^a	45.15 ± 2.19 ^b
Docosadienoic acid (C22:2)	1.01 ± 0.30 ^a	2.78 ± 1.39 ^a	0.96 ± 0.45 ^a

Values are means ± standard deviations of triplicate determinations. ^{abc}Means across rows with the same superscript are not significantly ($p>0.05$) different. ¹FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, ²FYSC = Frozen yogurt with sodium caseinate, ³PFY = plain frozen yogurt. *Only major fatty acids were reported.

Similar results were observed for the extrapolated onset temperatures, which are the temperatures at which the frozen yogurts start melting. Differential scanning calorimetry thermograms in all frozen yogurt samples showed an endothermic peak around 0 °C (Fig. 12) which has been attributed to ice melting (Alvarez et al. 2005). The addition of a NPRBO did not affect the melting point of the frozen yogurt or the freezing behavior of the yogurt mix.

Table 4.14 Differential scanning calorimetry analysis for frozen yogurts

Sample	ΔH^1 (J/g)	Extrapolated onset temperature (°C)	Endothermic peak temperature (°C)
FYNRO ²	211.45 ± 12.23 ^a	-8.35 ± 0.13 ^a	-1.91 ± 0.36 ^a
FYSC ³	218.25 ± 8.70 ^a	-8.07 ± 0.22 ^a	-1.00 ± 0.06 ^a
PFY ⁴	220.55 ± 7.14 ^a	-7.91 ± 0.30 ^a	-1.74 ± 0.37 ^a

Values are means ± standard deviations of triplicate determinations. ^aMeans within the same column with the same superscript are not significantly ($p>0.05$) different. ¹ ΔH = Overall enthalpy. ²FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, ³FYSC = Frozen yogurt with sodium caseinate, ⁴PFY = plain frozen yogurt.

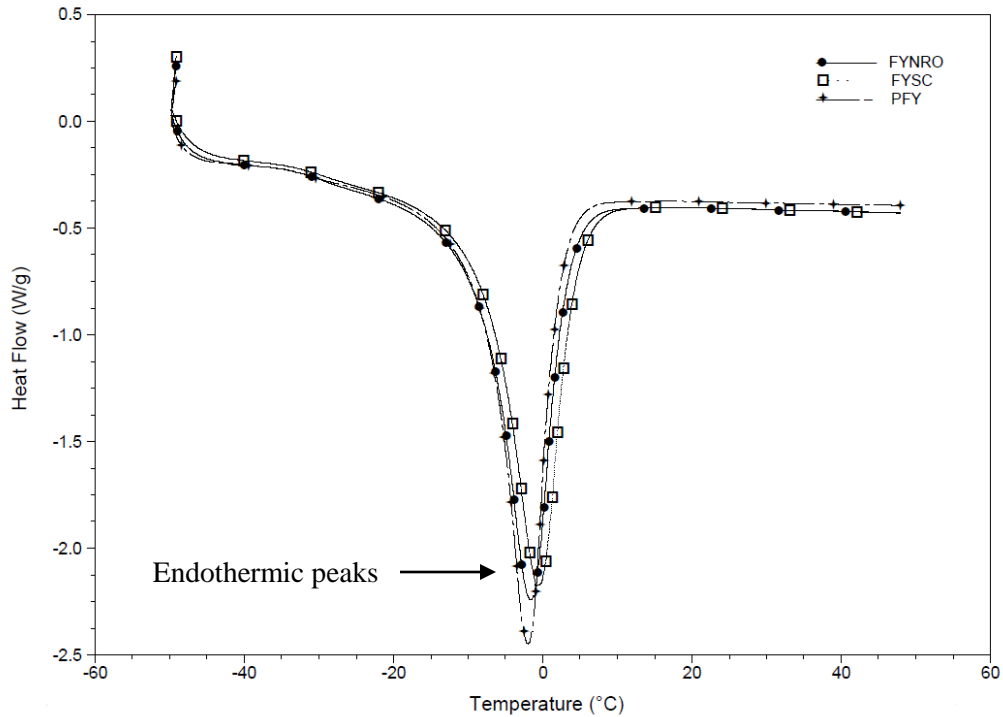


Figure 4.18 Differential scanning calorimetry melting curves for frozen yogurt samples. FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, FYSC = frozen yogurt with sodium caseinate, PFY = plain frozen yogurt

4.16 Confocal Scanning Laser Microscopy (CSLM) of FYNRO, FYSC, and PFY structure

CSLM has been used to examine fully hydrated yogurt samples (Hassan *et al.*, 1995; Andersen *et al.*, 2003). Casein micelles can be observed with CSLM since they have the ability to reflect laser beams, additionally, proteins can be stained by protein-specific fluorescent dyes (Tamime *et al.*, 2007). The CSLM micrographs after production of FYNRO, FYSC, and PFY are presented in Fig. 4.19. Large masses of casein aggregates are observed in all samples (orange color) separated by non-reflective black spaces (serum cavities). Lee and Lucey (2004) analyzed the structure of yogurt gels by CSLM and reported a microstructure with cross-linked and uniformly distributed protein network with the presence of larger pores. The FYNRO micrograph showed a more compact and dense structure with not many spaces as in FYSC and

PFY. From these results it could be observed that the NPRBO mixed well with the rest of ingredients after homogenization of the yogurt mix.

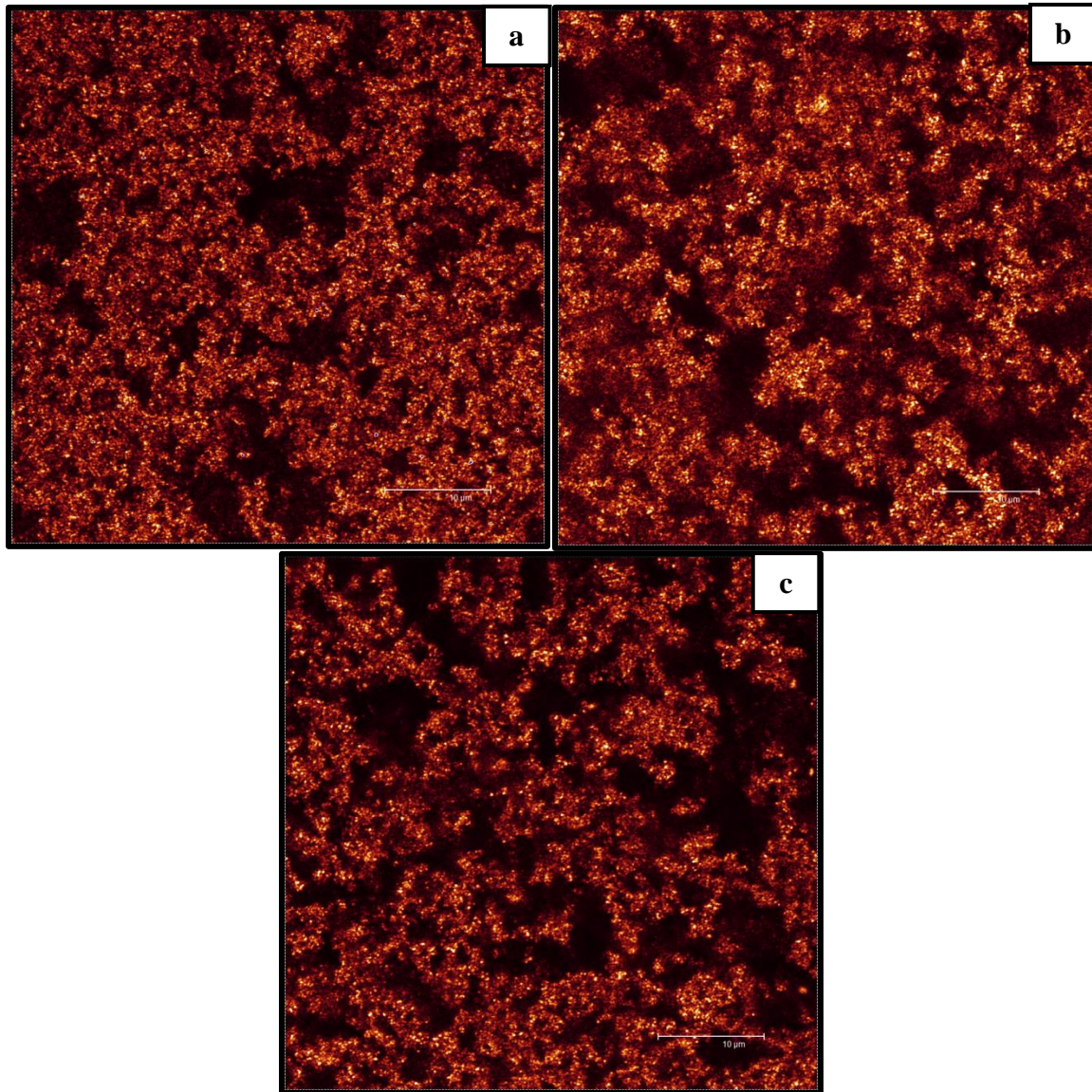


Figure 4.19 Confocal scanning laser micrographs of FYNRO (a), FYSC (b), and PFY (c). Bar = 10µm, FYNRO = frozen yogurt with a nano-emulsion containing purple rice bran oil, FYSC = frozen yogurt with sodium caseinate, PFY = plain frozen yogurt

CHAPTER 5. SUMMARY AND CONCLUSIONS

Rice bran oil (RBO) is extracted from rice bran, which is a byproduct of the milling processing of rice. It is known that RBO has a good balance of saturated, monounsaturated, and polyunsaturated fatty acids and meets the American Heart Association (AHA) recommendations for health improvement. In addition, RBO is rich in nutraceutical compounds such as tocopherols, tocotrienols and gamma oryzanol and it is known for its antioxidant and hypocholesteromic effects in humans. RBO from a new variety called purple rice (purple rice bran oil) is gaining interest due to the bioactive compounds found. Cholesterol levels in the US population are high and cardiovascular diseases are the number one cause of death in the United States. As a result, there is a big market for food products with added nutraceutical compounds that helps improve health. Recently there is an interest in nano-emulsions due to their benefits such as better stability and functional performance in food processing. Considering the above, the production of a functional frozen yogurt containing a nano-emulsion of purple rice bran oil is a new alternative to broaden the frozen yogurt market to health conscious consumers. The aims of this study were to produce a nano-emulsion containing purple rice bran oil (NPRBO), and to develop a frozen yogurt containing a NPRBO.

The specific objectives of the study were to: (1) determine the physicochemical characteristics of extracted purple rice bran oil (PRBO); (2) produce and characterize a nano-emulsion containing purple rice bran oil (NPRBO); and (3) determine the effect of addition of NPRBO on the physicochemical characteristics of frozen yogurt during 6 weeks of frozen storage. The physicochemical characteristics of PRBO were analyzed. FFA%, PV, and moisture of PRBO were in values acceptable for edible oils. α -Tocopherol, γ -tocotrienol and γ -oryzanol were the highest antioxidants found in the PRBO compared with α -tocotrienol and γ -tocopherol. The fatty

acid composition was found to be similar to brown RBO (conventional) with oleic (C18:1), linoleic (C18:2), and palmitic (C16:0) as predominant fatty acids. A nano-emulsion containing purple rice bran oil (NPRBO) was produced by ultrasonication followed by ultrashearing. NPRBO was more stable than control emulsion since had less oil recovery. The size of NPRBO was found as 199.2 nm and it was significantly lower than that of a control emulsion produced by sonication only. The microstructure of NPRBO had many very small droplets in the range of nano-scale, whereas the control emulsion had fewer droplets of larger sizes. The differences in microstructure corresponded to the differences in size found. Apparent viscosity of NPRBO was significantly ($p < 0.05$) lower than control emulsion. FAMES of emulsions indicated that the processing steps to develop NPRBO and the control emulsion did not produce significant changes in the FAMES of the rice bran oil since non-significant ($p > 0.05$) differences were found in the FAMES of NPRBO, control emulsion and PRBO. The oxidative stability analyses of emulsions indicated that NPRBO and the control emulsion had lower oxidation during storage at 4 °C. However, NPRBO had more oxidation than control emulsion when stored at 40 °C. This increase in oxidation was due to the higher surface area exposure of the oil droplets in the nano-emulsion because the high amount of very small droplets, thus, were more susceptible to high temperatures.

Frozen yogurt was produced and NPRBO was added to the rest of ingredients before homogenization and pasteurization of the yogurt mix. The addition of NPRBO did not affect the rheological properties of the yogurt mix of the FYNRO compared to PFY. Melting % of FYNRO was significantly lower than FYSC and PFY at min 45, which indicated that the addition of NPRBO helped in the cohesion of the frozen yogurt. No significant differences ($p > 0.05$) were found for overrun and instrumental hardness of all frozen yogurts. The addition of NPRBO did

not affect lactic acid bacteria (LAB) survival during the 6 weeks of frozen storage at -22 °C as evidenced by counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* being stable for all frozen yogurts. Similar results were observed for pH and TA%, with no significant changes during storage. A significant increase in PV of FYNRO was observed compared with FYSC and PFY at the end of storage time. On the other hand, no significant differences in TBARS within treatments were observed for all frozen yogurts during storage. Vitamin E vitamers and γ -oryzanol contents of lipids from FYNRO were found to be significantly lower compared with values obtained from purple rice bran oil. This indicated that losses in these compounds were influenced by heating and lipid oxidation reactions occurring during processing. L*, a*, and b* values were converted to total color difference (ΔE^*). FYNRO had significant changes in ΔE^* at the end of storage with an increase from 0.37 to 3.41 at the 6 week storage time. FAMES of the frozen yogurts indicated that FYNRO had significantly higher contents of palmitic, oleic, and linoleic acid (39.92 ± 0.07 , 26.93 ± 0.04 and 4.67 ± 0.88 % respectively) compared with FYSC and PFY. . CSLM micrographs showed that the addition of NPRBO produced a more dense structure in the FYNRO compared with FYSC and PFY, which indicated that NPRBO mixed well with the ingredients of the frozen yogurt. This study demonstrated that frozen yogurt could be fortified with a nano-emulsion containing purple rice bran oil (NPRBO) to create a product with unique marketing potential in the dairy industry and analyzed significant properties of the resulting frozen yogurt.

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