

Effect of Application of Stabilizers on Gelation and Syneresis in Yoghurt

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Abstract

Yoghurt and fermented milks were among the most common dairy products consumed in the world, which were estimated to be 8.3 million tonnes in 1999. In Ethiopia, a significant proportion of milk is consumed in its fermented form as Ergo. Ergo (naturally fermented sour milk) has similar properties with yoghurt; it is a semi-solid product and has pleasant aroma and taste. The fermentation process, usually natural, is affected through spontaneous proliferation of the initial raw milk flora at ambient temperature. However, modern yoghurt production is a well-controlled process that utilizes ingredients of milk, milk powder, sugar, fruits, flavoring agents, coloring agents, emulsifiers, stabilizers, and pure cultures of lactic acid bacteria, which are responsible for the fermentation process. Yoghurt is a product of lactic acid fermentation of milk by addition of a starter culture consisting of *Streptococcus salivarius* sub spp. *thermophilus* and *Lactobacillus delbrueckii* sub spp. *Bulgaricus*. The addition of sweeteners in yoghurt has made it more palatable. Yoghurt with no added flavor is predominantly sour due to the lactic acid produced during fermentation. For better acceptance of such yoghurts by the consumer, fruits, flavoring agents and sweeteners have been added to improve the flavor balance or to mask partially the acetaldehyde flavor characteristics. Therefore the application of stabilizers in yoghurt manufacturing will bring a positive effect on product stability and consumer acceptance. The review of yoghurt manufacturing and associated stabilizers will help for further research study and development work. The objectives of this review is to address The objective of the paper Yoghurt manufacturing, Common principles in yoghurt manufacturing, Ingredients used in yoghurt manufacturing, Starter culture used in yoghurt manufacturing, Sweeteners used in yoghurt manufacturing, stabilizers used in yoghurt, the science of gelatinization and syneresis in yoghurt, nutraceutical properties of yoghurt, Physico-Chemical Properties of Yoghurt and Sensory Properties of Yoghurt.

Keywords: stabilizers, gelation, syneresis and yoghurt

INTRODUCTION

Milk is a major component of the traditional diet in many regions of Africa. In these communities, most of the milk produced is consumed in the home and is rarely sold. However, high temperatures and lack of refrigeration facilities have led to the inability to process and store fresh milk (Ogwaro *et al.*, 2002). Hence, conversion of any surplus liquid milk to relatively shelf stable products such as yoghurt, cheese, acidified milk, butter, and ghee has traditionally been done at the household level. Acidification of milk by fermentation is an old method of milk preservation. There are different methods of carrying out fermentation in various part of the world and these give rise to a range of fermented milk products including *Kumiss*, *Kefir*, acidophilus milk and yoghurt (Tamime and Deeth, 1980).

Product quality and consumer satisfaction are important for increasing the demand of various types of yoghurt. The increase in the per capita annual consumption of yoghurt in the majority of the countries has been attributed to increased knowledge regarding the health benefits of yoghurt, the ever-increasing availability of fruit or flavored yoghurt, and the diversity of presentations of the product (Küçüköner and Tarakçı, 2003; Van de Water and Naiyanetr, 2008). The healthy food image of yoghurt is due to the probiotic effect of yoghurt bacteria. According to Guarner *et al.* (2005) yoghurt bacteria are considered as “probiotics”, (i.e., live microorganisms which when administered in adequate amounts confer a health benefit to the host). The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhanced digestion of lactose by maldigesters, decreased risk of cancer, lower blood cholesterol, improved immune response and help the body assimilate protein, calcium and iron (Perdigón *et al.*, 1998; Van de Water and Naiyanetr, 2008).

Background of yoghurt

Yogurt is derived from Turkish word “Jugurt “ reserved for any fermented food with acidic test ,Yogurt in different forms with appropriate local names is made throughout the world, in Botswana it is called Madila, Lesotho Amasi, Namibia Omashikwa, Omaze Uozongombe, Zambia MabisiSawa and in Ethiopia Ergo (Mburude Wagt,2011). Currently yogurt of many types including kefir, Greek style yogurt, Swiss and fruit yogurts can be found. Yogurt is one of the most popular fermented dairy product widely consumed all over the world. It's obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* (Fadela *et al.* , 2009) and Ergo a traditional fermented milk in Ethiopia is semi-solid, smooth and uniform appearance and usually has a white milk color with pleasant odor and taste when prepared carefully. It constitutes primarily sour milk from which other products may be processed (Bereda *et al.*, 2014). Yogurt has assumed different forms like stirred, set and frozen liquid yogurt (Anjum *et al.*, 2007) . The nutritive value of yogurt is attributed to the fat content, sugar and casein (El-Malt *et al.* , 2013). Proteins in yogurt are of excellent biological quality, as are that in milk, because the nutritional value of milk proteins is well preserved during the fermentation process. Therefore, yogurt is recommended for sick and convalescent people (Ebringer *et a l.*, 2008).

Proteins in yogurt are of excellent biological quality, as are that in milk, because the nutritional value of milk proteins is well preserved during the fermentation process (Gursoy *et al.*, 2010).It has been argued that protein from yogurt is more easily digested than is protein from milk, as bacterial predigestion of milk proteins in yogurt may occur (FAO, 2013).Both the caseins and the whey proteins in yogurt are rich source of Amino acids (93%) and high in nitrogen availability.Amino acids like proline and glycine are present in free form and higher contents in yogurt than in milk (Damian, 2013). Although much of the yogurt sold in industrialized countries is produced from skimmed milk, traditional yogurt has always contained some 3-4 g per100 g milk fat; indeed concentrated yogurt or Greek-style yogurts will contain 9-10 g per100 g fat (Tamine and Robinsion , 1999). Conjugated linoleic acid , a type of essential fatty acid found almost exclusively in the fat of dairy products, can be obtained only through the diet because it is not produced by the human body (Miller *et al.*,2006). CLA has been shown to be a powerful natural anti-carcinogen that also can reduce the risk for cardiovascular disease, help fight inflammation, reduce body fat, especially abdominal fat, lower cholesterol and triglycerides, increase metabolism, lower insulin resistance and enhance the immune system (Lee and Lucey , 2010).

In the case of natural yogurt, a number of mono and disaccharides are present in trace amounts, but lactose remains the dominant sugar in natural yogurt; even after fermentation, the product may contain some 4-5 g per 100 g lactose. The reason for this residue is that the processed milk is often fortified to 14-16 g per 100 g total solids (i.e. up to about 8 g per 100 g lactose), so that the lactose content of the end product is little different from normal milk (Tamine and Robinsion,2000).What is different, however, is the effect that these apparently identical levels of lactose can have on people who are so called lactose intolerant or lactose maldigestors and the nature of this reaction is of considerable medical interest (Damian, 2013).

Yogurt contains appreciable quantities of sodium and potassium which may not be suitable for feeding babies less than 6 months but, as shown in, the mineral salts in milk can be reduced prior to the production of yogurt (FAO, 2013). According to the report made by Mbaeyi (2010), a product called dahi which resembles plain yogurt in appearance and consistency and differ in having lesser acidity,100 g of dahi contains 0.8 g mineral matter, 149 mg Ca, 93 mg P, 102 IU Vitamin A, 49 µg Thiamine, 157 µg Riboflavin, 86 µg Nicotinic acid, 3.2 µg Biotin, 183 µg Pantothenic acid, 178 µg Folic acid and 1.3 mg Ascorbic acid. The cow milk *dahi* (1 day old) has 0.57 % total nitrogen, 0.497% protein nitrogen, 0.073 % non protein nitrogen, 0.027% dialyzable nitrogen and 9.79% ammonia nitrogen (Vasiljevic and Shah, 2008)

Yoghurt probiotics, and probiotics association

Probiotics are dietary supplements or food products that contain beneficial, friendly and good bacteria or yeasts normally found in human body (Roberfroid *et al.* , 2010), and according to the definition of (Gursoy *et al.*, 2010), probiotics are selected viable microorganisms used as dietary supplements having potential for improving health or nutrition of man or animal following ingestion (Mazahreh and Ershidat, 2009) . Milk or milk products provide an excellent carrier for these probiotic organisms.Most of them can readily utilize lactose as an energy source for growth (Mckinley, 2005). Thus, an important requirement for the growth in the intestinal tract is provided by the milk. Milk proteins also provide important protection to the probiotic bacteria during passage through the stomach (Pereira *et al.*, 2013).

The primary probiotic bacteria associated with dairy products have been *Lactobacillus acidophilus*, *Lactobacillus casei* and *bifidobacteria*. Species of *Lactobacillus* and *Bifidobacterium* are most commonly used as probiotics, but the yeast *Saccharomyces cerevisiae* and some *E. coli* and *Bacillus* species are also used as probiotics (Kun *et al.*, 2008). Lactic acid bacteria, including *Lactobacillus* species, which have been used for preservation of food by fermentation for thousands of years, can serve a dual function by acting as agents for food fermentation and in addition, potentially imparting health benefits (Mazahreh and Ershidat , 2009) .Strictly

speaking, however, the term “probiotic” should be reserved for live microbes that have been shown in controlled human studies to impart a health benefit (Roberfroid *et al.*, 2010). Probiotics are widely used in fermented food production and are considered as generally recognized as safe organisms which are safely applied in medical and veterinary functions (Trachoo, 2002). In the food industry, probiotics are widely employed as starter cultures and have been indexed as part of human microbiota (Vasiljevic and Shah, 2008). A number of dairy products are marketed as containing probiotic bacteria. However, the most widely encountered one is yogurt (Touch and Deeth, 2009). There are a number of potential benefits that might be derived from consuming dairy products containing probiotic (Roberfroid *et al.*, 2010).

Prebiotics are food components, non-digestible by humans, that selectively stimulate the growth and activity of certain bacterial species already existing in the human colon, and inductively improve the health of the host (Law, 2006) and according to (pereira *et al.*, 2013), Prebiotics are non-digestible but, fermentable food ingredients that confer a health benefit on the host associated with modulation of microbiota in the colon (Bailey *et al.*, 2010). Most prebiotics are oligosaccharides in general, fructooligosaccharides in particular. Most common oligosaccharides with prebiotic character are inulin, *trans*-galactooligosaccharide, lactulose, isomalt and oligofructose (Bano *et al.*, 2011). They have been shown to stimulate the growth of endogenous Bifidobacteria, and make them the predominant species in human feces (Sfakianakis *et al.*, 2014). Prebiotics promote the growth and proliferation of beneficial bacteria in the digestive system (Chowdhury *et al.*, 2011). Unlike probiotics, which are live organisms, prebiotics are components of food that are not otherwise easily digested by humans and these food components essentially feed beneficial bacteria in the gut (Roberfroid *et al.*, 2010).

Yoghurt Manufacturing

There are two main types of yoghurt, set and stirred, based on method of production and physical structure of the coagulum. Set yoghurt is the product formed when fermentation/coagulation of milk is carried out in the retail container, and the yoghurt produced is in a continuous semi-solid mass. By contrast, stirred yoghurt results when the coagulum is produced in a bulk and the gel is broken before cooling and packing (Robinson and Tamime, 1993). Manufacturing methods of yoghurt vary considerably depending up on country, raw materials used, size of operation, product formulation and type of product being manufactured, but there are a number of common principles, which determine the nature and quality of the final product (Staff, 1998).

Preparation of base milk

To make a good quality product, raw milk used must be of low bacterial count, free from antibiotics, sanitizing chemicals, mastitis, milk colostrum, and the milk also should be free from contamination by bacteriophages (Haj, 2007). The use of lactoperoxidase-thiocyanate - hydrogen peroxide (LP) activated milk and antibiotic (penicillin/streptomycin) containing milk should be avoided due to retarded metabolic activity of yoghurt (Sarkar and Misra, 1994).

In order to meet legal requirements and to obtain consistent product quality, standardization of milk total solids content (mostly milk protein content) and milk fat content are important. The level of total solid in the milk is significant for both the consistency and aroma of the manufactured yoghurt (Tamime and Deeth, 1980). The total solids level in milk for yoghurt manufacture can vary from as low as 9% in skim yoghurt to over 20% in other types of yoghurt. According to Tamime and Robinson (1999), consistency improves when the content of milk total solids increases from 12–20 % especially protein content. The viscosity of yoghurt is almost wholly dependent on the protein content of the milk. Hence, a high protein concentration is essential for production of viscous yoghurt. Penna *et al.* (2006) concluded that the optimum yoghurt consistency could be achieved from milk containing 14-16% total solids. Although small difference in consistency is achieved when the content of total solids varies from 16–20 %, there is little interest in the use of concentrations above 16 % and, most low-fat yoghurt fall within the range of 14-15%. The protein or SNF content of milk can be increased by concentration of milk or by dry-matter enrichment. The sources of dry-matter are usually SMP, WPC or (occasionally) Na caseinate depending on what is legally permitted to be added to yoghurt, which varies from country to country. Commonly, the SNF content is increased by 1-3%, which corresponds to evaporation by 10-25% (Zourari *et al.*, 1992). In general, total solid level in excess of 25% is not recommended as it adversely affects the availability of moisture and hinders starter culture activity.

Yoghurt can have fat contents ranging from zero to 10%, with most common values comprised between 0.5% and 3.5% (Lucey and Sigh, 1998). The content of fat in yoghurt is often dictated by quality standards of the country. The legal or proposed standards for the chemical composition of yoghurt in various countries are based on three possible types of yoghurt classified according to fat content (full, medium and low) of the product (FAO/WHO, 1973). Such a classification is used in compositional standards to facilitate standardization of product and to protect the consumer. The effect of fat content on yoghurt texture is linked to the role of homogenization, a treatment that is almost systematically used in yoghurt manufacture. Fat provides a perception of creaminess and improves the mouth feel of yoghurt products (Sodini *et al.*, 2004).

Homogenization

Homogenization is an integral part of the yoghurt manufacturing process. It is usually carried out before heat treatment. However, in some cases it may take place after heat treatment (Tamime and Deeth, 1980). It is carried out chiefly to effect a homogeneous dispersion of the milk mix constituents and to increase the viscosity and coagulum stability of the yoghurt. Homogenization of milk for yoghurt manufacture is considered to prevent fat separation (cream layer formation) during storage, improve consistency, increase whiteness and reduce whey separation (Tamime and Robinson, 1999). It also improves the 'mouth-feel' of the product and thus increases the organoleptic quality of yoghurt.

Milk is usually homogenized at pressures in the range 10-20 MPa, at temperatures in the range 55-65 °C and prior to heat treatment of the mix. The tendency for cream layer formation is reduced in homogenized milk because of the formation of small fat globules (< 2 µm). During homogenization, casein and some whey protein adsorb at the fat globule interface and this effectively increases the number of structure-building components (Walstra *et al.*, 1999). Since protein was adsorbed onto the surface of homogenised fat globules, higher levels of fat increased the ability of the protein to immobilise water (Keogh and O'Kennedy, 1998).

Heat treatment

As with almost all heat processes employed in food processing, the primary aim of heating the base milk in yoghurt manufacture is destruction of microorganisms which may be pathogenic or which may adversely affect the quality of the product. Almost all organisms with the exception of spore formers in the vegetative form are destroyed during yoghurt manufacture. According to Soukoulis *et al.* (2007), milk heat treatment is considered a critical factor for texture formation. Heating induces whey protein denaturation so that whey proteins can associate with casein micelles. Whey proteins are bound to caseins through disulfide linkages and hydrophobic interactions (Law, 1996). The application of a time-temperature profile ranging from 80 to 85 °C for 30 min, to 90 to 95 °C for 5 min is regarded to be adequate for producing high quality yoghurt (Lucey and Singh, 1998). Lucey *et al.* (1997) reported that the increase in heating intensity induced an extensive denaturation of whey proteins (primarily β-LG), which in turn was correlated to increased viscosity during acid gelation. In general, an increase in denaturation of whey proteins (primarily of β-LG) leads to a reduction in the gelation time and an increase in the pH at gelation (Shaker *et al.*, 2000). However, yoghurt prepared with unheated or inadequately heat-treated milk, is characterized by poor texture, weak gel and firmness, and increased susceptibility to wheying off (Tamime and Robinson, 1999). Heating before yoghurt preparation would decrease watery part of milk produced when raw milk sours and coagulates. According to Lucey (2004), severe heat treatment has many effects on milk and the resulting yoghurt, including:

- ✚ destruction of microorganisms and most milk enzymes;
- ✚ denaturation of whey protein and its association with caseins (at the pH of heating or during subsequent acidification), which results in: increased gel firmness and viscosity;
- ✚ reduced gelation time (facilitate the rate of gelation)
- ✚ reduction in oxygen levels (starter bacteria are sensitive to oxygen); destruction of some inhibitors;
- ✚ production of some stimulatory compounds, which can accelerate the fermentation process (e.g formic acid);
- ✚ Hydration of stabilizers that require a high temperature for adequate hydration.

Inoculation and incubation

The incubation temperatures for yoghurt are usually in the range 40 to 45 °C and fermentation often takes up to 4 h, depending on the amount of starter addition. The inoculation amount can vary between 0.5-5percent but the recommended value is ≥ 2% (Tamime and Robinson, 1999). The *bacilli* to *cocci* ratio in yoghurt starter culture is 1:1 (Wouters *et al.*, 2002; Sokolinska and Pikul, 2004). After the pH of yoghurt has decreased to the desired values (usually 4.6), the gel is then cooled to < 10 °C. The final pH of most yoghurt varies from 4.6-4.0. An excessive rate of acid development at a high incubation temperature contributes to the 'whey-ing-off' defect and poor gel formation (Lucey and Sigh, 1998). Several researchers have recommended lowering the incubation temperature from 44 °C to 38 °C to improve gel firmness, viscosity and reduce whey separation (Lucey and Sigh, 1998). At lower incubation temperatures (e.g. 30 °C) the fermentation time can be extended for up to 12 h (overnight incubation) but good quality yoghurt can be produced. At cold room temperature incubation may take more than 24 hour.

Cooling and storage

Cooling is a critical step in yoghurt production. It is carried out directly after product reaches the desired acidity. The cooling process can take place either in the incubation vessel (in-tank cooling) or in tubular or plate heat exchanger. The former system requires a longer period. For example, in the Wincanton tank 4 h are needed to cool yoghurt from 42 to 5 °C compared with 20-30 min for the plate/tubular coolers to cool yoghurt to 15 °C and further cooling to 5 °C takes place in the cold store (Robinson and Tamime, 1993).

The objective of fat cooling is to reduce the metabolic activity of the starter culture and hence to control the acidity of the yoghurt otherwise it may lead to over acidification, shrinkage of the protein gel and whey separation. From a microbiological point of view, the yoghurt starters (*S. thermophilus* and *L. bulgaricus*) have limited growth at around 10-15 °C (Tamime and Deeth, 1980). However, in most industrial installations, yoghurt is cooled to less than 20 °C, mixed with fruits, packaged and finally cooled at 5 °C (or less) in the refrigerated store. During cold storage at 4 °C, viscosity of yoghurt increases for 1-2 days. Hydration leads to, much firmer gels and casein micelles continue to stabilize. According to Tamime and Robinson (2000), the ideal pH of the finished product of yogurt shall be between 4 and 4.1. However this pH also depends on the addition of fruits or flavoring agent to the yogurt. But after fermentation when the yogurt is ready to eat, the pH is 4. The pH of yogurt is decreased if the amount of skimmed milk powder is increased. The yogurt mixture coagulates during fermentation due to the drop in pH (Bönisch *et al.*, 2007).

Ingredients of Yoghurt

Stabilizers

Stabilizers are commonly used in cultured products to control texture and reduce whey separation since they impart good resistance to syneresis and a smooth sensation in the mouth by binding water to reduce water flow in the food matrix space (Amatayakul *et al.*, 2006). Some may interact with protein in the food matrix and hence further increase hydration behaviour (Duboc and Mollet, 2001). Two of the most frequently used stabilizers are gelatin and starch (Tamime and Robinson, 1999). The use of modified food starch helps to create a creamier texture but the type should be selected based on the processing conditions (shear, heat, pressure, and pH) that will be encountered. Usage levels in cultured products are usually 0.1–0.4% for gums, < 0.8% for modified starches and up to 2 % for starch. Starch is also used in yoghurt to increase its viscosity, improve its mouth-feel, and prevent syneresis. Starch granules absorb water and swell to many times their original size, resulting in increased viscosity of the solution. Starch granules act as non-interacting fillers and cause the concentration of the other ingredients in the continuous phase because of the exclusion effect (Verbeke *et al.*, 2006).

According to Baziwane and He (2003), of all the hydrocolloids in use today, none has proven as popular with the general public and found favor in as wide a range of food products as gelatin. Gelatin is a proteinaceous material obtained from animal connective tissue (collagen) using hydrolysis in acidic (type A) or basic (type B) solution followed by hot water extraction (Stevens, 2009). It is usually derived from sources of collagen that are available both in quantity and at a reasonable price for the manufacturer. Commercially viable sources include mainly demineralized cattle bone (ossein), bovine hide and pigskin. Gelatin is one of the hydrocolloids or water-soluble polymers that can be used as a gelling, thickening or stabilizing agent; it is a highly digestible protein, containing all the amino acids except tryptophan and have low methionine, cystine and tyrosine due to the degradation during hydrolysis (Jamilah and Harvinder, 2002). The amino acid composition and sequence in gelatin are different from one source to another but always consists of large amounts of glycine, proline and hydroxyproline (Gilsenan and Ross-Murphy, 2000).

Moreover, gelatin is an ingredient compatible with the milk proteins and contributes good palatability to the final product, giving a fat-like sensory perception because of its unique property of melting at mouth temperature. It eliminates syneresis and considerably reinforces the mechanical resistance of the gels making it possible to obtain a wide range of texture (Fizman and Salvador, 1999). Potentially, the use of different concentrations of gelatin would make it possible to obtain a wide range of textures including creamy, slightly gelled and firm, “moldable” gel of yoghurt (Fizman and Salvador, 1999). One of the most important properties of gelatin is its ability to form thermo-reversible gels. Gelatin swells in cold liquid absorbing 5-10 times its volume of water. When heated to temperatures of approximately 50 to 60 °C it dissolves and forms a gel when cooled. This sol- gel conversion is reproducible and can be repeated several times. The gelling power of gelatin is determined by its Bloom value, which is a standardized measurement of the firmness of a standard gel under precisely determined conditions. The Bloom values of gelatin range from 80 to 300 g. In general, viscosity increases with increasing Bloom strength (Schott, 2001).

Addition of the right type and the right amount of stabilizer to fermented milk improved viscosity of yoghurt, prevented whey separation and did not require fortification with dry skim milk powder, which in turn did not affect the flavor of the yoghurt (Bassett, 1983). Since low concentration of stabilizer may not satisfy the functional requirements, too high concentration may result in unacceptable appearance and texture (mouth feel, surface sheen, rubbery texture). Controlling the concentration of stabilizer addition is important to produce good quality yoghurt (Staff, 1998). According to Ares *et al.* (2007) addition of gelatin into stirred yoghurt at a level of 0.6% prevented syneresis whereas addition of starch only reduced the degree of syneresis. Moreover, yoghurt samples produced with 6 mg/g of gelatin showed the highest sensory viscosity, creaminess and mouth-feel scores thus might have a positive effect on consumers' acceptability.

Although, various non-milk additives are used in yoghurt processing in order to improve the sensory properties of yoghurt milk and milk derived products (cream, skim milk, skim milk powder, whey concentrate proteins, casein) are the major ingredients in yoghurt production. These additives become part of the food, in

order to provide some very specific and precisely defined sensory characteristics such as taste, appearance, consistency, or shelf life (Spreer, 1998).

The science of gelation and Syneresis in yoghurt

Leakage of whey (syneresis) from yoghurt is a common defect and must be controlled. Wheying-off may be indicative of faulty fermentation and off-flavors. Common reasons for the occurrence of whey on cultured products include the use of a high incubation temperature, excessive whey protein to casein ratio, low solids content (protein and fat if the milk is homogenized) and physical mishandling of the product during storage and retail distribution (Lucey, 2002). The type and concentration of added flavoring material and the storage time influence syneresis of yoghurt. Salvador and Fiszman (2004) concluded measured syneresis increased with storage time. Syneresis was observed to increase during storage of fruit flavored yoghurt (Küçüköner and Tarakçı, 2003). Similarly, Salwa *et al.* (2004) showed that as the concentration of carrot juice added into the yoghurt increased the tendency of the resulting product to syneresis increased.

The use of natural stabilizers

In recent days, natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Thaipong *et al.*, 2006; Temple, 2000). Thus, supplementation of yoghurt with products derived from fruit and vegetable is gaining the attention of both the processors to increase sales of yoghurt and the consumer for better health in addition to their role as flavoring agents. The defensive effects of natural antioxidants in fruits and vegetables are attributed to three major groups of compounds: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Halliwell, 1996).

Yogurt is also produced and consumed in flavored and supplemented forms. The flavoring pigment can be done by addition of natural ingredients or by addition of synthetic flavor compounds, adding fruit juices or fruit pulp (Vahedi *et al.*, 2008). Many of these fruits are known as very good sources of anthocyanins, which display a wide range of biological activities including antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic activities (Pereira *et al.*, 2013). Although, in a wide assortment of flavors, typically fruit flavors such as strawberry or blue-berry and more recently cream pie and chocolate flavors are used (Mbaeyi and Anyanwu, 2010). Fruits and vegetables are not only good sources of carotenoids but also an important source provitamin A, i.e., β and α -carotenoids that are an efficient provitamin-A. Beta and α -carotenoids are the predominant carotenoids in fruits and vegetables, which account for 90% of the total carotenoids. Thus, incorporation of fruits and vegetables (juice stabilizers) into yoghurt could contribute to the prevention of diseases (e.g. sight problem) related to vitamin A malnourishment in addition to improving the antioxidant property of the yoghurt.

In summary, researches have indicated the health risk of consuming traditional fermented milk products (e.g., *Ergo*) which is made from raw milk without any heat treatment. Thus, there is a need to produce fermented dairy products such as yoghurt, which is safe as it is made from heat-treated milk. In addition, yoghurt production is a well-controlled process in which not only safe but also consistent product quality could be assured. However, in our country there is no published information on yoghurt production in such a way that it can be applied under small scale and household level production while safety and product quality are considered. In addition, development of functional food from dairy products has proven to increase the demand of dairy products in many countries especially in the developed ones. However, there is no published research work in our country regarding development of functional dairy products, which are believed to stimulate the dairy market if in addition nutritional education is provided to the consumer.

Sweeteners

Sweetening compounds are usually added into yoghurt either via the addition of fruit concentrate or in the initial milk base and after addition of fruits and flavoring materials, yoghurt may contain anywhere from 4 to 20% sweetener (Wilson *et al.*, 1983). The main reason for addition of sweeteners in to yoghurt is to inhibit the level of acidity produced especially when high acid/low sugar content fruits are added (Staff, 1998). There are different types of sweetening compounds used for yoghurt preparation. They can be grouped into non-nutritional (such as; Saccharin, Cyclamate, Aspartame, Acesulfame-k) and nutritional (Sorbitol, Xylitol, High Fructose Corn Syrup, Glucose and Sucrose). Non-nutritive sweeteners (synthetic sweeteners) are mainly used to impart calorie reduction in fermented milks. Aspartame and Acesulfame-K individually and in combination have been used as sweeteners for the formulation of numerous low-calorie and sugar free yoghurts and cultured milk products (Lotz *et al.*, 1992). Among all types of sweeteners, sucrose (sugar) is the most commonly used sweetener in yoghurt production especially in flavored yoghurt preparation.

While selecting sweeteners for yoghurt production, properties of the sweetener such as; stability to

high temperature, the pH range at which the sweetener is stable, solubility in water, concentration at which it gives the desired sensory properties, inhibition of the starter culture, presence of lingering bitter after taste and stability towards microbial degradation by yoghurt starter during storage time should be considered (Pinheiro *et al.*, 2005; King *et al.*, 2000).

King *et al.* (2000) observed the stability of APM was influenced by pH, time and temperature. Fellows *et al.* (1991) reported sweetness perception (from Aspartame) decreases over time of storage and this was related to the growth and activity of the starter culture. Hyvoenen and Slotte (1983) investigated the effects of different sweeteners on yoghurt quality and they found that 15% Sorbitol added prior to incubation inhibited culture growth such that no acid, aroma, or coagulation is developed. The same results were obtained when yoghurt was sweetened with 7% Sorbitol. Saccharin-sweetened yoghurt was unacceptable due to an overpowering, bitter aftertaste. Similarly, Greig *et al.* (1985) demonstrated a slightly sweet, lingering aftertaste in yoghurt sample of 0.14% added aspartame which was not present in yoghurt samples of 2% added sucrose with equivalent degree of sweetness. McGregor and White (1986) concluded that High Fructose Corn Syrup (HFCS) did not adversely affect yoghurt quality and may have increased its acceptability. Nevertheless, due to its higher osmotic pressure, HFCS may inhibit the growth of the yoghurt starter culture (Keating and White, 1990).

Therefore, amount and type of sweeteners used may be critical to culture growth and consumer acceptance. According to McGregor and White (1986), regardless of the type of sweetener used, yoghurt made with 4 % sweetener was more liked by the consumer panelists. Even though, some workers showed the positive effect of sucrose levels on the growth of yoghurt starter and acetaldehyde production, Bills *et al.* (1972) reported that growth and acid production were inhibited in yoghurt containing 4% or more sucrose and lower acetaldehyde production was observed above 8% sucrose.

Good Physico-Chemical Properties of Yoghurt

Acidity

The acidity of yoghurt is the result of lactic fermentation of lactose into lactic acid by the yoghurt starter culture mainly during the incubation period and to some extent due to post acidification during storage (Dave and Shah, 1997). Development of lactic acid under controlled conditions during fermentation is essential for the formation of yoghurt gel network. On the contrary, development of acidity after the fermentation is not desired since it leads to wheying-off, textural defects, and excess sourness, which masks the perception of aroma compounds by the consumers (Mc Feeters, 2004). Especially, development of souring and bitter taste could determine the shelf life of yoghurt. During the storage period, the increase in titratable acidity and bitterness, and the decrease in pH of yoghurt depend on species and strain of starter culture used (Walstra *et al.*, 1999). Acid development may be monitored by measuring the acidity of the incubated yoghurt using volumetric titration method or by measuring pH. However, the current trend in yoghurt processing industries is to use pH measurements rather than titratable acidity. Yoghurt milk will become solid at an acidity of about 0.6% (expressed as lactic acid). The pH at this point is about 5.3 depending on the type of milk and the protein content (Lucey, 2004). In deciding the degree of acidity of yoghurt, the consumers' preference should be considered since the degree of sourness of yoghurt is related to the acidity. Codex Alimentarius suggests a minimum of 0.6% lactic acid for yoghurt in the retail product (Table 1).

Texture

Rheological properties for foods, such as fermented dairy products, are important in the design of flow processes, quality control, storage and processing and in predicting the texture of foods (Shaker *et al.*, 2000). The textural properties of acid milk gels can be assessed by a range of fundamental and empirical methods such as small-amplitude oscillatory rheology (SAOR), large-amplitude oscillatory shear, penetration, texture profile analysis, rotational viscometry, flow through an orifice such as a Posthumus funnel, and various sensory methods (Lucey, 2004).

Yoghurt exhibits a variety of non-newtonian effects, such as shear-thinning, yield stress, viscoelasticity, and time-dependency (Benezech and Maingonnat, 1994). Almost every processing step in the manufacture of yoghurt can affect the viscosity of the finished product (Keating and White, 1990). O'Neil *et al.* (1979) concluded that the consistency of yoghurt was influenced by the composition, storage time and acidity.

According to Marshall (2006), it is of paramount importance to measure the firmness of the set-type yoghurt, which represents its resistance to rupture, i.e., the yield point of the structure as determined by a compression-type test. Measurement of gel firmness in yoghurt is achieved by means of constant speed penetration on universal testing machines or similar instruments, using cylindrical plungers and crosshead speed values ranging between 10 and 100 mm min⁻¹ at <10°C. The force-response is monitored as a function of penetration depth (Jaros and Rohm, 2003). The plunger size and the penetration depth affect the force-response. Gel firmness can be measured at a predefined time or at a predefined depth or until a point of gel breakage occurs i.e. yield point (Fiszman and Salvador, 1999; Harte *et al.* 2007). According to Ares *et al.* (2006), yield stress would be the most appropriate to characterize the texture of yoghurt in quality control or product

development, since several methods that allow a quick and direct determination of yield stress are available. The main disadvantages of these empirical methods, when compared to fundamental measurement of rheological properties, are the use of relative scales and that results are for a given set of experimental conditions, making it hardly possible to compare results unless the same conditions are used (Benezech and Maingonnat 1994).

Sensory Properties of Yoghurt

Yoghurt can vary greatly in ingredient composition, so determining consumer acceptance for these products is necessary for commercial success. Therefore, high importance is placed on flavor and texture of yoghurt products. Consumer acceptance of yoghurt depends on acidity (sourness), sweetness, aroma perceptions, and textural properties of the product (Beal *et al.*, 1999). Yoghurt aroma is generally ascribed to acetaldehyde produced by *L. bulgaricus* and *S. thermophilus* from threonine (Marschall and Cole, 1983). According to Vedamuthu (1991), the optimum range for acetaldehyde in yoghurt is 10-15 ppm. In addition, other volatile organic aroma compounds have been identified for example, the diketones 2,3-butanedione (diacetyl) and 2,3-pentanedione are also contributors (Ott *et al.*, 1997). Nevertheless, the contribution of these compounds to yoghurt quality was not clearly demonstrated, and their role is minor when flavorings are added to the product.

According to Ott *et al.* (2000) the acidity of yoghurt plays a major role in yoghurt flavor and is perhaps more important than the concentration of acetaldehyde, diacetyl, or 2,3-pentanedione since the perception of acidity has influence on the perception of the other attributes. Barnes *et al.* (1991b) reported the overall liking of yoghurt by consumer was influenced by the degree of sweetness, sourness and above all, by the specific fruit flavor liking. Sensory attributes associated with plain yoghurt such as astringency, acetaldehyde and sourness were masked by the sweetener and fruit flavorings. Hence, to produce yoghurts having the highest overall liking rating, a balance between sweetness and sourness is necessary.

Texture is one of the main characteristics that define the quality of yoghurt and affect its appearance, mouthfeel and overall acceptability (Yoon and McCarthy, 2002). The consistency of yoghurt is probably as important as flavor. Adequate firmness without syneresis is essential for a top-quality product (Kroger, 1975). The textural characteristics of yoghurt are generally studied in the cup, using a spoon, or in the mouth. According to Cayot *et al.* (2008), in sensory evaluation of yoghurt, either consistency can be perceived visually or orally where oral consistency can be defined as Viscosity of the product on the tongue. The yoghurt sample is 'fluid' if it flows over the tongue immediately (non-viscous). The yoghurt sample is "thick" if the yoghurt sample stays on the tongue or flows slowly and is swallowed with difficulty (viscous) while visual consistency can be defined as Viscosity of the product when the spoon is slowly tilted up to 90°. The yoghurt sample is 'fluid' if the flow is easy, immediate and continuous from the spoon (non-viscous). The yoghurt sample is 'thick' if the yoghurt sample flows slowly, with difficulty (viscous).

Mouth feel is another important sensory property of yoghurt products. According to Lawless and Heyman (1999), mouth feel is defined as 'a category of sensations occurring in the oral cavity, related to the oral tissues and their perceived condition (e.g. drying, coating). Mouth feel, flavor, sweetness, sourness, and the balance between these factors have been shown to affect the overall preference for yoghurt (Barnes *et al.*, 1991a). These characteristics, and many others, are important attributes for the acceptance of yoghurt products.

Nutracutical properties Yoghurt

Foods containing probiotics, such as fermented milks, yogurts, and cheese, fall within the functional food category, which includes any fresh or processed food claimed to have health-promoting and/or disease-preventing properties beyond the basic nutritional function of supplying nutrients (Van de Water and Naiyanetr, 2008). Yoghurt for a long time has been accepted as functional food due to the presence of viable probiotic bacteria in it. The health benefit of yoghurt has been advocated since the early 1900s after Metchnikoff proposed that the lactic acid microbes of fermentation must be antagonistic to the putrefying microbes of the gut, and once introduced into the intestine, they would prevent the breeding of the noxious microbes that required an alkaline environment. Further, he supported his hypothesis by the fact that populations such as those living in the Balkans were regularly eating yogurt and were noted for their longevity. Until 2002, when FAO/WHO announced a new definition of probiotic bacteria, there were some controversies regarding the probiotic effect of yoghurt bacteria. According to FAO/WHO, probiotic bacteria are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Guarner *et al.*, 2005). Hence, yoghurt bacteria, which are proven to improve the health of lactose intolerant people due to their β -Galactosidase activity, are clearly now categorized as probiotic bacteria.

The increasing demand from consumers for dairy products with 'functional' properties is a key factor driving value sales growth in developed markets. Yoghurt is an increasingly popular cultured dairy product in most countries. For example, in USA probiotic drinking yoghurt was the fastest growing dairy product sector between 1998 and 2003. This is mainly because of an increased awareness of the consumers regarding possible health benefits of yoghurt (Küçüköner and Tarakçı, 2003; Van de Water and Naiyanetr, 2008).

Yogurt is being enjoyed everywhere in the world for its beneficial properties. For this reasons manufacturers are continuously investigating value added ingredients such as prebiotics and probiotics to entice

health-conscious consumers. Moreover, there are numerous advantages of consuming fermented dairy products containing probiotic bacteria (Huth *et al.*, 2006). A high population of probiotic organisms in the colon contributes to good intestinal health (Aswal *et al.*, 2012). The results of several clinical studies indicate that a regular administration of selected probiotics may reduce the concentration of serum cholesterol, especially of low density lipoprotein (Ebringer *et al.*, 2008). In addition to being a nutritious food for humans; yogurt provides a favorable environment for the growth of microorganisms (Lourens-Hattingh and Viljoen, 2001). There are genetic and environmental factors that increase the chances of cancer. Some factors, such as diet rich in cultured dairy products, may inhibit the growth of many types of cancer, including breast tumors and most recently proved its effect in colon cancer and cancer of the liver (Rayes *et al.*, 2008). According to (Van de Water *et al.*, 1999), many recent studies have focused on the possible effect lactobacilli may have on the immune system and the ability to fight off an infection.

The few studies to date indicate that lactobacilli activate both a systemic and a local immune response. Locally, yogurt may enhance the immune response by increasing the percentage of B lymphocytes and the phytohemagglutinin and lipopolysaccharide induced proliferative responses of Peyer's patches in the intestine (Mazahreh and Ershidat, 2009). Based on the report made by Kumar *et al.* (2012), in order to improve colonic function and quality of life of elderly, nutrition and its influence on intestinal ecosystem raises a great interest so that, two dietary intervention strategies are explored to regulate gut physiology consumption of fermented products containing viable micro-organisms resistant to digestion and metabolically active in the colon (probiotics) or and consumption of non-digestible substrates reaching the colon and selectively fermented by intestinal microbiota (prebiotics).

Shelf Life of Yoghurt

Product shelf life is the controlling factor in the distribution of dairy foods, which are highly perishable by nature. Shelf life dictates the total elapsed time allowed from production to consumption. The shelf life of non-sterile dairy products such as yoghurt and fermented milk products, is generally limited to one to 3 weeks (Salvador and Fiszman, 2004), depending upon the quality of the raw ingredients, processing conditions, and post processing handling. The keeping quality of yoghurt can be improved by various methods such as gas flushing (carbon dioxide and nitrogen), use of preservatives and application of heat after incubation (Tamime and Deeth, 1980). The drive to extend shelf life stems from increased distribution center demands due to consolidation of manufacturing facilities, product returns from code expirations, and interest in opening up new distribution channels and in expanding geographically.

Changes in the physical, chemical, and microbiological structure of yoghurt determine the storage and shelf life of the product. Alteration of these properties causes color, aroma, and texture deterioration of yoghurt, which are considered important quality criteria by consumers. Moreover, Salvador and Fiszman (2004) reported that studies of changes in these quality characteristics during storage would enable producers to predict the shelf life of the product more accurately.

Microbial activity is the first and most important limitation of a food's shelf life in general and yoghurt in particular. The presence of live starter bacteria and yeast and mold contaminants coupled with packaging/storage conditions lead to the formation of off-flavors and other undesirable physicochemical changes that eventually lead to product failure (Muir and Banks, 2000; Salji *et al.*, 1987). Yoghurt is notorious for its spoilage, especially in the development of harsh-acidic flavor and in thinning (low viscosity), when processing parameters, handling and cooling are not in control (Bille and Keya, 2002). According to Viljoen *et al.* (2003), mold and yeast growth and development of off-flavors can be a major determinant of shelf life of yoghurts. The off-flavor produced by those organisms can be described as yeasty, fruity, musty, cheesy or bitter and occasionally soapy-rancid which may be ascribed to their high proteolytic and lipolytic activities (Walstra *et al.*, 1999).

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