Whey Protein Concentrate as a Substitute for Non-Fat Dry Milk in Yogurt.

Thesis

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Abstract

Whey is a by-product liquid produced from cheese manufacturing. Whey used to be a waste product but its conversion through new technologies such as fractionation, modification, and filtration has made it possible to improve its utilization. Whey proteins have good nutritional properties and enhance the textural properties of food when they are used as ingredients. The objective of this study was to evaluate non-fat, low-fat and full-fat stirred style strawberry flavored yogurt formulated with whey protein concentrate 80 (WPC80) replacing non-fat dry milk. Levels of total solid were adjusted to 14.8%, 15.7%, and 17.3% for non-fat, low-fat and full-fat yogurts, respectively. Non-fat dry milk yogurt formulation was used as a control. Batches of 17 pounds of yogurts at 0% fat, 1% and 3.3% fat were made by mixing milk with powdered ingredients in a liquefier, homogenized at 2300psi, and pasteurized at 92^{o}C for 30 seconds. Following cooling, yogurts were fermented at a final pH of 4.5. Yogurts were analyzed for their chemical and physical properties following the standard methods of analyses. Sensory evaluation was done by descriptive method with hedonic and monadic scales. Whey protein yogurts at all fat levels showed better water holding capacities (*ca*. 10%) than controls with increased hardness (*ca*. 20%) and viscosity (*ca*. 40%). Sensory results revealed that whey protein yogurts had higher flavor and overall liking scores than controls, while controls

had better scores for the yogurts' texture. Results showed that WPC80 is a good alternative to replace NFDM completely in yogurt. Whey yogurts had equal or greater quality than yogurt products made with NFDM.

Dedication

Dedicated to my family.

Thank you for your unconditional love and always believe in me.

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Table of Contents

Abstract ii

Dedication iv

Acknowledgements v

List of Tables ix

List of Figures x

Chapter 1: Introduction 1

Chapter 2: Literature Review 4

2.1. Yogurt 4

2.2. Nonfat Dry Milk 13

2.3. Whey Protein Concentrate 15

2.4. Fat in Yogurt 18

2.5. Sweetener 20

2.6. Stabilizer 21

2.7. Yogurt Flavor 23

Chapter 3: Materials and Methods 25

Preliminary Work: 25

3.1. Formulation 26

3.2. Yogurt Processing and Pilot Plant scale-up. 28

3.3. Chemical, Textural and Sensory Analyzes 29

Chapter 4: Results and Discussion 34

4.1. Composition 34

4.2. Texture 41

4.3. Sensory 46

Chapter 5: Conclusion 51

References 53

Appendix A: Statistical Results 58

Total Solids Results 58

Fat Content Results 60

Protein Content Results 62

pH Results 64

Hardness Results 66

Water Holding Capacity Results 68

Viscosity Results 70

List of Tables

Table 3.1. Formulations for the developed control and whey protein products…….33

Table 3.2. Chemical analyses of experimental design………………………………37

Table 3.3. Physical analyses of experimental design………………………………..37

Table 3.4. Sensory tests of experimental design……….……………………………38

List of Figures

Figure 2.1. Total U.S. yogurt sales by years……………………………………14

Figure 4.1. Total solids content of yogurt products………………………….39

Figure 4.2. Fat content of yogurt products………..…………………………40

Figure 4.3. Protein content of yogurt products……….……………………42

Figure 4.4. pH of yogurt products……………………………………………44

Figure 4.5. Hardness values of yogurt products……………..………………47

Figure 4.6. Water holding capacities of yogurt products…………………….48

Figure 4.7. Viscosity values of yogurt products………………..…………….50

Figure 4.8. Monadic scale sensory scores of yogurt products………………..52

Figure 4.9. Hedonic scale sensory scores of yogurt products………………..53

Chapter 1: Introduction

Yogurt is a fermented milk product and carries a health halo due to its probiotic cultures and its reported effects in cancer studies. In theory, only milk and starter cultures activity is enough to produce a yogurt product. However, in practice, yogurt milks total solids content needs to be increased to produce a better product without syneresis. This can be achieved with longer heating times which will decrease the moisture in milk hence increasing the solids content. Similarly, fortifying milk with dry protein powders will increase the total solids content to desired levels. The common practice for today's yogurt industry is formulating yogurt milk with dry protein powders using enough heating and holding times to denaturate the milk proteins. Major milk protein casein denaturates at 160^{o}C, however whey proteins start to denaturate after 70^{o}C. Since protein denaturation enhances the gel structure of yogurt it will decrease the need for the dry protein powder addition. Therefore, fortifying milk with whey protein powders will result in better textured yogurt product.

Whey proteins are by products of the cheese making process and used to be a waste product. This was due to its high lactose content, acidity and whey flavor. However, novel production techniques such as drying and filtration systems made it possible to decrease the lactose content while increasing its protein content. The processes also reduce the relatively objectionable whey flavor. Therefore, newly manufactured whey protein concentrates can have a wide spectrum of usage in the food industry. Dairy, bakery and confectionary are the top three food applications for whey protein concentrate. However, there is not sufficient data reported about the use of whey protein concentrates as major ingredients. When compared with other proteins like egg, meat or milk proteins (casein), which are considered good quality proteins, whey protein has better nutritional properties. Whey proteins structure is rich in branched chained amino acid (BCAA) such as leucine, valine, and isoleucine. Whey proteins are available in the forms of acid whey, sweet whey, whey protein concentrate (protein content range 34% to 80%) and whey protein isolate (protein content >90%). It would be a waste to not to use this valuable ingredient as a major ingredient and benefiting its textural and nutritious properties.

Whey protein products are well known as replacements for egg proteins in confectionery and bakery products. Whey proteins are also used as functional ingredients and as milk replacers in dairy products such as ice cream and as a solid non-fat contributor in yogurt products in addition to non-fat dry milk. Whey protein can be used in ice cream up to 10%. However, the recommended range for yogurt is 0.4% to 2%. Since WPC is a dairy ingredient, which comes directly from milk, it can be used in dairy applications and labeled as a natural ingredient. Previous studies show that yogurt can be a good source of WPC supplementation since it acts as a stabilizer in yogurt's texture by improving its protein denaturation. However, the current regulations allow WPC to be used only as a secondary ingredient at yogurt applications. Regulations state that the solids should come from either evaporated milk or non-fat dry milk (NFDM) to increase the milk solids non fat levels to required 8.25% levels. Therefore, it is limiting the use of WPC as a major ingredient.

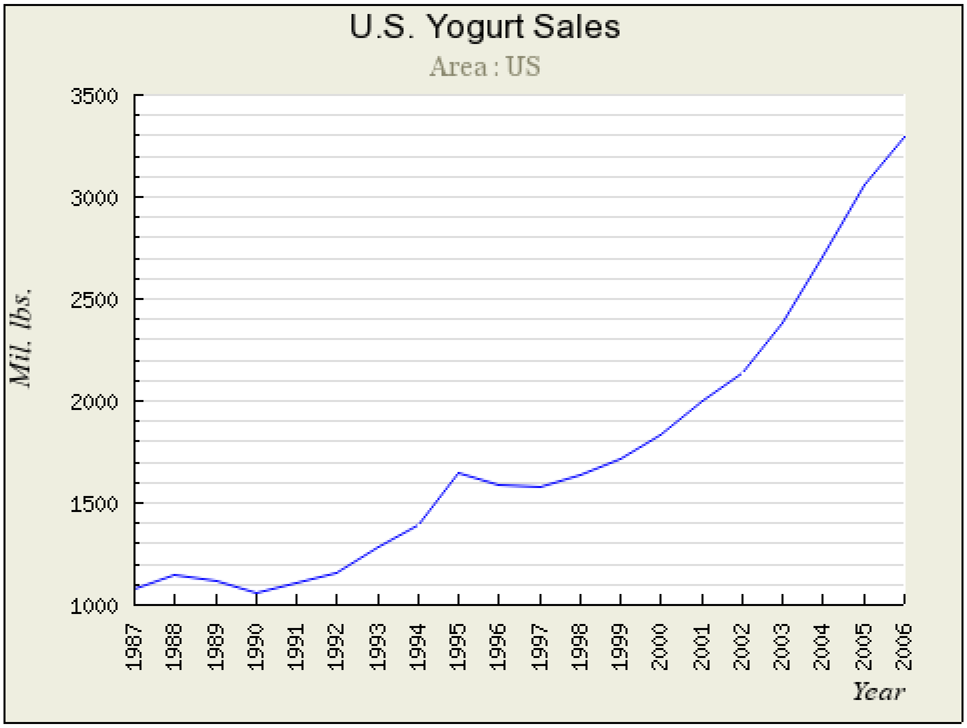
The objective of this study is to investigate the replacement of NFDM with WPC in yogurt formulations at three fat levels. Products were analyzed for their compositional, textural and sensory properties.

Chapter 2: Literature Review

2.1. Yogurt

Yogurt is made from the lactic fermentation of milk by associative growth of *Lactobacillus delbrueckii ssp. Bulgaricus* and *Streptococcus thermophilus* (Sandoval-Castilla et al., 2004). This mutuality gives yogurt its unique texture by increased amounts of lactic acid coming from its live cultures (Tamime and Robinson, 1999). Recently, addition of probiotic cultures as *Lactobacillus acidophilus* and *Bifidobacterium bifidus,* which are widely used in industrial applications, enhanced yogurts functional properties such as immune system boosting, digestion improvement, and anti-carcinogenic activity by reducing serum cholesterol levels (Lourens-Hattingh and Viljoen, 2001).

Yogurt holds 50% of the cultured product market sales in U.S. (Thompson et al., 2007) and according to figure (figure 2.1); this trend routine is increasing every year. Introduction of new sweetened flavor compounds especially strawberry reduced the amount of fat content and addition of probiotic cultures can be counted as major reasons for this increase (Barnes et al., 1991; Thompson et al., 2007).



(http://future.aae.wisc.edu/data/annual_values/by_area/2139?tab=sales)

Figure 2.1. Total U.S. Yogurt Sales by Years

Yogurt has a challenging and diverse market structure. Consumer needs and demands are the major attributes that help to shape the yogurt market. Newly developed or enhanced yogurt products kept entering the market place as consumer's dietary habits and lifestyles changed. The biggest change started with the entrance of flavored yogurts into the market. Moreover, other attributes such as different levels of fat, sugar content, live culture selection, etc. helped to create a more diverse product on market shelves (Chandan et al., 2006).

There are two general types of yogurts based on their manufacturing techniques, which are set and stirred type yogurts.

Set style yogurt is mostly known as plain yogurt. Plain yogurt is responsible for approximately 5% of total refrigerated yogurt sales for the United States market and is available at all fat levels. Set yogurt has a different manufacturing style than stirred style yogurt. The way it is fermented and processed is the part that makes the difference. Set yogurt fermentation style is called by cup incubation or sometimes by vat incubation. Plain style yogurts are manufactured with less added ingredients when compared to stirred type yogurts. Therefore, plain yogurts provide better option for consumers who seek a more natural yogurt flavor. Another type of set style yogurt is known as fruit on the bottom (FOB). In this yogurt, the fruit is placed at the bottom of the cup and the remaining space is filled with yogurt mix, which is incubated, and then cooled when the desired pH is reached (Singh et al., 1997).

The majority of the yogurt market is dominated by stirred yogurts also known as Swiss-style. Stirred yogurts have a stable growth curve in sales. The reasons for the domination of stirred yogurts are: First, the use of stabilization bases which enabled a smoother body with desirable less gel-like texture. Second, the culture diversity used in the manufacturing. Incorporation of different strains of yogurt cultures made an important contribution to yogurt flavor by producing more appealing milder taste that is preferred by consumers. In addition, cultures have also helped to overcome the post-acidification flavor problem. Combination of new strains made it possible to stop fermentation around pH of 4.5 by inhibiting their own activity (Lourens-Hattingh and Viljoen, 2001).

Stirred yogurts have different processing conditions than set yogurts. In stirred yogurt manufacturing, the gel is broke down after fermentation to produce a more homogenous product. However, this step is essential for final yogurt products texture since there will be another gel formation following the cooling. For this reason, stirred yogurts are always fortified with a stabilizer addition to help the gel formation (Tamime and Robinson, 1999).

Consumer demands due to increased awareness about cardiovascular health problems related to fat have made the yogurt industry switch production from full-fat to low-fat and non-fat (Isanga and Zhang, 2009). Different fat levels in yogurt are achieved by standardizing cream and skim milk to the desired fat levels. Following standardization, additional dry dairy ingredients, with or without stabilizer base, and sugar, are blended together in a mix tank by an agitation system to ensure that mix is blended homogenously (Chandan, 1997).

In addition to flavor, texture is another important attribute related to the quality of stirred yogurt (Sodini et al., 2002). Milk composition, starter culture selection, dry ingredient content, processing conditions like heating, homogenization, incubation temperature, and cooling are the major attributes that can affect the texture of finished yogurt product (Beal et al., 1999). Excess use of ingredients, high incubation temperatures, unbalanced ratio of whey protein to casein, different strains of starter cultures and their excess usage will result in textural defects such as gel-like, graininess, roughness, etc. (Lucey, 2004; Lucey and Singh, 1998; Kucukcetin et al., 2009)

Tamime and Robinson (1999) reported that yogurt texture is improved when total solids in the formulation are between 12% to 20% levels. However, the authors indicated that the most efficient total solids levels were between 14% to 16%.

Homogenization is an essential step for yogurt mixes in mixing the stabilizers and other ingredients. The homogenization step enhances the stability, consistency and body of the yogurt mix. After homogenization, the formation of small fat globules (< 2µm) result in a reduced tendency for cream layer formation, thus increased viscosity. This effect is more pronounced with high fat contents (Walstra and Jennes, 1984).

Yogurt mix with different fat levels are usually homogenized using high pressure values of 10–17 MPa (1500–2500 psi) first stage and 3.4 MPa (500 psi) second stage at 55–71°C (Soukoulis et al., 2007).

During homogenization, casein and some whey protein absorb at the fat globule interface, which effectively increases the number of structure-building components (Walstra, 1998). Homogenization pressures higher than 2000 psi result in increased firmness and viscosity of final yogurt product. This is due to an increase in surface area as a result of formation of a larger number of smaller fat particles. Homogenization also helps to reduce whey separation and increases the whiteness of the product (Tamime and Robinson, 1999).

Yogurt mixes were batch pasteurized at 63°C for 30 min, however, nowadays most yogurt mixes are high-temperature short-time (HTST) pasteurized at 85–98°C for period of times between 20 s and 7 min (Chandan et al., 2006). However, severe heat treatment can affect milk properties and the resulting yogurt such as most of the milk enzymes and microorganisms will be destroyed.

Pasteurization temperatures higher than $70^{o}C$ will denaturate the whey protein in yogurt milk. Whey protein denaturation and its interaction with casein during acidification will result in: viscosity increase and firmer gel formation, decreased fermentation time, oxygen levels reduction which will promote the growth of starter culture sensitive to oxygen, inhibitors destruction and stimulatory components production, which can increase the speed of the fermentation (Tamime and Robinson, 1999).

The incubation temperature for yogurt is usually around 40–45°C and the process typically takes between 3 and 6 hours (Walstra and Jennes, 1984). Acidification during fermentation impacts some properties of casein, which affects their gelation properties during the formation of cultured products. Activity of LAB will result with increased buffering around of pH 5 because of buffering caused by lactic acid (pKa 3.95) (Singh et al., 1997).

Buffering aids to slow down the pH decline help to some extent but as the starter cultures are mostly in their growth phase, the pH will continue decreasing until most bacteria start to become inhibited due to effects of the low pH (Beal et al., 1999).

Acid production by LAB results in several changes in the physicochemical properties of casein micelles (Lucey and Singh, 1998). Surface charge decreases in milk which comes from the originally negative charged values when isoelectric point for casein is reached to pH 4.6. Thus aggregation of casein molecules takes place through hydrophobic and positive/negative electrostatic interactions. Static attraction remains from macro peptide hairs. When the pH of isoelectric point is reached, the aggregation takes place (Chandan et al., 2006).

Heat treatment at 78°C for at least 15 min results in sufficient whey protein denaturation which will have a major affect on the gelation properties (Lucey and Singh, 1997). The main whey protein β-lactoglobulin (b-lg) in heated milk has a high isoelectric pH of 5.3 hence coagulation and gelation is shifted to higher pH values. The denatured whey proteins resulting from milk heat treatment become mostly associated with casein micelles during heat treatment or they do so during the acidification process. Denatured whey proteins in heated milk are insoluble at pH 4.6 and are precipitated along with caseins (Larson and Rolleri, 1955). High heat treatment of milk leads to firmer and more viscous gels.

When the milk environment gets more acidic and the pH is decreased to 4.6 at or above 20°C, casein becomes insoluble and precipitates out. Whey proteins remain in the solution. As the pH arrives at 4.6, calcium phosphate is cleaved from the micelle. In the mean time, casein is reached its isoelectric point and there is no charge to keep micelle suspended by repelling forces. Therefore this aggregation results with dense coagulum. This reaction gives yogurt its unique coagulated texture (Fox, 2001).

Cooling is an important step to prevent post-acidification defect which caused by starter culture activity. If the cooling rate is slow than final yogurt products pH will keep decreasing. More acid production will make yogurt product undesirable if $pH < 4.2$ is achieved. Stirred type yogurts are cooled in the jacketed vat or by tubular cooler with gentle agitation. Set type yogurts are cooled by transferring the container to a cold store. Blast chilling is also commonly used to cool both type of yogurts (Chandan et al., 2006).

Reformation of bonds is an important step for stirred yogurt's re-gelling properties (Zoon, 2003). Fruit preparations are added when product is cooled and filtered to breaking lumps and finally filled in to cups.

The temperature is decreased to around 5°C and yogurts stored after to slow down physical, chemical and microbiological degradation. It is also common at some countries to do the filling at refrigeration temperatures. Extra care should be taken at pumping and filling process since they affect the final texture of yogurt product (Tamime and Robinson, 1999).

Many methods are being used to assess textural properties of yogurt products as hardness / firmness (penetration), viscosity (resistance to flow), texture profile analysis and various sensory methods (Lucey and Singh, 2003). Textural properties have direct effect on consumer acceptability for sensory evaluations since it affects the attributes like mouth-feel and creaminess (Muir and Hunter, 1992).

The main processing parameters affecting the texture of cultured products include: addition of dry protein powder materials and level being used; type of stabilizer is used at what quantities; homogenization settings with fat levels; pasteurization temperatures and holding time of milk; starter culture strain selection; proper incubation temperatures for selected starter cultures; fermentation ending pH; how fast cooling will be handled; post manufacture conditions as transportation or keeping cold chain (Lucey and Singh, 1997; Walstra, 1998; Tamime and Robinson, 1999).

Increased amounts of casein particles will result with enhancement in the texture and viscosity in many cultured products. Unhomogenized milk fat globules (native) do not react with the matrix of casein, however, when homogenized fat globules interact with protein membrane which will result as pseudocasein particles. In addition, homogenization will affect the textural properties like firmness and viscosity at increased fat levels (Chandan, 1997).

Following homogenization, yogurt mixes are exposed to heat treatment. Maximum temperature achieved and holding times depend on desired final yogurt products textural properties. During heating, whey proteins are denatured after 70^{o}C and they associate with the casein micelles. Degree of denaturation depends on available whey protein content with temperature holding times. An excessive amount for whey protein in formulation will result with grainy texture depending on the degree of denaturation (Mangino, 1984).

Pasteurized yogurt mixes are cooled to room temperatures immediately if inoculation will take place otherwise, they are cooled to refrigeration temperatures and placed in coolers for overnight storage. Since most current starter cultures are thermophilic, incubation temperatures lower than 40^{o}C will result with longer gelation times. The common inoculation temperature recommended by suppliers is around 42^{o}C which will decrease the fermentation time while maintaining desired textural properties (Lucey, 2002a). In addition, lower incubation temperatures will result with slow aggregation and gelation processes for proteins which will have tendency to softer gel production for final yogurt product. Increase in gel strength is more pronounced for yogurt products between pH of 5.1 and 4.6 since whey proteins and casein starts to precipitate (Fox, 2001). On the other hand, pH values lower than 4.0 will result with decreased gel strength due to excessive charge repulsion (Parry, 1974).

Yogurt has good properties to be a functional product. Fortification with nutrients like calcium, proteins, vitamins, and prebiotics with addition of probiotic cultures enhances its healthy benefits. Moreover, emerging approaches also focus on processing conditions to maximize the effects of added ingredients. For this purpose, denaturation of proteins has been a major interest point to enhance the textural properties of yogurt product while decreasing the need for other ingredients (Tamime and Robinson, 2007).

2.2. Nonfat Dry Milk

Extensive research has been conducted to overcome the textural defects of yogurt such as expulsion of serum also known as syneresis and variations in viscosity, which are counted as the two primary defects of the finished product. These defects also contribute to overall likeness of yogurt (Keogh and Kennedy, 1998).

Commercial yogurts include milk proteins and stabilizers in their formulations to minimize texture defects. Non-fat dry milk is the most common milk protein source used for the formulation of yogurt mixes (Puvanenthiran, 2002). Whey protein concentrate is another source of milk protein and is a very good alternative to replace NFDM (de Wit, 1998).

Milk powder is widely used in the dairy industry to fortify yogurt mixes to increase viscosity and get better texture in the finished product (Mistry and Hassan, 1992). Increased awareness of personal health care and artery blocking effects of fats made NFDM first choice for dairy applications when compared to whole milk powder which has a fat content between 26% – 42% (Tamime and Robinson, 1999).

The addition of solids non-fat is recommended to make yogurt with good textural properties. The increase of these solids is achieved by adding NFDM. The rate of solid non-fat addition can be as little as 1% and as high as 6% but the recommended level is between 3-4%. Textural defects like weak body, graininess and powdery taste can be observed when non-fat solids are used out of these percent levels (Tamime and Robinson, 1999). Commercial yogurt formulations contain around 2% NFDM with additions of stabilizers to minimize textural defects (Soukoulis et al., 2007) .

Casein and whey are the most important milk proteins and they are present at 80% and 20%, respectively. Casein has unique characteristic structure, charge, biological, physical and nutritional properties. The interaction between calcium phosphate and various caseins is responsible for formation of large colloidal complex particles named casein micelles. The light scattering effect of colloidal micelles is the reason of the whitish color of milk (Guggisberg et al., 2006).

Casein micelles are stable under most heating and homogenization conditions, however, the ε-amino group of lysine in caseins interacts with the aldehyde group of lactose at increased temperature, causing the formation of brown pigments which is called Maillard reaction. (Chandan, 2006).

2.3. Whey Protein Concentrate

Whey is the soluble fragment of milk, which separates from the curds during cheese manufacturing. Whey is an opaque liquid with a greenish-yellow color (Magenis, 2006). Cheese whey also named as sweet or acid whey depends on the pH and it is production technique. Sweet whey is produced at a pH of 5.6 and with rennet coagulation while acid whey is manufactured at a pH of not higher than 5.1 and with acid coagulation (Tunick, 2006). Whey consists of lactose, calcium, proteins, and soluble vitamins therefore considered as a good source for nutrients (González-Martínez et al., 2002).

Whey proteins are well known for their high nutritional value and versatile functional properties in food products. Estimates of the worldwide production of whey indicate that about 700,000 ton of true whey proteins are available as valuable food ingredients (Tunick, 2006).

The worldwide production of liquid whey is estimated at 118 million tonnes, a quantity that is equivalent to about 7 million tones of whey solids. Compared with 4.3 million tones of whey solid, about 62% of the world wide whey production appears to be gainfully utilized. By taking the (true) whey protein content of this surplus whey solids at 10%, it appears that about 270,000 tonnes (38%) of the 700,000 tonnes of true whey proteins are still available as valuable food ingredients (Tamime and Robinson, 2007).

Nutritional and functional characteristics of whey proteins are related to the structure and biological functions of these proteins. Interest in the nutritional efficacy of whey proteins used in infant formula, in dietetic and health foods has grown in recent decades (de Wit, 1998).

Whey proteins are composed of four main proteins; β-lactoglobulin (b-Lg), α-lactalbumin (a-La), blood serum albumin and immunoglobulins. In contrast to the caseins, the whey proteins possess high levels of secondary, tertiary and quaternary structures (Fox, 2001). Additionally, when compared to caseins, whey proteins have comparatively better ordered globular structure, which includes disulfide linkages. As a result, unlike caseins, whey proteins have better hydrophilic properties and they are not susceptible to precipitation under acidic situations (Chandan, 2006).

Different from caseins, whey proteins need adequate heat treatment to be able to assist in strengthening the gel network as called denaturation (Guggisberg et al., 2007). The functional properties of the whey proteins become more apparent after heating the milk. At temperatures above 80°C, they are denatured and react/bind with k-casein to form a more stable micelle. Denaturation takes place between a-La and b-Lg at the rate of 70% when yogurt milk is heated to 95^{o}C for 22 seconds (Tamime and Robinson, 1999). The effect of heat on the proteins, according to Parry (1974), is a two-stage process: first, the structure is altered causing denaturation, and second, aggregation takes place followed by coagulation, depending on the level and duration of heating; b-Lg undergoes such a process when the —SH groups are reactivated as a result of heating (Walstra and Jenness, 1984).

Casein and whey proteins of milk products complement each other in the quantitative distribution of amino acids. Casein, which is used as a reference protein, is given an arbitrary protein efficiency ratio (PER) value of 2.5. Whey proteins have a PER value of 3.2. Whey proteins have a protein digestibility corrected amino acid score (PDCAAS) of 1.14. The reported score for casein is 1.0, which is the maximum value allowed by the USDA for reporting purposes. The PDCAAS is the USDA's officially approved method of scoring protein quality (de Wit, 1998).

Biological value (BV) is another measure of protein quality. BV measures the amount of protein that is retained from the absorbed protein for maintenance and growth. The value is calculated by measuring the fraction of the nitrogen in the diet that remains after the nitrogen losses in the waste products have been subtracted. Whey proteins have a biological value of 100, which is higher than the value for casein, soy protein, beef, or wheat gluten (Chandan, 1997).

2.4. Fat in Yogurt

Milk fat, though quite bland in taste, imparts richness and smoothness to fat-containing dairy products. Fat content of milk varies from 3.4% to 5.1%, depending on the breed of the cow (Aziznia et al., 2008). Most of the milk used for yogurt production typically contains an average of 3.5-3.6% fat. Variability of milk fat also depends upon the individuality of animal, stage of lactation, feed, environmental factors, and stage of milking (Keogh, 1998).

The milk fat of cows consists chiefly of triglycerides of fatty acids, which make up 95-96% of milk fat. The remaining milk fat is composed of diglycerides, monoglycerides, free fatty acids, phospholipids, and cholesterol (Chandan, 2006).

The functional properties of milk fat are attributed to its fatty acid makeup. More than 400 distinct fatty acids have been detected in milk. Typical milk fat consists of 62% saturated, 29% monounsaturated, and 4% polyunsaturated fatty acids (Tamime and Robinson, 1999).

Milk fat functions as a concentrated source of energy as well as a source of fat-soluble vitamins. A, D, E, K and essential fatty acids, linoleic acid, and arachidonic acid (Parry, 1974).

Milk fat occurs in milk as an emulsion of fat particles suspended in aqueous phase. The spherical particles are called fat globules. The average size of fat globules in raw cow milk varies from 3.4 to 4.5 um, depending on the breed of the cow. Jersey milk fat globules tend to have larger diameters than Holstein milk fat globules (Sandoval-

Castilla, 2004). Fat globules are lighter (less dense) than the surrounding water phase and rise to the surface when milk is left undisturbed.

The use of a cream separator in dairy plants permits fractionation of whole milk into skim/low-fat milk and cream. Processed milk products, namely homogenized milk, ultra-high temperature (UHT) milk, ice cream, yogurt, light cream, half and half, evaporated milk, and condensed milk, which have undergone homogenization have diameters of their globules of the order of 0.3-0.7 um (Sandoval-Castilla et al., 2004). The fat globules of unhomogenized products like whipping cream show an average diameter of 4.0 um. Skim milk has smaller fat globules left over as a result of separator action and their diameter is around 1.3um. Cream layer is observed in products with relatively large fat globules, while the homogenized dairy products show virtually no cream layer during the shelf life of such products (Chandan, 2006).

During yogurt making, the mix is homogenized and the fat becomes coated with casein, which causes the homogenized and size-reduced fat globules to behave as very large casein micelle-coated spheres. Thus, there is an increase in the consistency, and a decrease in syneresis (Keogh and Kennedy, 1998).

Low-fat and fat-free yogurts have gained popularity because of increasing demands of consumers who seek healthy options across product categories. However, fat solids reduction in yogurt has been associated with poor texture, where commonly the fat removed is substituted by skim milk powder, sodium caseinate, or whey protein concentrates. The amounts required of these ingredients to achieve the total solids content similar to that of the full-fat yogurt can lead to a powdery taste, excessive acid development from lactose fermentation, excessive firmness, higher whey expulsion, and grainy texture (Tamime and Robinson, 2007). Therefore, production of low-fat and non fat yogurt demands careful control of texture and flavor attributes (Haque and Ji, 2003; Isleten and Karagul-Yuceer, 2006).

One of the most important steps in production of low-fat and fat-free yogurts are to increase the total solids content to prevent specific textural defects such as poor gel firmness and surface whey separation (Lucey, 2002). It is common to use NFDM to fortify yogurt milk, but other dried dairy ingredients such as calcium caseinate, sodium caseinate, whey protein concentrate, and other milk protein-based ingredients have gained acceptance as a viable way to increase total solids in fat-free or low-fat yogurts (Tamime and Robinson, 1999).

2.5. Sweetener

Sweetener is a common name used for agents which have sweet taste. They can consist of mono, di and polysaccharides. Sucrose is used for the yogurt applications, which is a disaccharide derived from glucose and fructose (Chandan, 2006). Sucrose is a white, odorless, crystalline compound with a pleasing sweet taste. In addition, sucrose has a strong water binding capabilities (Tamime and Robinson, 1999). These sensory and textural properties make sucrose suitable for yogurt applications. However, the addition of sucrose should not exceed 15%, otherwise it will change the osmophilic balance and will suppress the growth of starter cultures (Chandan, 2006).

Common amount of sucrose used in yogurt mixes usually ranges between 2% and 7%. It should be noted that sucrose is not the sole compound which affects the sweetness of the final yogurt product. Lactose, which comes from milk and other dry dairy powders and flavoring agents also, can increase the overall sweetness of the product.

Mistry (1992) explained that addition of sucrose enhanced the yogurt's body and texture by binding available free water in yogurt milk. Moreover, it increases the overall sweetness of product and makes it more appealing to consumers. However, excess usage levels of sucrose more than 10% can cause a starter culture inhibition by changing the osmophilic balance of the environment (Beal, 1999).

2.6. Stabilizer

Stabilizers are usually added to food products to improve texture and reduce cost. Starches and hydrocolloids are the most known examples for food applications. They can be used alone or in combination with each other.

For the yogurt applications, it is common to mix starches with gelatin or pectin. Stabilizers perform two simple functions in yogurt, which are binding of water and increase the viscosity (Tamime and Robinson, 1999). In addition, they aid in texture by producing smoothness in body and imparting gel structure. Stabilizers are usually bland in flavor and should be effective at low pH values.

Starch can be derived from many sources as potato, corn, wheat and tapioca (Schmidt et al., 2000). Corn starch is the most used stabilizer since it offers low price, good water holding capacity and texture in the finished product. However, corn starch alone is not well suitable for yogurt applications. The reason is that yogurt is affected when it goes through high processing temperatures and high shearing conditions during stirred type yogurt making. To prevent possible deformations starches are modified by cross-linking process. Cross-linking strengthens the hydrogen bonds in starch with chemical bonds thus acts as a bridge between molecules (Wurzburg, 1987).

Gelatin is another stabilizer used in yogurt formulations. Gelatin is isolated by irreversible hydrolysis of collagen and ossein from cattle, pigs and selective fish. Levels of 0.3 to 0.5% are suitable for yogurt applications because at these levels, the finished product has a smooth and shiny appearance with good gel properties (Tamime and Robinson, 1999). Gelatin forms clear thermo-reversible gels with a melting point close to human body temperature (Tribby, 2009). However, at temperatures below 10^{o}C, the yogurt can form a pudding like consistency (Chandan, 2006).

Previous studies (Lucey, 2004; Soukolis, 2007; Smithers, 2008) explained that the need for stabilizer was decreased when whey proteins are used with NFDM due to textural enhancing properties of whey protein powders. On the other hand, Tamime

(1999) reported that it was essential to use stabilizers in stirred yogurt applications due to breaking down the gel structure after fermentation.

2.7. Yogurt Flavor

Yogurt is a nourishing and flavorful food compound. Refreshing flavor is an important sensory attribute for the consumer acceptance and it requires good combination of acidity and flavors without any after taste (Karagul-Yuceer et al., 1999). Since yogurt is a fermented milk product, its taste might present some variations based on the starter culture's activity. Thus, using flavor compounds will be helpful to maintain a stable end product.

For good flavor purposes, fruit flavorings are widely used in stirred type yogurts. Vanilla, strawberry, mixed berry, blueberry, peach, raspberry, strawberry/banana, cherry, lemon and key lime are the top ten flavors used for yogurt applications, respectively. The top five flavors of this category constitute around 80% of all flavored yogurt sales (Tribby, 2009).

With the addition of fruit and flavors, yogurt products gained more acceptances from consumers who do not like the sour plain taste. Common approach for fruit preparation is mixing fruit chunks with syrup which consist of sugar, water, thickeners and flavor compounds (Nongonierma et al., 2006).

Strawberry is the most used and preferred fruit for the consumer liking in yogurt applications. Strawberry has a pleasant taste with good flavor masking properties (Tamime and Robinson, 1999). However, flavor compounds also can interact with food components and can influence textural and sensory properties of the final product.

Nongonierma et al. (2006) reported that lipids tend to act as solvents hence reducing the volatile content of flavoring compounds. Undenaturated b-lg, which is the major whey protein compound also can decrease the volatility of flavoring compounds.

Chapter 3: Materials and Methods

Preliminary Work:

Preliminary study was conducted to determine the feasibility of using whey protein products in three different types of yogurts. The yogurt categories were high protein, high resistance, and low cost yogurt. Different whey protein powders were used to formulate fortified yogurt products with acceptable texture and flavor. The high protein yogurt was developed by increasing the protein content as much as possible with WPC and WPI. High resistance yogurt formulation products were developed to provide very good resistance to transportation conditions and prevent syneresis. Low cost yogurt formulas included the lowest cost ingredients but also were expected to maintain acceptable texture and flavor properties.

Bench top formulations were conducted to establish the pilot plant trials for selected formulations. After numerous laboratory trials, working formulations were selected for each type of yogurt. High protein yogurts were formulated with only WPC80. This was the major whey ingredient since it contains high amount of protein. High resistance yogurts were produced with a combination of WPC80 and WPC34. This combination was useful because WPC80 proteins denature during the heat treatment process and provide good texture properties. WPC34 offer similar results but they are less expensive and reduce the cost of the formulation. Low cost yogurt was produced with sweet whey, WPC34, and WPC80 at decreasing usage levels, respectively. Sweet whey and WPC34 were used as major ingredients since they provide a significant cost savings when compared to other protein powders. WPC80 was used mainly as stabilizer to maintain the texture of yogurt.

These results showed that it was possible to incorporate various whey protein products at different levels in yogurt formulation while maintaining acceptable texture and flavor in the finished products. Therefore, these data served as the basis for the current study, which focused on replacing the non-fat dry milk with whey protein products at three different fat levels.

3.1. Formulation

Control non-fat, low-fat and full fat products were produced with similar ingredient formulations used in commercial products. Whey protein products were developed by replacing NFDM completely in control products with whey protein concentrate. Control and whey protein product formulations and ingredients are presented in Table 3.1. Both control and whey protein products were formulated at the same total solids content for comparison purposes.

Table 3.1: Formulations for the developed control and whey protein products.

Ingredients / Products	Control Non fat	Control Low fat	Control Full fat	Whey Non fat	Whey Low fat	Whey Full fat
Cream	-	4.8	15	-	4.3	14.5
Skim milk	79.25	74.45	64.25	79.25	74.95	64.75
WPC80	-	-	-	2	2	2
NFDM	2	2	2	-	-	-
Sugar	3	3	3	3	3	3
Stabilizer	0.75	0.75	0.75	0.75	0.75	0.75
Flavor (strawberry)	15	15	15	15	15	15
Totals (%)	100	100	100	100	100	100

The ingredients for yogurt formulation were the following: whey protein concentrate (WPC80, Agrimark, Lawrence, MA), yogurt stabilizer (Crest 41-1444, Crest Foods Inc., Ashton, IL), direct-vat-set yogurt culture (*S. thermophilus, L. bulgaricus, L. acidophilus*, and *Bifidobacterium spp.*) (Yo-Mix 205, Danisco, Madison, WI), strawberry base (FRD-12-25794, Fruitcrown Products Co., Farmingdale, NY), non-fat dry milk solids (NFDM), and sucrose.

3.2. Yogurt Processing and Pilot Plant scale-up.

Three batches of yogurt were made for each formulation. All batches were processed in the same day and left in the cooler ($4 \pm 2^{o}C$) overnight. Next day all batches were fermented and filled in to cups. This procedure was repeated three times.

Raw milk (4% milkfat), obtained from a dairy farm (The Ohio State University, Columbus, OH) was used for yogurt processing. Milk was kept at $4 \pm 2^{o}C$ at all times. Milk was separated into skim milk (<0.5% milkfat) and cream (20% milkfat) using an Alfa-Laval 29AI separator (Stockholm, Sweden). Yogurt mix was standardized at 0%, 1% and 3.25% milkfat using the ingredients listed in Tables 1-3. Each yogurt mix was subsequently homogenized at 2300 psi using a Lab 100 M-G homogenizer (Lubeck-Schlutut, Germany), and then pasteurized at $92^{o}C$ for 30 sec in an AVP Junior HTST system (Tonawanda, NY). Pasteurized yogurt mix was cooled to ($25 \pm 1^{o}C$) and kept at refrigeration conditions ($4 \pm 1^{o}C$), overnight. The next morning, yogurt mix was placed into 19lbs fermentation baths and warmed up to $42 \pm 1^{o}C$. Each yogurt batch was inoculated following the manufacturer's (Danisco Co. Inc., Madison, WI) recommended inoculation rate (0.02% w/w). Yo-Mix 205 starter culture frozen pellets were poured directly into the pasteurized mix. The mixture was agitated for 10 minutes to distribute the culture evenly. Temperature was maintained at $42 \pm 1^{o}C$ during the fermentation process. The fermentation was stopped when the pH of the mixture reached 4.6 in about 4 to 4.5 hours. Strawberry base flavor (Fruitcrown, Farmingdale, NY) was added to the final yogurt at a rate of 15% (w/w). The finished product was placed into 8 oz sanitized (200 ppm sodium hypochloride) plastic containers, labeled and stored at $4 \pm 1^{o}C$.

3.3. Chemical, Textural and Sensory Analyzes

Chemical Composition Analyses: Yogurt samples were analyzed for moisture content using a CEM Lab Wave 9000 moisture/solids analyzer (Matthews, NC). Fat content was determined by the Babcock method (AOAC method number 989.04). Protein content of yogurt samples was measured using a micro Kjeldahl total nitrogen (TN) analyzer (Horwitz, 2002; AOAC method number 991.20;33.2.11). All analyses were conducted in triplicate.

pH: pH was measured using a pH meter WTW-pH 330 (Weilheim, Germany) with a glass electrode standardized at 25°C over the range 7.0 to 4.0.

Hardness: An Instron 5542 series single column testing system (Norwood, MA) was used to measure yogurt hardness. Samples were removed from refrigeration (4 ± 1^{o}C) just before analysis. Gels were penetrated using a 35 mm diameter flat probe at a crosshead speed of 0.83 mm/s. Hardness (N) was defined as the maximum mean force necessary to penetrate up to 50% compression of the gel's anvil height. Anvil height of yogurt samples was set at (6.0 cm), and anvil diameter was set at (6.8 cm).

Water holding capacity: A sample of about 20g of yogurt (Y) was centrifuged for 10 min at 4000 rpm at 4^{o}C. The whey expelled (W) was removed and weighed. The water holding capacity (WHC, g kg-1) was calculated as:

$$WHC = (Y – W) / Y \times 1000$$ (Sodini, 2002).

Brookfield Viscosity: Viscosity was measured using a viscosimeter (Brookfield DV-II+, Middleboro, MA) with a LV spindle number 3 rotated at 1.5 rev min^{-1} for 1 min. Samples are kept in a water bath to maintain room temperature 23^oC .

Sensory Analysis: The sensory panel consisted of 5 members who had previous experience in sensory evaluation of yogurts. Products were presented in 3-digit coded, white plastic isothermal cups stored at 4^oC. The samples were at approximately 10 °C when they were tested. Panelists were provided with mineral water for palate cleansing between samples. The sessions were carried out in a temperature controlled room at 20°C under white lighting in individual booths. Data acquisition was assisted by CompuSense five software (CompuSense Inc, 2010). Both monadic and hedonic scales are being used to rate the flavor and the texture attributes of products. The attributes were evaluated in the following order: visual texture with a spoon, odor, aroma, taste, and texture-in-mouth.

Experimental Design: The experimental design was a randomized complete block, performed in triplicate on separate days (runs), with run as the blocking variable. Variables were the fat content and the day effect for homogenous results. All analyses were performed in triplicate and were analyzed by 1-way and 2-way ANOVA with sample and run as the main effects, using SPSS statistical software (Release 18.0.2). Means comparisons were made when the effect was significant ($P < 0.05$) using Tukey's HSD procedure. All results were reported as the combined means of 3 repeated measures from each of the 3 runs made.

For the one-way anova, the equation below was used:

$$y_{1i\ell} = \mu_1 + \alpha_i + \varepsilon_{ii\ell} \qquad \varepsilon_{ii\ell} \sim N\ (0, \sigma^2)$$

For the two-way anova, the equation below was used:

$$y_{1i\ell} = \mu_1 + \alpha_i + \mu_1\alpha_i + \varepsilon_{ii\ell} \qquad \varepsilon_{ii\ell} \sim N\ (0, \sigma^2)$$

where:

μ = Overall mean,

α_i = Effect of treatments at same fat level,

$\mu_1\alpha_i$ = Effect of treatments at different fat levels,

$\varepsilon_{ii\ell}$ = Experimental error.

Table 3.2. Chemical Analyses of Experimental Design for Control and Whey Protein Yogurts.

Chemical Analyses	NFDM (control)			WPC		
	Non Fat[i]	Low Fat	Full Fat	Non Fat	Low Fat	Full Fat
Fat[1]	-*	1.1000	3.2556	0.1444	1.1000	3.2889
Protein	3.6800	3.5952	3.4555	4.6167	4.5565	4.3973
Total Solids	14.6878	15.5867	17.3689	14.6744	15.5356	17.3989
pH	4.4556	4.4278	4.3878	4.3678	4.3889	4.3678

*indicates that results are the mean of triplicate analyses of three different batches.

Table 3.3. Physical Analyses of Experimental Design for Control and Whey Protein Yogurts.

Physical Analyses	NFDM (control)			WPC		
	Non Fat[i]	Low Fat	Full Fat	Non Fat	Low Fat	Full Fat
Hardness[1]	0.7189*	0.7244	1.4033	1.1078	1.1211	1.4789
Water holding capacity	582.0667	622.2874	725.5492	682.4383	730.3251	768.9269
Viscosity	19355.5556	21340.0000	47722.2222	31847.7778	33011.1111	56977.7778

*indicates that results are the mean of triplicate analyses of three different batches.

Table 3.4. Sensory Evaluation Results of Experimental Desing for Control and Whey Protein Yogurts.

Sensory Tests	NFDM (control)			WPC		
	Non Fat[i]	Low Fat	Full Fat	Non Fat	Low Fat	Full Fat
Appearance[1]	6.6*	7.1	6.2	5.9	4.7	5.0
Texture	6.3	7.0	6.0	6.2	4.3	5.4
Flavor	6.0	6.7	6.6	7.0	6.2	6.0

*indicates that results are the mean of triplicate analyses of three different batches.

Chapter 4: Results and Discussion

4.1. Composition

Total solids include fat, protein, carbohydrate and minerals for yogurt product. Total solids content were similar in yogurts with the same levels of fat but it was significantly different among products with different levels of fat (Fig.4.1).

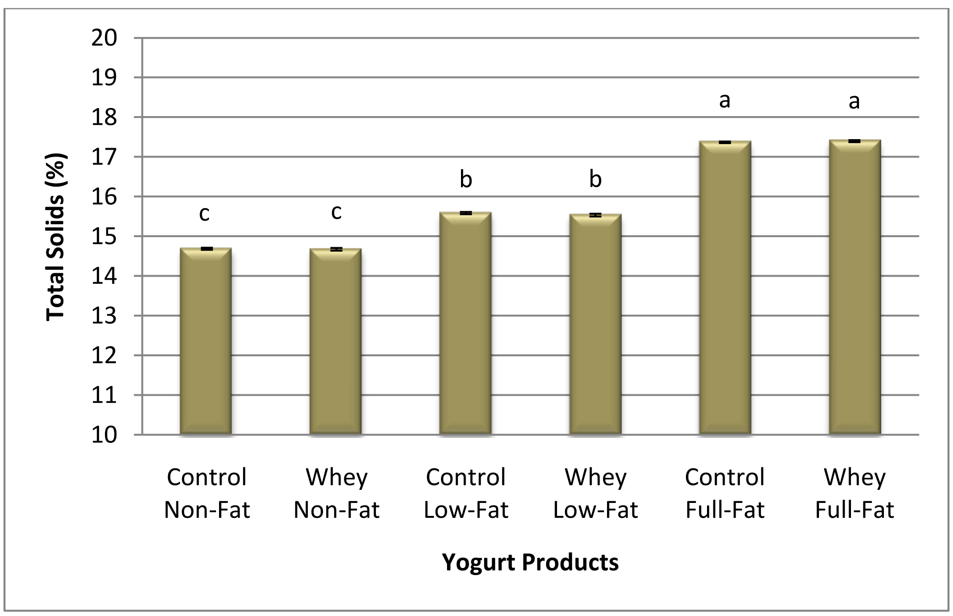


Fig. 4.1. Total Solids Content of Yogurt Products.
[a-c]Different letter among the bars indicates significant difference (P < 0.05) (Tukey's test).
*Results are mean of triplicate analyses of three different batches.

Non-fat control and non-fat whey protein yogurts had the same 14.8% total solids content. Low-fat and full-fat yogurts showed the same levels of total solids content, in both control and whey samples, 15.7% and 17.3%, respectively. The effect of total solids on the properties of yogurt will be discussed at later in the corresponding sections.

The code of federal regulations require that yogurt products should have levels of 0% to 0.5%, 0.5% to 2% and more than 3.25% in order to be named as non-fat, low-fat and full-fat, respectively (USDA, 2009).

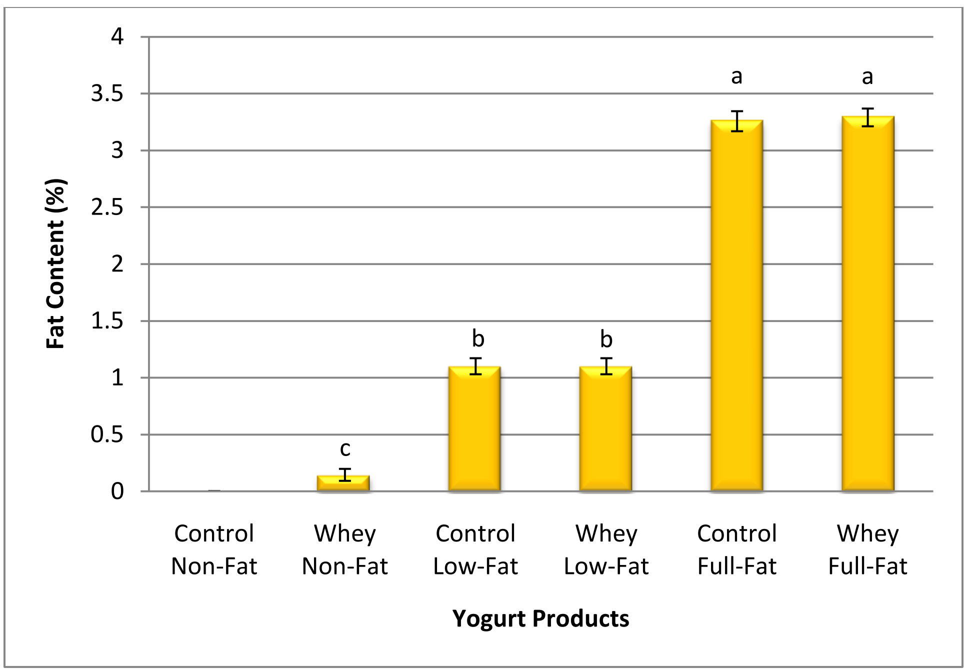


Fig. 4.2. Fat Content of Yogurt Products.
[a-c]Different letter among the bars indicates significant difference ($P < 0.05$) (Tukey's test).
*Results are mean of triplicate analyses of three different batches.

Figure 4.2 shows fat contents of the control and whey protein yogurt products. Low-fat control and whey protein yogurts had around 1.1% fat content while full-fat had 3.3% fat content. There were no significant differences on fat content between control and whey samples of both low-fat and full-fat yogurts. However, whey non-fat yogurt had 0.1% fat content while control non-fat yogurt had no detectable fat content. The reason for this difference is the fat content of the used whey protein concentrate in the formulation. Whey protein concentrates contain around 5% of fat and this level made it detectable in the final product when used at 2%. However, 0.1% difference was not significant to change the fat group of non-fat.

Presence of fat is important on both texture and flavor of yogurt. Non-fat yogurts had lower texture and sensory results than low-fat and full fat yogurts. This might be explained by the absence of fat in yogurt products. Other studies (Sandoval-Castilla et al., 2004; Keogh and Kennedy, 1998) also reported that homogenized fat globules contributed to texture and flavor of yogurt by enhancing its body and imparting richness to the flavor.

In addition to the presence of fat, the interactions of fat globules with protein molecules are important for the textural properties of finished product. Recent studies (Aziznia et al., 2008; Isleten and Karagul-Yuceer, 2006) reported that reformed casein micelles with homogenized fat globules during acidification affected textural and sensory properties of yogurt products. Higher sensory and texture scores of control low-fat yogurt (1.1% fat content) (Fig. 4.9) can be explained with this interaction. Because control low-fat yogurt was fortified with casein and homogenized at 2300 psi, this would result with reformed casein micelles.

Protein is the one of major contributors for the composition of the yogurt products and total solids. Figure 4.3 shows that the protein content was significantly different for all control and whey yogurts.

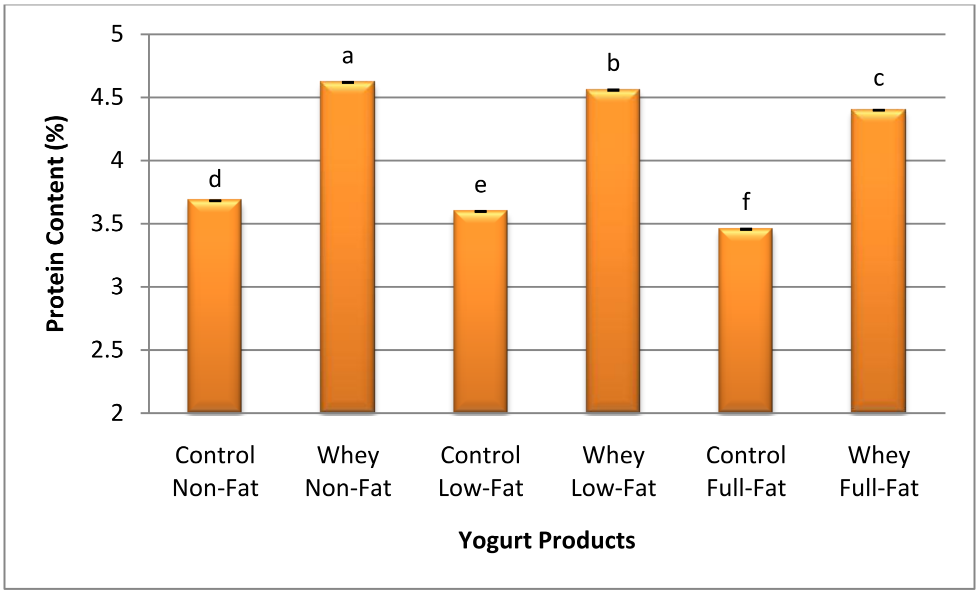


Fig. 4.3. Protein Content of Yogurt Products.
[a-f]Different letter among the bars indicates significant difference ($P < 0.05$) (Tukey's test).
*Results are mean of triplicate analyses of three different batches.

Control non-fat yogurt had 3.68% protein content and whey non-fat yogurt had the highest protein content of 4.61%. This was due to high protein content of WPC (80%). Likewise, the reason for the low protein content for the control yogurts was the protein source, which is NFDM (34%). Similar results were observed for low-fat and full-fat control and whey products. This suggests that high protein concentration in WPC was the factor for whey protein yogurts to have higher protein content than control yogurts.

The presence of protein is essential for good body and texture of yogurt products. The need for proteins is the main reason for the addition of milk solids non-fat to yogurt formulations. Increased levels of protein resulted in higher values in hardness, WHC and viscosity scores for whey protein yogurts than controls. Mistry and Hassan (1992) also reported that increased protein content improved the textural properties of yogurt products. This finding may explain the higher texture results for whey protein yogurts due to the high protein content in the formulation.

Whey low-fat yogurts had 4.55% protein content while control low-fat yogurts had 3.59%. When compared with non-fat yogurts, the difference in protein content was significantly different. The reason for the lower protein content was the increased fat content in the low-fat yogurt products. Another reason for the differences in protein values is that milk cream has lower protein content (2.6%) than skim milk (3.3%).

Protein denaturation is another condition that affects texture, flavor and chemical properties of yogurt products (Parry, 1974; Walstra and Jenness, 1984; de Wit, 1998). Better results of whey protein yogurts texture could have been the result of the denaturation. However, increased protein content with protein denaturation in whey protein yogurts might be the reason of their lower sensory evaluation scores than the controls.

Full-fat whey yogurt had 4.39% protein content while full-fat control yogurt had 3.45%. The variation was significantly different and full-fat yogurts had the lowest protein content when compared to non-fat and low-fat yogurts. It was due to the highest fat content of full-fat yogurts which is around 3.3%.

When compared the pH results between yogurt products with the same fat level, the variations for pH were significantly different (P < 0.05) for all non-fat, low-fat and full-fat control and whey protein yogurts. As shown in Fig 4.4, groups with different fat levels also showed significant differences between each other with the exception of whey low-fat and control full-fat.

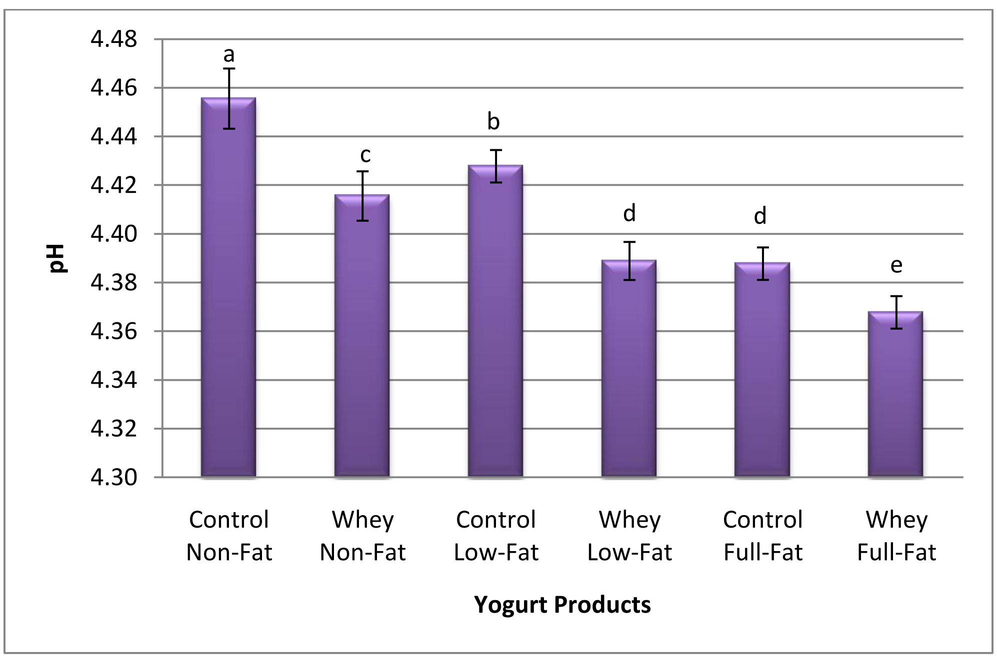


Fig. 4.4. pH of Yogurt Products.
[a-e]Different letter among the bars indicates significant difference (P < 0.05) (Tukey's test).
*Results are mean of triplicate analyses of three different batches.

Control non-fat yogurt had the highest pH of 4.46 while whey non-fat had the pH of 4.42. A study reported that high pH affects the flavor of yogurt products by increasing its acidity (Soukolis et al., 2007). The sensory evaluation results also indicated the effect of pH on flavor since whey full-fat yogurt with the high pH got lower scores for the flavor (Fig. 4.9). The pH differences may be explained by the findings of Modler et al. (1983). They reported that the greater protein content would end up with greater buffering capacity on the yogurt products that would affect the pH. Other effect of high protein content in yogurt mixes was reported by, Amatayakul et al., (2006). The authors stated that the increased nutritional components from the whey proteins might influence the growth of the starter culture. *Lactobacillus Bulgaricus* is a fastidious microorganism which needs available amino acids coming from *Streptococcus Thermophilus* (Ji et al., 2004).

Control low-fat yogurt had higher pH of 4.43 than the pH 4.39 of whey low-fat yogurt. The decreased pH of whey low-fat yogurt may be due to the increased fat content. This pH decrease can be associated with the anaerobic environmental conditions being provided by fat globules for the *Lactobacillus Bulgaricus*, which works better under anaerobic conditions (Chandan, 2006).

Similar results were observed for the full-fat yogurts. Control full-fat yogurt had the pH of 4.39 while whey full-fat yogurt had the pH of 4.37.As in the case of low-fat yogurt results, pH of full fat products decreased as fat content increased. Low pH is reported to cause textural defects in yogurt products since it decreases the gel strength due to excessive charge repulsion (Parry, 1974). The effect of low pH was observed through the sensory evaluation of control and whey full-fat yogurts. They had the lowest sensory texture scores (Fig 4.8.). Effects of pH on texture and flavor will be discussed in detail later in this chapter.

4.2. Texture

Hardness was used to measure the textural properties of yogurt products. Fig.4.5 shows that hardness values of whey protein yogurt samples at non-fat, low-fat and full-fat were significantly different than the controls. Control non-fat and low-fat yogurts had the same lowest hardness value of 0.72 Newton while whey non-fat and whey low-fat yogurts had values of 1.11 Newton and 1.12 Newton, respectively. The increase difference of hardness between control and whey protein yogurts has been associated with the denaturation of whey proteins. Whey proteins form irreversible gels when they are heated above 70^{o}C by reforming into extended three dimensional networks. This newly formed structure can entrap fat and water better than its former shape (Mangino, 1984).

These observations are supported by the result of a recent study reported that yogurt containing whey proteins had firmer texture than casein fortified yogurt (Guggisberg et al., 2007). Similar effect was observed in control full-fat yogurt that had a hardness value of 1.40 Newton. This hardness was lower than 1.47 Newtons for whey full-fat yogurt. Higher fat content also affected hardness. Increased levels of fat content produced greater increase in the hardness value of both control (1.40 N) and whey protein yogurt (1.47 N). Other studies reported similar results and concluded that the increase in yogurt hardness was directly related to fat and total solids content (Puvanenthiran, 2002; Walstra, 1998).

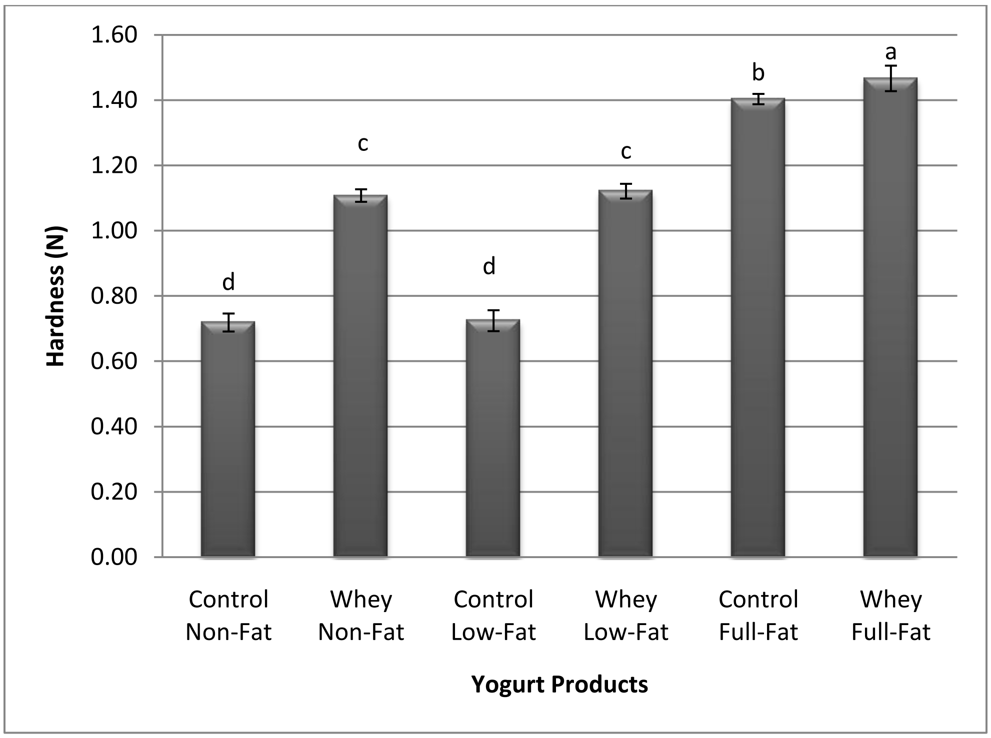


Fig. 4.5. Hardness Values of Yogurt Products.
[a-d]Different letter among the bars indicates significant difference ($P < 0.05$) (Tukey's test).
*Results are mean of triplicate analyses of three different batches.

As indicated earlier, total solids content also influenced the hardness of both control and whey protein yogurts. Non-fat and low-fat yogurts had 14.8% and 15.7% total solids content and hardness values for them were 0.72 Newtons and 1.12 Newtons, respectively. These total solids values are lower than those for full-fat product that had 17.3% total solids and 1.47 Newtons. Tamime and Robinson (1999) reported that the optimum total solids level was in the range of 14 to 16% to produce acceptable products. Later studies reported similar results regarding total solids content and their effects on hardness of yogurt products (Chandan, 2006; Lucey, 2007).

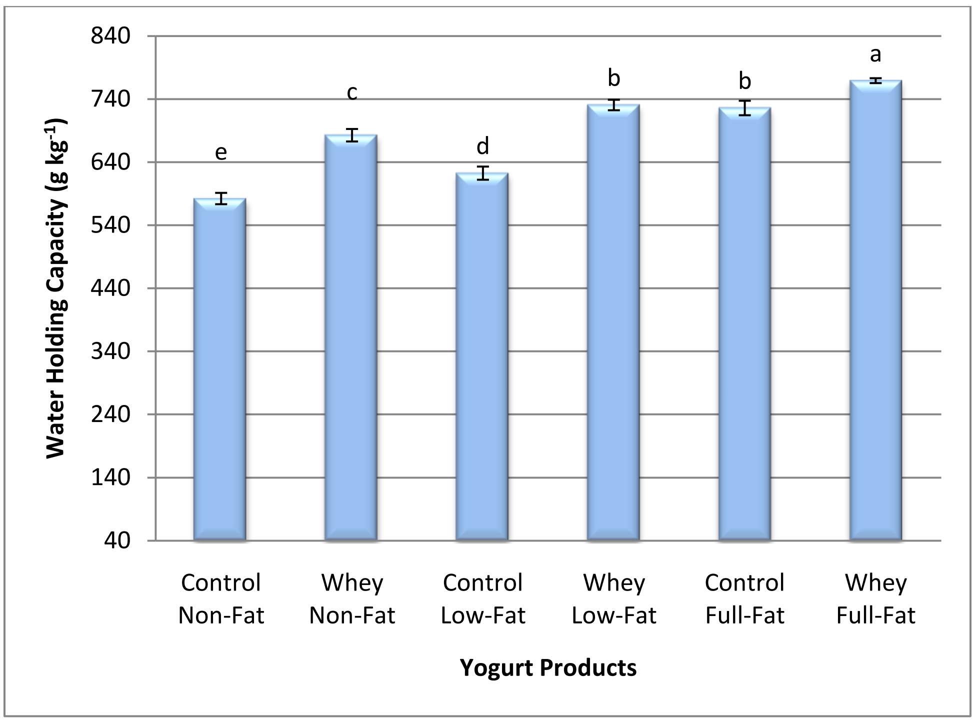


Fig. 4.6. Water Holding Capacatites of Yogurt Products.
[a-e]Different letter among the bars indicates significant difference ($P < 0.05$) (Tukey's test).
*Results are mean of triplicate analyses of three different batches.

Results in figure 4.6 showed that control non-fat yogurt had the lowest water holding capacity of 58.2% while whey full-fat had the highest moisture preservation of 76.8%. Water holding capacity is an indicator of how much water can be kept in a yogurt product. There are many factors associated with yogurt's water holding capacity. Total solids content, protein and fat content, source of protein, selection of starter culture (either ropy or non-ropy) and processing conditions are important factors to decrease or increase the water holding capacity in yogurts (Chandan, 2006).

Whey non-fat yogurt had higher WHC score of 68.2% than control non-fat, which had 58.2%. Since both products have the same fat and total solids content, the higher WHC can be attributed to proteins. Sodini (2006) reported that protein denaturation was responsible for the increase of water holding capacity in yogurt products. Similar studies (Akhtar and Dickinson, 2003; Mangino et al., 1987) also stated that whey protein denaturation enhanced the gelling properties and hence the WHC with adequate heat treatment.

Whey low-fat yogurt had higher water holding capacity of 73.0% than control low-fat, which had 62.2%. Similarly, the water holding capacity score of 76.8% for whey full-fat yogurt was higher than the control full-fat yogurt, which had a WHC score of 72.5%. Increase in total solids and fat content resulted in an increase on water holding capacity for all yogurt products. Previous studies by Parnell-Clunies et al. (1985) and Yazici et al. (1997) also concluded that increasing fat and total solids content increased the water holding capacity of yogurt products.

An additional factor that influences the WHC of yogurt is the homogenization of the mix during yogurt making. Homogenized fat globules interact with protein molecules and entrap free water of homogenized yogurt mix during coagulation (Isanga and Zhang, 2009).

The viscosity values of all yogurts increased with increased fat content as shown on figure 4.7. Control non-fat yogurt had the lowest viscosity score while whey full-fat yogurt had the highest viscosity score. Whey proteins also increased viscosity of yogurts at all fat levels.

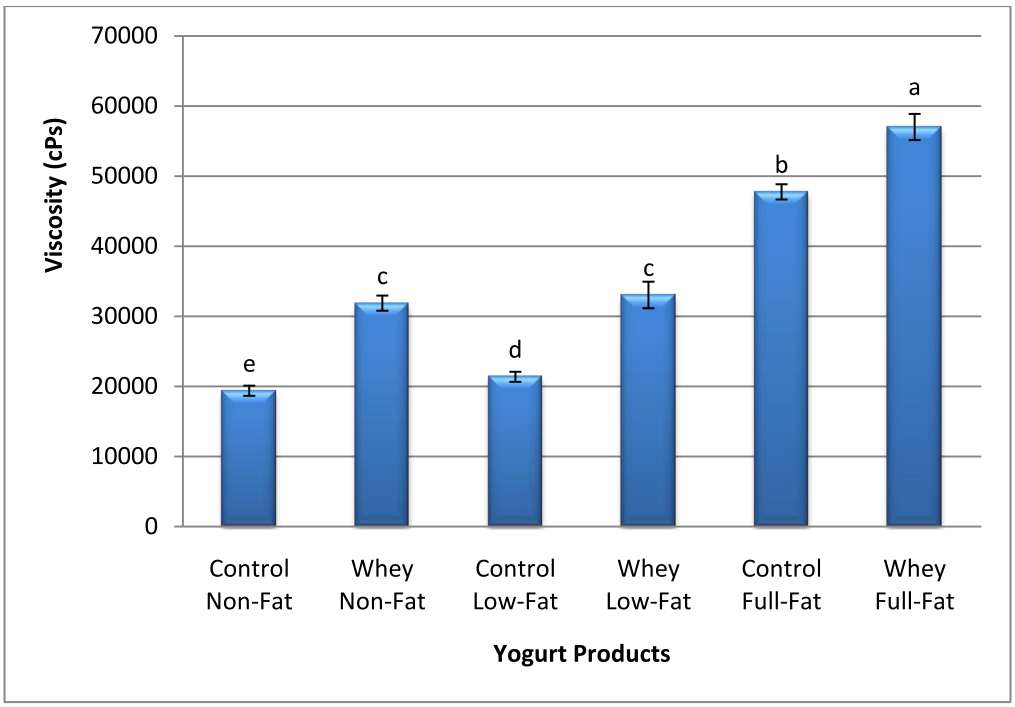


Fig. 4.7. Viscosity Values of Yogurt Products.
[a-e]Different letter among the bars indicates significant difference ($P < 0.05$) (Tukey's test).
*Results are mean of triplicate analyses of three different batches.

Control non-fat yogurt had a score of 19,355 cps that was lower than 31,847 cps value for whey non-fat yogurts. Similar results were observed in low-fat yogurt products.

Control low-fat yogurt had 21,340 cps while whey low-fat yogurt had 33,011 cps. These viscosity results may be due to the high total solids content and whey protein denaturation of the yogurt mix. Mistry and Hassan (1992) reported that increased total solids resulted with the increase in viscosity of yogurts. Other studies reported that whey protein denaturation increased viscosity and thus affected yogurt texture (Mottar and Basier, 1989; Guzman-Gonzalez et al., 1999; Lucey and Singh, 1999).

Whey full-fat yogurt had the highest viscosity score of 56,977 cps when compared with control full-fat score of 47,722 cps. The increase in viscosity was significantly different from low-fat and non-fat yogurts.

The increase of viscosity was due to increase in fat. Similar results were reported by Brauss et al. (1999) and Shaker et al. (2000). The authors found that the increase in fat led to an increase in viscosity. van Vliet and Dentener-Kikkert (1982) had similar findings related to fat and viscosity in yogurt.

4.3. Sensory

Sensory results on a monadic scale (1: less amount to 5: excess amount) showed diversity between products (Fig. 4.8.). Another name is for monadic scale is metric scale and it is used to measure non-comparative purposes. Texture scores had the most variations among others. Control low-fat yogurt had the most appealing texture score of 2.8 compared to other yogurts. Both control and whey protein full-fat yogurts had the lowest score of 2.0 for texture. Previous studies on sensory of yogurt (Muir and Hunter, 1992; Karagul-Yuceer et al., 1999) reported that full-fat yogurts had better texture scores than other fat levels due to their creamy structure. However, they didn't use whey protein in their formulation so the effect of whey protein denaturation was less pronounced.

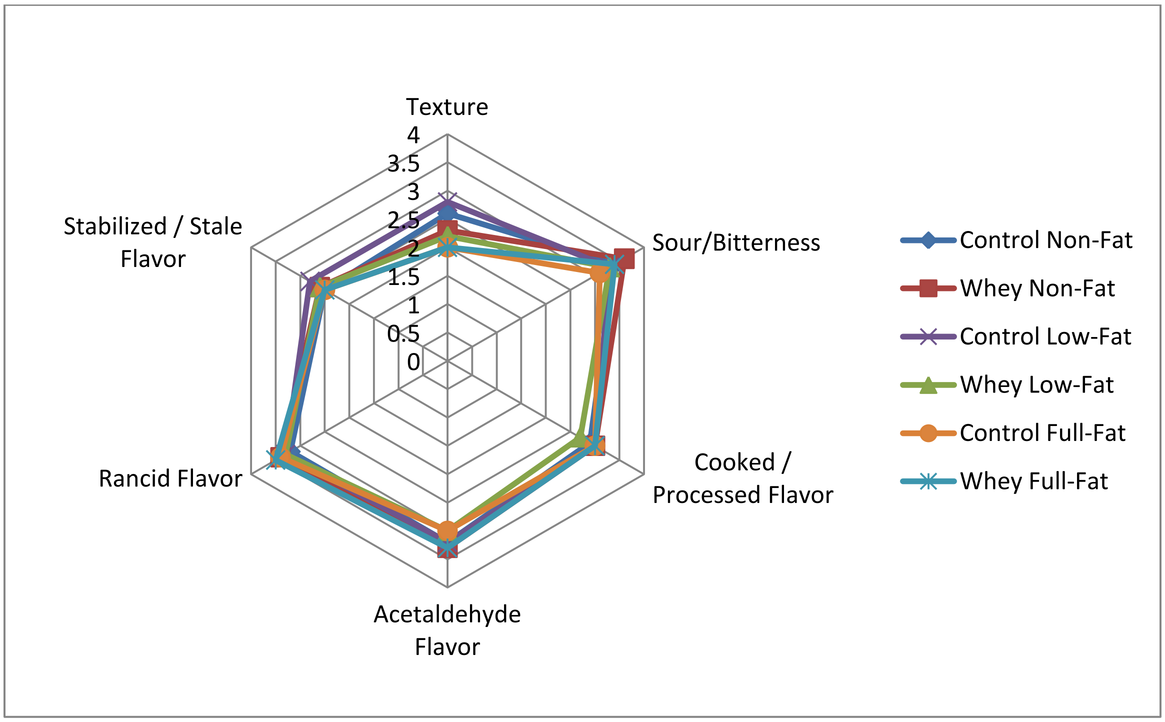


Fig. 4.8. Monadic Scale Sensory Scores of Yogurt Products.
*Results are mean of triplicate analyses of three different batches.

Whey low-fat and control full-fat products had the best scores for the acetaldehyde flavor with the score of 3.0. This might be due to higher pH of these yogurts (Fig. 4.4) among others. Acetaldehyde is the product of fermentation process in yogurt applications and gives yogurt to its famous tarty flavor. Moreover, acetaldehyde is the major flavor compound in yogurt products (Chandan, 2006). Stale, rancid and cooked/processed flavors were not significantly notable in all the yogurts.

For sour/bitterness scores, control and whey protein products scored between 3.1 and 3.6. The results showed that the acidity of yogurts was well developed. The pH of yogurts was also in agreement with the sour/bitterness results since they ranged between pH of 4.36 and 4.46. Yogurt fermentation was suggested to cut at pH 4.6 to avoid post-acidication and the values below that point would result with increased acidity and sourness (Rosell, 1932).

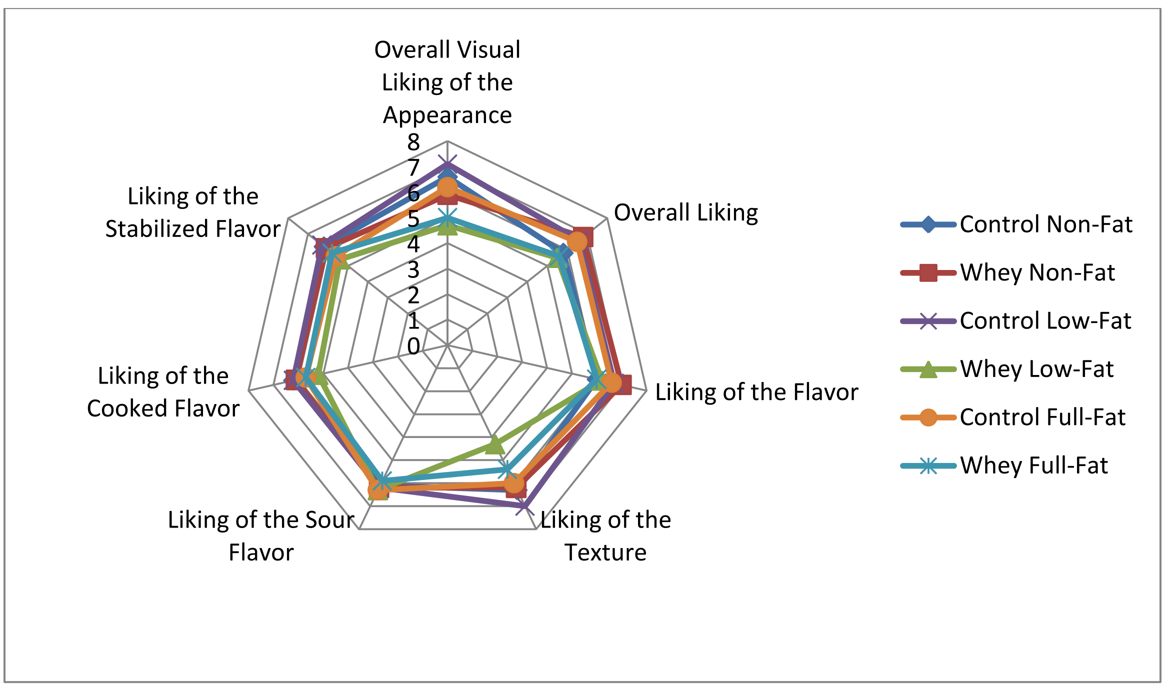


Fig. 4.9. Hedonic Scale Sensory Scores of Yogurt Products.
*Results are mean of triplicate analyses of three different batches.

The most widely used measuring scale for food acceptability is the 9-point hedonic scale. This scale starts with dislike extremely (scale 1) and ends with like extremely (scale 9). Fig. 4.9 showed that whey protein non-fat product had the highest overall liking score of 6.8 compared to other yogurts followed by control low-fat (6.6) and control non-fat yogurt (6.5). This result is a good indicator for the yogurt industry when today's low fat and high protein diets are taken into account.

Whey protein non-fat yogurt can deliver the need for a protein on a healthy product without compromising the overall acceptability. However, for the overall visual liking of the appearance whey protein products had lower scores of 5.2 than control yogurts of 6.3. This might be related to firmer texture of whey protein products than control yogurts. Control low-fat yogurt had a highest score of 7.1 in this category which was in agreement with monadic texture score.

Liking score of the texture varied from 4.3 to 7.0. Whey low-fat and full-fat yogurts had the lowest scores of 4.3 and 6.0, respectively. Control low-fat had the highest liking score of texture (7.0) followed by control non-fat (6.3) and whey non-fat (6.2). This result indicates that textural issues might be related to whey protein denaturation which was caused more firm gel formation of yogurt.

Similar to overall liking, whey non-fat yogurt had highest score of 7.0 in the flavor liking category followed by control low-fat (6.7) and control full-fat (6.6). This result may be related with high acetaldehyde volatile composition of whey non-fat yogurts.

Liking scores of the stabilized, cooked and sour flavor were not significantly different. Sensory scores revealed that control low-fat yogurt had better textural properties than others, while whey non-fat yogurt had higher scores than others for the flavor.

Chapter 5: Conclusion

The results of this study have shown that it is possible to completely replace NFDM in yogurt formulations with WPC. Whey protein yogurt products had acceptable sensory and textural properties when compared with control samples. Whey protein products showed better water holding capacities at all fat levels. Therefore syneresis that is the number one problem associated with yogurts texture was reduced significantly. Same results observed for the hardness and viscosity for yogurt samples. Whey protein yogurts had higher hardness and viscosity scores than control yogurts. This was mainly due to denaturation of whey proteins at higher pasteurization temperatures (92^{o}C for 30 seconds) thus resulted with firmer gel structure than controls.

Total solids content was not significantly different for control and whey protein yogurts at similar fat levels since it has been adjusted at formulation step. However, protein content was different due to higher protein content of WPC than NFDM. pH was higher for whey protein yogurts than controls. This might be related with higher amino acid content of whey proteins that would benefit the fastidious *Lactobacillus bulgaricus* which takes the lead at the late stages of fermentation.

Sensory tests also showed that whey protein yogurts had better scores for flavor and overall liking than controls. On the other hand, texture scores for the whey protein yogurts were lower than controls. This might be explained with the relatively higher usage levels of WPC in order to completely replace NFDM at same levels.

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Appendix A: Statistical Results

Total Solids Results

Oneway

Descriptives

value

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
cn	9	14.6878	.02539	.00846	14.6683	14.7073
cl	9	15.5867	.02784	.00928	15.5653	15.6081
cf	9	17.3689	.01900	.00633	17.3543	17.3835
wn	9	14.6744	.03283	.01094	14.6492	14.6997
wl	9	15.5356	.03432	.01144	15.5092	15.5619
wf	9	17.3989	.02472	.00824	17.3799	17.4179
Total	54	15.8754	1.13657	.15467	15.5651	16.1856

Test of Homogeneity of Variances

value

Levene Statistic	df1	df2	Sig.
.895	5	48	.492

ANOVA

value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	68.428	5	13.686	17669.436	.000
Within Groups	.037	48	.001		
Total	68.465	53			

Homogeneous Subsets

Tukey HSD[a]

degisken	N	Subset for alpha = 0.05			
		1	2	3	4
wn	9	14.6744			
cn	9	14.6878			
wl	9		15.5356		
cl	9			15.5867	
cf	9				17.3689
wf	9				17.3989
Sig.		.910	1.000	1.000	.220

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Fat Content Results

Oneway

Descriptives

value

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
cn	9	.0000	.00000	.00000	.0000	.0000
cl	9	1.1000	.07071	.02357	1.0456	1.1544
cf	9	3.2556	.08819	.02940	3.1878	3.3233
wn	9	.1444	.05270	.01757	.1039	.1850
wl	9	1.1000	.07071	.02357	1.0456	1.1544
wf	9	3.2889	.07817	.02606	3.2288	3.3490
Total	54	1.4815	1.34866	.18353	1.1134	1.8496

Test of Homogeneity of Variances

value

Levene Statistic	df1	df2	Sig.
3.271	5	48	.013

ANOVA

value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	96.188	5	19.238	4328.467	.000
Within Groups	.213	48	.004		
Total	96.401	53			

Post Hoc Tests

Homogeneous Subsets

value

Tukey HSD[a]

degisken	N	Subset for alpha = 0.05			
		1	2	3	4
cn	9	.0000			
wn	9		.1444		
cl	9			1.1000	
wl	9			1.1000	
cf	9				3.2556
wf	9				3.2889
Sig.		1.000	1.000	1.000	.894

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Protein Content Results

Oneway

Descriptives

value

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
cn	6	3.6800	.00358	.00146	3.6762	3.6838
cl	6	3.5952	.00392	.00160	3.5911	3.5993
cf	6	3.4555	.00464	.00189	3.4506	3.4604
wn	6	4.6167	.00393	.00161	4.6125	4.6208
wl	6	4.5565	.00505	.00206	4.5512	4.5618
wf	6	4.3973	.00273	.00112	4.3945	4.4002
Total	36	4.0502	.48912	.08152	3.8847	4.2157

Test of Homogeneity of Variances

value

Levene Statistic	df1	df2	Sig.
.392	5	30	.851

ANOVA

value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.373	5	1.675	102421.812	.000
Within Groups	.000	30	.000		
Total	8.373	35			

Post Hoc Tests

Homogeneous Subsets

value

Tukey HSD[a]

		Subset for alpha = 0.05					
degisken	N	1	2	3	4	5	6
cf	6	3.4555					
cl	6		3.5952				
cn	6			3.6800			
wf	6				4.3973		
wl	6					4.5565	
wn	6						4.6167
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

pH Results

Oneway

Descriptives

value

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
cn	9	4.4556	.01236	.00412	4.4461	4.4651
cl	9	4.4278	.00667	.00222	4.4227	4.4329
cf	9	4.3878	.00667	.00222	4.3827	4.3929
wn	9	4.4156	.01014	.00338	4.4078	4.4233
wl	9	4.3889	.00782	.00261	4.3829	4.3949
wf	9	4.3678	.00667	.00222	4.3627	4.3729
Total	54	4.4072	.03056	.00416	4.3989	4.4156

Test of Homogeneity of Variances

value

Levene Statistic	df1	df2	Sig.
1.223	5	48	.313

ANOVA

value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.046	5	.009	122.356	.000
Within Groups	.004	48	.000		
Total	.049	53			

Post Hoc Tests

Homogeneous Subsets

value

Tukey HSD[a]

		Subset for alpha = 0.05				
degisken	N	1	2	3	4	5
wf	9	4.3678				
cf	9		4.3878			
wl	9		4.3889			
wn	9			4.4156		
cl	9				4.4278	
cn	9					4.4556
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Hardness Results

Oneway

Descriptives

value

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
cn	9	.7189	.02759	.00920	.6977	.7401
cl	9	.7244	.03206	.01069	.6998	.7491
cf	9	1.4033	.01581	.00527	1.3912	1.4155
wn	9	1.1078	.01922	.00641	1.0930	1.1226
wl	9	1.1211	.02261	.00754	1.1037	1.1385
wf	9	1.4667	.03905	.01302	1.4366	1.4967
Total	54	1.0904	.29620	.04031	1.0095	1.1712

Test of Homogeneity of Variances

value

Levene Statistic	df1	df2	Sig.
3.055	5	48	.018

ANOVA

value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.614	5	.923	1245.844	.000
Within Groups	.036	48	.001		
Total	4.650	53			

Post Hoc Tests

Homogeneous Subsets

value

Tukey HSD[a]

		Subset for alpha = 0.05			
degisken	N	1	2	3	4
cn	9	.7189			
cl	9	.7244			
wn	9		1.1078		
wl	9		1.1211		
cf	9			1.4033	
wf	9				1.4667
Sig.		.998	.902	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Water Holding Capacity Results

Oneway

Descriptives

value

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
cn	9	582.0667	8.97353	2.99118	575.1690	588.9643
cl	9	622.2874	10.43410	3.47803	614.2671	630.3078
cf	9	725.5492	11.47897	3.82632	716.7257	734.3727
wn	9	682.4383	9.90193	3.30064	674.8270	690.0496
wl	9	730.3251	8.33750	2.77917	723.9163	736.7338
wf	9	768.9269	3.89540	1.29847	765.9326	771.9211
Total	54	685.2656	66.09321	8.99415	667.2256	703.3056

Test of Homogeneity of Variances

value

Levene Statistic	df1	df2	Sig.
1.387	5	48	.246

ANOVA

value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	227489.360	5	45497.872	541.751	.000
Within Groups	4031.181	48	83.983		
Total	231520.541	53			

Post Hoc Tests

Homogeneous Subsets

value

Tukey HSD[a]

degisken	N	Subset for alpha = 0.05				
		1	2	3	4	5
cn	9	582.0667				
cl	9		622.2874			
wn	9			682.4383		
cf	9				725.5492	
wl	9				730.3251	
wf	9					768.9269
Sig.		1.000	1.000	1.000	.877	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Viscosity Results

Oneway

Descriptives

value

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
cn	9	19355.5556	725.10536	241.70179	18798.1902	19912.9209
cl	9	21340.0000	718.05292	239.35097	20788.0557	21891.9443
cf	9	47722.2222	1087.12669	362.37556	46886.5827	48557.8618
wn	9	31847.7778	1078.49175	359.49725	31018.7756	32676.7799
wl	9	33011.1111	1896.44697	632.14899	31553.3729	34468.8493
wf	9	56977.7778	1865.62173	621.87391	55543.7340	58411.8216
Total	54	35042.4074	13663.50116	1859.36700	31312.9895	38771.8253

Test of Homogeneity of Variances

value

Levene Statistic	df1	df2	Sig.
2.490	5	48	.044

ANOVA

value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.811E9	5	1.962E9	1125.169	.000
Within Groups	8.371E7	48	1743903.704		
Total	9.895E9	53			

Post Hoc Tests

Homogeneous Subsets

value

Tukey HSD[a]

degisken	N	Subset for alpha = 0.05				
		1	2	3	4	5
cn	9	19355.5556				
cl	9		21340.0000			
wn	9			31847.7778		
wl	9			33011.1111		
cf	9				47722.2222	
wf	9					56977.7778
Sig.		1.000	1.000	.433	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.