IRON FORTIFICATION OF YOGURT AND PASTEURIZED MILK

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ABSTRACT

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Both yogurt and pasteurized liquid milk was made from whole cow's milk which was fortified with ferrous bisglycinate, ferrous lactate and ferrous sulfate microencapsulate. Yogurt was stored for 7 days and milk for 2 days before consumer acceptance sensory test was done. Chemical analysis was done every 5 days for yogurt and every 3 days for pasteurized milk. Sensory mean scores show that there were no significant differences between the control yogurt and yogurt fortified with ferrous sulfate microencapsulate in appearance, flavor, mouthfeel and overall preference. Significant differences ($p<0.05$) were observed between control yogurt and yogurt fortified with ferrous bisglycinate and yogurt fortified with ferrous lactate. The observation was different in milk where no significant differences were observed in appearance and flavor in all treatments while control milk and milk fortified with ferrous lactate showed no significant differences in taste. Both thiobarbituric acid (TBA) and peroxide value (PV) numbers were highest in yogurt and pasteurized milk fortified with ferrous sulfate microencapsulate. Control yogurt had the lowest TBA value. PV was lowest in yogurt fortified with ferrous lactate while pasteurized milk has low PV in control. Therefore ferrous sulfate microencapsulate was the best option for fortifying both yogurt and pasteurized liquid milk.

I would specifically dedicate this work to my beloved wife, Beatrice Chalinda Nkhata and my lovely daughter Trinity Nkhata for enduring my absence when I went for my studies abroad.

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KEY TO ABBREVIATIONS

CFU:	Colony Forming Units
FDA:	Food and Drug Administration
g:	gram
GoM:	Government of Malawi
GRAS	Generally Regarded as Safe
Hb:	Hemoglobin
HTST:	High Temperature Short Time
ID:	Iodine deficiency
IDA:	Iron deficiency anemia
IDD:	Iodine deficiency disorder
LAB:	Lactic acid bacteria
MDA:	Malondialdahyde
MDHS:	Malawi Demographic Health Survey
Meq:	Milliequivalent
ml:	millimeter
MRS:	Man Rogosa and Sharpe
N:	Normality
NaFeEDTA:	Ferric sodium ethylenediaminetetraacetic acid
NDM:	Non-fat dry milk
Nm:	nanometer (a billionth of a meter,10^{-9})
pH:	power of hydrogen

PV:	Peroxide value
RDA:	Recommended daily allowance
TA:	Titratable acidity
TBA:	Thiobarbituric acid
TBARS:	Thiobarbituric reactive substances
UCRIHS:	University Committee on Research Involving Human Subject
UHT:	Ultra heat treatment
FAS:	Food Standard Agency
VAD:	Vitamin A Deficiency
WHO:	World health organization
Wt.	Weight

1.0 INTRODUCTION

Iron is essential micronutrient in human nutrition. It is also a component of heme in hemoglobin and myoglobin in which it plays important role in the transport, storage and utilization of oxygen. Iron deficiency induces anemia, alters mental development, decreases immunity (Gaucheron 2000) impairs cognitive scores in children and leads to poor pregnancy outcome and lowers working capacity in adults (Martinez-Navarreta and others, 2002). In cases in which anemia is severe and not corrected, blood transfusion may become necessary. Anemia has been reported to contribute significantly to maternal mortality and both maternal and fetal morbidity (Van de Broek and Letsky, 2000).The iron found in food can be highly bioavailable, as is the case with heme iron which is found in red meat. However, the cost of these products is too high for many people. The iron present in other products of vegetable origin, is non-heme and has the disadvantage of interacting with substances in food that inhibit its absorption such as tannins, phytates, and polyphenols hence it has low bioavailability. Much of this kind of food is consumed by people in the lower socioeconomic classes, who thus cannot meet their physiological needs for iron (Van de Broek and Letsky, 2000). Therefore it is widespread in less industrialized countries as in developing countries. Iron deficiency is also caused by either insufficient dietary intake of iron, poor absorption of iron or both (Gaucheron 2000), insufficient absorbable iron, hookworm infection, malaria or Vitamin A deficiency (Richard, 1997). Hence all these factors should be addressed simultaneously in any food fortification strategy.

The best way to prevent problems associated with iron deficiency is through iron fortification of food for the whole population or only for certain groups. Compounds used in food fortification provide nonheme iron, so it is important to select fortification

compounds and foods vehicles that will not diminish iron bioavailability (Van de Broek and Letsky 2000). Though food fortification may increase unit cost of food being fortified, it is the most cost-effective technique than other interventions that have the potential to achieve the same health or nutritional outcome, such as supplementation (Allen and others, 2006). Iron fortification of flour, bread and cereals is practiced to correct iron deficiency (ID). Iron fortification of milk and dairy product is considered as a potential approach to prevent the ID disorder (Gaucheron 2000), since dairy foods are an important part of the daily diet in most parts of the world; also, in the diets of those most susceptible to iron deficiency primarily women and children.

Dairy products are an important group in human nutrition. Direct addition of iron to milk or dairy product might be effective means of increasing the dietary intake of iron to the general population. Malawi has a tropical climate and milk cannot be kept for more than three hours at ambient temperature immediately after milking. Cooling equipment are not available in many parts of the country and if available is not affordable to rural people. The new scientific and efficient method to overcome this problem is use of Ultra high temperature (UHT) pasteurization of milk. While yogurt cannot store without refrigeration for long period of time aseptically processed UHT pasteurized milk is shelf stable and can store for months without spoiling. Both pasteurized milk and yogurt are excellent sources of vitamins, minerals and proteins but like any other dairy product they contain very little iron (approximately 0.2mg/kg (Gaucheron, 2000) which makes it impossible to meet iron Recommended Daily Allowance (RDA). Therefore dairy products are logical vehicle for iron fortification (El-Kholy and others, 2011) and considered as practical and cost-effective long term solution (Abasi and Azari, 2011). Milk, either pasteurized or sterilized, is the most

commonly consumed dairy product in Malawi because of the relatively low cost, availability and its readiness to drink. It was estimated that 80% of the total dairy products consumed is pasteurized or sterilized liquid milk. Yoghurt and Chambiko are also frequently consumed, taking up at least 15% of sales by volume. (Anonymous, 2004) These foodstuffs are distinguished as suitable vehicles because of their high consumption by children, high risk group with regard to iron deficiency (Abbasi and Azari, 2011)

Fortification with iron is technically more difficult than fortification with other nutrients because iron is a prooxidant and therefore promotes lipid oxidation (El-Kholy and others 2011). Therefore, the ideal iron compound for food fortification should be one that supplies high bioavailability of iron and does not affect the nutritional value or sensory properties of the food, should be stable during food processing and of low cost (El-Kholy and others 2011). It is therefore proposed that iron salts should be microencapsulated to reduce or prevent these negative effects. Microencapsulation is the technology of packaging solid, liquid and gaseous materials in small capsules that release their contents at controlled rates over prolonged period of time (Abbasi and Azari, 2011). The choice of iron compounds also depends on its solubility in gastric juice and on the presence of activators or inhibitors in the fortification food (Boccio and others 1997).

2.0 LITERATURE REVIEW

2.1. Milk Composition

Milk composition tends to differ from one species to another. There are also variations within the same species depending on season, feeds, lactation stage, intervals between milking, etc (O'Mahony 1988). The composition of milk varies according to the animal from which it comes, providing the correct rate of growth and development for the young of that species, thus for human infants, human milk is obviously more suitable than cow's milk. Indeed, the popular consensus among health care professionals is that ordinary cow's milk, goat's milk, condensed milk, dried milk, evaporated milk, or any other type of milk should not be given to a child under the age of one as a breast milk substitute. (O'Mahony, 1988). This is because of differences in the composition of milk that have been revealed by research over the last decade or so. While cow's milk and human milk contain a similar percentage of water, the relative amounts of carbohydrate, protein, fat, vitamins and minerals vary widely (O'Mahony, 1988)

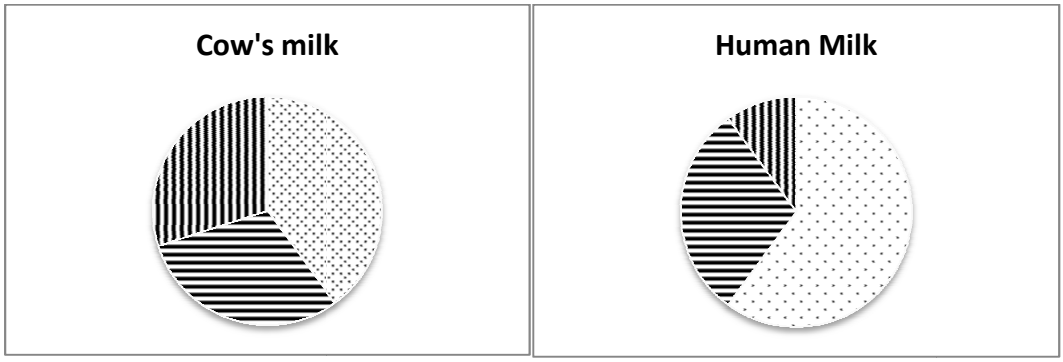

Figure 1: A comparison of the carbohydrate (dotted), protein (vertical) and fat (horizontal) components of whole cow's milk and human milk. Source: FSA, 2002.

Table 1: Comparison of the mineral and vitamin components of cow's milk and human milk. Source: FSA 2002

Micronutrients	**Cow's Milk (per 100g)**	**Human Milk (per 100g)**
Sodium (mg)	43	15
Potassium (mg)	156	58
Calcium (mg)	120	34
Phosphorus (mg)	94	15
Iron (mg)	**0.02**	**0.07**
Zinc (mg)	0.4	0.3
Iodine (µg)	30	7
Folate (µg)	9	5
Vitamin C (mg)	2	4

2.2. Heat Treatment of Milk

Heat treatment in the production of long life product is called sterilization. Product is exposed to high heat treatment that the microorganism and most of the enzymes are inactivated and the product can be kept several months under ambient temperature if sterilized in the container or packaged under aseptic conditions. Although, products are manufactured with UHT and aseptic processing in most countries, the market share of UHT milk varies considerably by countries (Chavan and others, 2011). Aseptic processing has great potential to increase dairy consumption in tropical countries as there is a low milk trend due to high temperatures and limited refrigerated distribution (Gedam and others, 2007). Production of long life milk can be done in two ways; a) in-

container sterilization with product in package being heated at about 115-120 ^{0}C for 20–30 minutes. This can be stored at ambient temperature, b) UHT treatment with the product heated at 135-150 ^{0}C for 4-15 seconds followed by aseptic packaging in packages protecting the product against light and atmospheric oxygen and can be stored at ambient temperature for long (Gedam and others 2007). However in most cases milk is prepared and processed in the following ways:

2.2.1. Pasteurized Milk

Pasteurization is heat process that is designed to kill pathogenic bacteria in milk and may cause spoilage of milk products. During pasteurization the milk is heated to a minimum of 72^{O}C (161℉) for a minimum of 15 seconds or 63 ^{O}C (145℉) for 30 minutes and packaged under clean and sanitary conditions.The shelf-life of pasteurized milk held under proper refrigeration, usually less than 7.2^{O}C (45℉) can range from 12 to 21 days post processing. Holding pasteurized milk at temperatures above 45℉ will shorten the shelf life significantly. The majority of U.S. fluid milk is pasteurized using a high temperature short time (HTST) continuous process of at least 161℉ (72℃) for 15 seconds to be legally pasteurized.

2.2.2 Ultrahigh Temperature (UHT) Pasteurized Milk

UHT is the sterilization of milk by heating it for a short time135-150 ^{0}C for 4-15 seconds. Advantages of UHT processing include extended shelf life, lower energy costs, and elimination of required refrigeration during storage and distribution if aseptically packaged. Milk maybe packaged either before or after sterilization. Desirable changes that take place during UHT processing of milk include destruction of microorganisms and inactivation of enzymes, while undesirable changes include browning reactions, loss of nutrients, sedimentation, fat separation, cooked flavor that

take place. Gelation of UHT milk during storage (age gelation) is a major factor limiting its shelf life (Gedam and others, 2007). Based on sensory work, Oupadissakoon (2007) reported butyric acid, sour aromatics, and lack of freshness as negative attributes with UHT milk.

2.3. Yogurt fortification

Yogurt is a dairy product produced by use of lactic acid bacteria (LAB) through the process of fermentation. Yogurt has gained widespread consumer acceptance. It is an excellent source of calcium and protein but contains very little iron (El-Kholy and others 2011). Therefore fortification of yogurt with iron would help solve this nutritional need. However, before any such fortification is undertaken, the effects of iron addition on microbial physiology during manufacture and shelf life of yogurt, oxidation of milk fat, and the effect of iron on the taste and acceptance of a fortified yogurt must be ascertained (Sharareh 1996). Yogurt fortified with 10, 20 and 40 mg of iron/kg showed no difference in counts of *Lactobacillus delbruckii* ssp. *Bulgaricus* and *Streptococcus thermophilus* after one day of storage from unfortified yogurt. But there was a significant difference after 30 days of storage (Gaucheron 2000) but no increase in chemical oxidation was detected. Many studies have been carried out on iron fortification of yogurt. It is well known that major off-flavor is associated with fortified dairy product due to catalyzed lipid oxidation by iron salts. Fresh yogurts fortified with iron from different iron compounds are affected differently (El-Kholy and others 2011).

2.4. Iron requirements by the body

Recommended daily intake of dietary iron for normal infants are 1 mg iron per kg per day and for children, male and female adolescents are 10, 12 and 15 mg per day respectively, and adult men and postmenopausal women require only 10 mg per day

(Martinez-Navarrete and others, 2001). While pregnant and lactating mothers require 27mg and 10mg per day respectively. At birth, most term infants have 75 mg of elemental iron per kilogram of body weight, found primarily as hemoglobin (75%), body storage (15%) and tissue protein (10%) (Oski,1982). Infants of mothers with poorly controlled diabetes and small-for-gestational-age infants have approximately 10% and 40% of normal storage iron, respectively, meaning that they may have less of a buffer for protection from postnatal iron deficiency (Petry and others, 1992; Georgieff and others, 1995). Because more than 80% of the iron of the newborn term infant is accreted during the third trimester of gestation, infants born before term must accrete more iron postnatally to catch up to their term counterparts during the first year. Thus, the requirements for preterm infants range from 2 mg/kg per day for infants with birth weights between 1500 and 2500 g (American Academy of Pediatrics, 1976) to 4 mg/kg per day for infants weighing less than 1500 g at birth (Siimes and Jarvenpaa, 1982).

2.5. Iron fortification compounds

The most common iron fortification compounds can be classified into three groups according to solubility: group 1, freely water-soluble iron (ferrous sulfate, ferrous gluconate, ferrous lactate); group 2, poorly water-soluble iron or soluble in diluted acids (ferrous fumarate, ferrous succinate); and group 3, water-insoluble iron or poorly soluble in diluted acids (ferric orthophosphate, ferric pyrophosphate, elemental iron (Boccio and others1997). Group 1 compounds can be completely dissolved and thus provide very high bioavailable iron. However, they have the disadvantage of freely interacting with the fortified food, which may alter its sensory properties. This can happen because iron catalyzes oxidative processes and thus provokes fat rancidity. This catalytic oxidation process may occur with other nutrients such as vitamins and amino acids, thus

decreasing the nutritional value of the food (Boccio and others 1997). Group 2 compounds have good solubility and thus good bioavailability. However, they have the disadvantage of being used only in solid dehydrated food because they do not dissolve in neutral liquids, in which they precipitate. In this last situation, the free fraction of iron interacts with the constitutive elements of the food to decrease its nutritional value and alter its sensory characteristics. Their advantage is that they cause far fewer negative effects on food sensory attributes than freely water-soluble compounds and they still readily enter the common iron pool during digestion. They have been suggested for use in infant cereals (Hurrell, 1997a) and chocolate drink powders. Group 3 compounds have a very low solubility. Thus, although they do not change the sensory properties or nutritional value of the food, they have the disadvantage of having very low bioavailability (Hurrell, 1997a; Boccio and others 1997).

Highly soluble compounds of iron like ferrous sulfate are desirable for food fortification but cannot be used in many food vehicles hence less absorbed forms of iron are commonly used in food fortification (Boccio and others 1997). Inorganic iron compounds added to whole cow's milk are poorly absorbed, because the compounds attach extensively to whey proteins, casein micelles, salts, and fat droplets, reducing its solubility. However, organic compounds of iron like ferrous lactate and ferrous gluconate absorb more easily to the water phase of milk than ferrous sulfate (Villalpando and others 2006). When the diet does not satisfy the body's iron requirements, nutritional deficiency of this element may occur. If this situation is not reversed, anemia may result therefore it is important to select fortification compounds and foods that will not diminish iron bioavailability to the body (Boccio and others 1997). This necessitates the careful selection of both the food product to be fortified and the

iron fortification compound to be added. Clearly, the iron compound must be first optimized with respect to relative bioavailability. Ferrous bisglycinate, ferrous lactate and ferrous sulfate microencapsulate have the following iron content ; 23%, 19% and 18% respectively. Ferrous bisglycinate (brown) and ferrous lactate (white) are found in powdery form while ferrous sulfate (granulated cream white) is sometimes microencapsulated with approximately 50% of vegetable fat. Microencapsulation is the technology of packaging solid, liquid and gaseous materials in small capsules that release their contents at controlled rates over prolonged period of time (Selaiman and Sara, 2011). This keeps the iron from coming into contact with food, reducing the chances of interactions that happens when conventional iron compounds are used (Gaucheron 2000).

However, if the food vehicle contains potent inhibitors of absorption, the added iron, like the native iron, will be poorly absorbed and will have little or no impact on the iron status of the consumer. The success of a food fortification program thus depends heavily on the absorbability of the added iron and its protection from major dietary absorption inhibitors. For example, phytate, polyphenols and a satisfactory iron status in an individual will diminish absorption, whereas vitamin C or low iron status will enhance absorption (Hurrell 1997b). World Health Organization (WHO) Guidelines 2005 recommend the following fortificants in order of preference: ferrous sulfate, ferrous fumarate, encapsulated ferrous sulphate, encapsulated ferrous fumarate, electrolytic iron (added at twice the level of ferrous sulphate), ferric pyrophosphate (added at twice the level of ferrous sulphate) and (ferric sodium ethylenediaminetetraacetic acid (NaFeEDTA). The recent efficacy studies which have followed the guidelines have

demonstrated that prolonged consumption of iron fortified foods greatly improves iron status of the consumers (Richard 1997b).

Table 2: Summary of iron salts used in food fortification.

classification	iron compound	advantage	disadvantage
1. Freely water soluble	ferrous sulfate ferrous gluconate ferrous lactate	high bioavailability	Freely interact with fortified food altering sensory properties because iron catalyzes oxidation.
2. Poorly water soluble or soluble in dilute acid	ferrous fumerate ferrous succinate	good solubility and bioavailability, cause far fewer organoleptic problems	suitable for solid dehydrated foods because they don't dissolve in neutral liquid, they precipitate
3. Water insoluble or poorly soluble in dilute acids	ferric orthophosphate ferric pyrophosphate elemental iron	Don't change sensory or nutritional value of the food.	low solubility and bioavailability

2.6. Effects of Iron deficiency

Iron deficiency is most common in poor countries of the developing world. About half of these iron deficient individuals develop iron deficiency anemia (IDA). Iron

deficiency is therefore a major health problem ranked by World Health Organization (WHO) as 7th out of 10 major preventable risks for diseases and death that together account for 40% of the 56 million deaths that occur world-wide each year (Hurrell 1997b). Iron fortification offers a more cost effective approach to providing additional iron to some segment of the population. Studies of 3 month old infants have shown that iron fortification of infant milk powder with ferrous sulfate significantly reduces anemia. After 15 months the prevalence of anemia in a group receiving non-fortified milk was 35% and 13% in infant consuming fortified milk. Iron status was significantly improved when ferrous bisglycinate was added to flavored milk in Saudi Arabia. Pilot fortification trials in developing countries have given promising results but there are no major success stories except for Chile due to lack of political commitment, insufficient funding, too little technical support from local or multinational industry, poor distribution network, or lack of nutrition education program for the consumers which are necessary for successful fortification program (Hurrell, 1997a). There are also other factors that affect the success of any fortification program. However if low bioavailability of food iron is the determinant of iron deficiency anemia in developing countries, increasing the supply of absorbable iron should decrease the prevalence of iron deficiency anemia (Hurrell and Sean, 1997).

Many food vehicles for iron fortification contain substances that inhibit iron absorption. Cereals contain phytic acids and polyphenols, milk contains calcium and caseins while chocolate drinks contain polyphenols (Hurrell 1997a). The presence of phytate, the major phosphorus storage compound in grain, has been associated with reduced mineral absorption due to the structure of phyate which has high density of negatively charged phosphate groups which form very stable complexes with mineral

ions causing non-availability for intestinal absorption (Walter and others, 2002). Polyphenols also forms insoluble complexes with iron thereby reducing its bioavailability to the body. The inhibitory effect of calcium on iron absorption was recognized many years ago. Different studies have been conducted but they often give conflicting results because several factors influence the interaction between calcium and iron absorption (Allen, 1996). These factors include molar ratio of calcium to iron, forms of calcium and iron and iron status of the subject. Calcium inhibits absorption of both heme and nonheme. It enters into the mucosal cells by different pathways and leave in the same form which implies that calcium inhibit the intracellular transport of iron (Hallberg 1992). It also competes for iron binding sites in mobilferrin, a protein in the duodenal mucosa that assists iron transport through the cell (Conrad and Umbreit 1993), and inhibits the release of iron from mucosal cells into circulation (Wienk and others 1996). In addition, many diets in developing countries to which fortified salt, sugar and other condiments are added are high in phytic acid and polyphenols especially cereal and legume foods. To ensure a level of absorption that is high enough to improve or maintain iron status, it is necessary to prevent the fortification iron from reacting with the absorption inhibitors that are inherently present in those foods. This can be accomplished by adding absorption enhancers. The most common enhancer is vitamin C. Alternatives would be bovine hemoglobin and NaFeEDTA where iron is in a protected form. Vitamin C can increase absorption of both native iron and fortification iron several folds due to both its reducing power and chelating action (Hurrel 1997). It can reduce ferric to ferrous iron and/or maintain ferrous iron in the ferrous state and so prevent or decrease the formation of insoluble complexes with absorption inhibitors or with hydroxide ion in the gut. In addition, it can form soluble complexes with iron at low pH that remains soluble

and absorbable at the more alkaline duodenal pH. Layrisse and others (1974) reported a sixfold increase in iron absorption (1.4% to 7.9%) by adult peasants in Venezuela who consumed 100 g maize containing 2.8 mg iron and 70 mg added vitamin C. Similarly, Cook and Monsen, (1976) reported that iron absorption in young men fed a liquid formula meal containing 4.1 mg iron increased from 0.8% to 7.1% as vitamin C was increased from 25 to 1000 mg. More recently, Siegenberg and others, (1991) reported that the effect of vitamin C on phytate and polyphenols was dose dependent and that as little as 30 mg vitamin C could completely overcome the effect of phytic acid (58 mg phytate phosphorus) in maize bran added to white bread, whereas greater than 50 mg vitamin C overcame the negative effect of meals containing greater than 100 mg polyphenols added as tannic acid.

In a milk-based infant formula fortified with 15 mg iron as ferrous sulfate per liter, iron absorption by infants was only 3% in the absence of vitamin C but increased to 5% with 100 mg vitamin C per liter and to 8% with 200 mg per Liter (Sketel and others, 1986) The relatively low iron bioavailability from milk products can be assumed to be due to the presence of two inhibitory factors, calcium (Hallberg and others, 1991) and the milk protein casein (Hurrell and others, 1989). In a series of fortification trials in Chile in which iron-fortified formulas were fed to infants, the improvement of iron status was only modest in the absence of vitamin C but improved considerably when it was added to formula (Walter and others, 1990). The widespread consumption of iron-fortified and vitamin C-fortified formulas by infants in the United States is regarded as the reason for the dramatic fall in the prevalence of anemia over the last 30 years (Yip and others, 1987).

2.7. Sensory effects

Iron catalyzes lipid oxidation that results in rancidity and development of off flavor. Fortification with $FeCl_3$, $FeSO_4$ or ferric/ferrous ammonium sulfate causes off flavor and high TBA numbers (Gaucheron 2000). However these effects can be reduced significantly. Ferric phosphate, ferric pyrophosphate or ferric ammonium citrate produced slight flavor change when added to milk followed by pasteurization. Different iron fortificants affect the food product differently. For example ferrous bisglycinate is advantageous over NaFeEDTA because it has GRAS status. However it readily promotes fat oxidation especially in cereals and undesirable color reactions occur in some foods. Ferrous bisglycinate is suitable for fortification of commercial food products such as liquid milk (Gaucheron 2000).

Many iron compounds are colored and cannot be used to fortify light-colored foods. In addition, the more soluble iron compounds often react with substances in foods, causing discoloration. It is reported that ferrous sulfate, ferrous lactate, ferrous gluconate, and ferric ammonium citrate, as well as the less soluble ferrous fumarate and ferric citrate, produce off-colors when added to a chocolate milk drink (Hurrell 1997b). Whole milk could also be considered as a vehicle for iron fortification, but because of the presence of calcium and casein, an absorption enhancer should be added to improve absorption. Unfortunately, it is difficult to add vitamin C to fluid milk and it has been reported to degrade rapidly to diketogluconic acid leading to changes in flavor (Hegenauer and others, 1979). Many soluble iron compounds rapidly produce off-flavors when added to milk, owing to the promotion of lipolytic rancidity, oxidative rancidity by the oxidation of free fatty acids, and the partial or complete loss of vitamins A, C, and ß-carotene (Cocodrilli and others, 1985).

Pentane, a product of fat oxidation, is the major hydrocarbon formed by the oxidative degradation of linoleic acid, and its formation correlates with the production of off-flavors. Ferrous sulfate and ferrous gluconate rapidly generated pentane and were judged unacceptable by a sensory panel after 4 to 6 weeks of storage. Ferric pyrophosphate and reduced elemental iron generated far less pentane and still had acceptable sensory characteristics after 7 weeks of storage (Hurrell 1997b).

Both ferrous sulfate and ferrous fumarate are available commercially in encapsulated form. The coating usually made from partially hydrogenated oil from soybean and cottonseed, or ethyl cellulose can prevent fat oxidation in infant formulas fortified with the easily oxidized long chain polyunsaturated fatty acids (Hurrell 1997b).This keeps the iron from coming into contact with food, reducing the chances of interactions that happens when conventional iron compounds are used (Gaucheron 2000).

2.8. Determination of Lipid Oxidation

Lipid oxidation is a complex process following free radical chain reactions. Lipids do oxidize by a radical chain mechanism through initiation, propagation, and termination stages. This reaction can be catalyzed my metals like iron and copper, light, heat, enzymes like lipoxygenase and other factors. The initiation stage is characterized by formation of highly reactive free radicals. The free radicals react with oxygen to form hydroperoxides (ROOH) and more free radicals. Hydroperoxides formed degrade and form aldehydes, secondary compounds of lipid oxidation. Some hydroperoxide branch and form more free radicals during propagation stage. In the final part of the reaction free radicals react with each other to form polymers, non-radical monomer products like

ketones, ethers, alkanes (Schaich 2005). This lipid auto-oxidative degradation results in products' change in food quality, e.g. aroma, flavor, texture and also the nutritive value.

Lipid oxidation can be detected using different ways. There is no single test available to measure all oxidative events at once, at all stages of oxidative process and applicable to all types of foods. Some of the most commonly used methods to determine lipid oxidation are Peroxide Values (PV) and Thiobarbituric Acid (TBA) tests.

2.8.1 Peroxide Value (PV) test.

Peroxide Value (PV) is one of the most commonly used methods to determine lipid oxidation due to its simplicity. It is based on the reduction of hydroperoxide group hence it is more sensitive in detecting early stages of oxidation. The amount of iodine liberated is proportional to the amount of peroxide present in food sample. Released iodine (I_2) is assessed by titration against a standardized solution of $Na_2S_2O_3$ using a starch indicator. The major disadvantage of this method is that it does not measure low PV due to difficulty in determining end point in a titration procedure.

2.8.2 Thiobarbituric Acid (TBA) test

Hydroperoxides are labile species which undergo changes and deterioration with the radicals. Their breakage causes secondary products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (MDA) (Fernandez and others 1997). MDA is a three-carbon dialdehyde with carbonyl groups at the C-1 and C-3 positions and is produced during lipid oxidation in oils containing linolenic or arachidonic acid.

MDA production is partially due to the secondary oxidation of primary carbonyl compounds e.g. 2-nonenal (Sinnhuber & Yu, 1977). TBA test is based on the MDA reaction with TBA reagent to obtain a red/pink pigment (chromagen), which results from

the condensation of two molecules of TBA with one molecule of MDA and the probable elimination of two molecules of water with absorbance at 530 nm. The major disadvantage of this method is that it measures later stages of oxidation as MDA is one of the byproducts of hydroperoxides breakdown. TBA can also be interfered with a number of compounds like amino acids and carbohydrates in the presence of iron (Fernandez and others 1997).

The reaction with TBA occurs by attack of the monoenolic form of MDA on the active methylene groups of TBA. The intensity of color is a measure of MDA concentration and has been organoleptically correlated with the rancidity. The mechanism of malonaldehyde liberation from linolenic acid has been suggested. However secondary products from linoleic acid also form a red pigment with the TBA test (Asakawa and Matsushita, 1979)

3.0. RATIONALE AND SIGNIFICANCE

Iron deficiency is one of the three major micronutrient deficiency disorders in Malawi. Others are Vitamin A deficiency (VAD) and Iodine deficiency (ID). The Government of Malawi (GoM) is currently implementing fortification of sugar and encouraging use of iodized salt to reduce VAD and ID respectively. There are reported cases of anemia in Malawi. Nationally prevalence of anemia in both children and mother are still high. Malawi Demograpic Health Survey (MDHS) 2010 reveals that 64 percent of children ages 6-59 months are anemic; 24 percent have mild anemia, 37 percent have moderate anemia, and 3 percent have severe anemia. Children in rural areas (65 percent) have a higher anemia prevalence compared with children in urban areas (53 percent). Among the districts, anemia prevalence ranges from a high of 77 percent in Chikhwawa to a low of 46 percent in Chiradzulu. It also indicates that 29 percent of women are anemic; 22 percent have mild anemia, 7 percent have moderate anemia, and 1 percent has severe anemia. Although there is moderate variation by urban-rural residence and region, differences vary greatly by district, ranging from a high of 51 percent having anemia in Mangochi to a low of 18 percent in Chitipa. This has been a serious problem for a long time as indicated by one study that was conducted more than 10 years earlier and showed a similar trend. It showed that between July 1997 and June 1998, the prevalence of all anemia (Hb < 11g/dl) in a population of urban women (n=4708) attending antenatal clinic at Queen Elizabeth Hospital in Blantyre was 57.1% and the prevalence of severe anemia (Hb< 7g/dl) was 3.6%. In a rural area (Namitambo Health Centre in Chiradzulu District) prevalence of anemia and severe anemia in pregnant women (n=2293) was 72% and 4%, respectively. A second study specifically measuring prevalence in an unselected group of women attending rural (Chiradzulu) and semi-

urban (Mangochi) antenatal clinics reported a prevalence of 58% (n=729) (Munasinghe and Van deBroek 2006). Other interventions like promotion of dietary diversification and iron supplementation, provision of iron tablets to pregnant women at antenatal clinic, has been used for a long time but prevalence of iron deficiency still remains high especially in young children, pregnant and lactating women (MDHS, 2004). Iron found in human milk is far more bioavailable, resulting in much lower rates of iron-deficiency anemia in children that are exclusively breastfed compared to children that rely on low-iron cow milk formula. Nevertheless, 6% to 20% of exclusively breastfed infants remain at risk for reduced iron stores. A higher rate (20%–30%) of iron deficiency has been reported in breastfed infants who were not exclusively breastfed (Anonymous, 1976) MDHS 2010 reported 53.2 % and 65.2% of urban and rural children respectively and 25.3% and 30.0% of urban and rural women respectively were anemic. This recent report shows that anemia is still a serious problem in Malawi despite all the interventions taking place.

Iron nutrition is particularly important during the weaning period, when the infant is growing rapidly and has a high demand for iron. Cereal porridges are common complementary foods during the weaning period and often provide much of the dietary iron intake because the iron contribution from human milk is low (Hurrell and others, 2003). Because of the high phytate content of cereal porridges, iron absorption of native iron and fortification iron may be very low (Hurrell and others, 2003; Lorenz and others, 2007)). One mole of phytic acid binds 6 moles ferric irons so that even relatively small quantities of residual phytate are still strongly inhibitory (Hurrell and others, 2003). Studies indicated that adding 10 mg/100 g phytic acid to bread rolls decreased iron absorption by 20% and that adding 20 mg/100 g decreased iron absorption by 40%

(Hurrell and others, 2003). Phytate: iron molar ratios greater than 0.15 are regarded as indicative of poor iron bioavailability.

Iron supplementation is the main strategy used in developing countries to combat IDA. Although supplementation is a reliable strategy to prevent anemia, the problems with poor compliance, low bioavailability, and the often poorly managed distribution systems have reduced the effectiveness of this approach (Bovell-Benjamin and Guinard 2003). The recommended daily allowance (RDA) for iron for a normal infant is 1 mg per kg per day and for children and male and female adolescents are 10, 12, 15 mg per day. A pregnant mother requires 27mg iron per day while breast feeding mother requires 10mg iron per day. In the United States of America use of iron fortified infant formulas from 1970s to the late 1980s was a success. During this period formulas were fortified with 10-12mg/L of iron. The rate of iron deficiency anemia dropped from 20% to less than 3% (Anonymous, 1989). Determination of acceptable range of iron concentration depends on standard used to assess iron sufficiency. In the US, iron concentration of iron fortified formulas range from 10mg/L to 12mg/L. the US Food and Drug Administration (FDA) recommends that iron fortification should not be less than 6.7mg/L. In Europe, infant formula tends to contain 4mg/L to 7mg/L (Anonymous, 1999). Malawi has experienced an increasing number of people consuming milk and milk products both in urban and rural areas with 80% consumed in the form of pasteurized liquid milk (Anonymous, 2004). However, the milk contains little iron with no impact on reducing iron deficiency. Therefore fortifying milk with iron will increase iron intake to the populace which may, in the long run, contribute to reducing the prevalence of iron deficiency disorder (IDD). Therefore the outcome of this study will help the GoM and its development partners to strategize ways to reduce iron deficiency through fortification of

milk. Its seeks to increase the iron content of iron fortified pasteurized liquid milk and yogurt by 20-30% per 200mL serving which has a potential to increase dietary iron intake.

There have been extensive studies on iron fortification of both fermented and unfermented milk and milk products. Different iron compounds, their advantages and disadvantages have been clearly documented. Most of the iron fortification that have been done focused much on solid and semi-solid milk products. Little had been done on fortification of pasteurized liquid milk and yogurt. This research seeks to establish how the quality of pasteurized liquid milk and yogurt fortified with ferrous bisglycinate, ferrous lactate and ferrous sulfate microencapsulate will change over a given storage time.

4.0. OBJECTIVES

This study hypothesizes that fortification of pasteurized milk and yogurt will enhance milk and yogurt iron content and result in increased dietary iron intake and consequently reduce prevalence of iron deficiency.

4.1. Long term objective:

To increase dietary iron intake through increased consumption of iron fortified milk and yogurt.

4.2. Specific objectives:

1. To determine effects of microencapsulated ferrous sulfate, ferrous bisglycinate, and ferrous lactate on yogurt culture growth.
2. To evaluate the sensory quality and storage stability of iron fortified pasteurized milk and yogurt.

5.0. MATERIALS AND METHOD

5.1. Materials:

Whole cow's milk (Michigan Milk Producers Association, Ovid, MI, USA) for yogurt production was provided by Michigan State University Dairy Plant, East Lansing, MI. Milk for production of pasteurized milk was purchased from Bunda College Student Farm, Lilongwe, MW. Ferrous bisglycinate, ferrous lactate and microencapsulated ferrous sulfate with 50% vegetable fats were provided by Dr. Paul Lohmann Inc, US. Commercial Hansen's DVS yogurt culture Yo-fast containing *Lactobacillus delbruckii* ssp *Bulgaricus* and *Streptococcus thermophilus* was purchased from Chris Hansen Milwaukee, WI. MRS agar (Difco, USA) was obtained from Microbiology Laboratory at Michigan State University, MI USA. Chloroform, glacial acetic acid, Thiobarbituric acid (TBA), starch, sodium hydroxide, toluene, sodium thiosulfate (Sigma-Aldrich, USA) were also used. However some of chloroform and glacial acetic acid used for milk analysis were purchased from Lab Enterprises, Blantyre, MW. Additional ingredients that were used to manufacture yogurt were sucrose from Michigan Sugar Company (Saginaw, MI, USA), stabilizer (Continental Custom Ingredients, Chicago, IL, USA), non-fat dry milk solids (Michigan Milk Producers Association, Ovid, MI, USA) and strawberry puree (Kraus & Co., Walled Lake, MI, USA)

5.2. Starter culture growth and activity

Reconstituted skim milk (12% w/v) was fortified with ferrous bisglycinate (63mg/kg), ferrous lactate (79mg/kg) and sulfate dried microencapsulate (83mg/kg) (Dr Paul Lohmann Inc, US). The fortification levels were different because ferrous bisglycinate, ferrous lactate and ferrous sulfate microencapsulate have different iron content so there was a need to harmonize iron in the final products. Ferrous

bisglycinate has approximately 23% iron. Ferrous lactate and ferrous sulfate microencapsulate have approximately 19% and 18% iron respectively. In order to obtain a product that has approximately 15mg/kg of iron, which can contribute 20-30% towards RDA for different categories of people when 200ml of fortified product is consumed, there was a need to compute how much of each iron salt should be used for fortification. Using same levels of fortification for all iron salts would mean that more iron would be present in one sample and less iron in the other sample. This would undoubtedly affect both the sensory and chemical analysis results. Iron compound was omitted from control treatment. The reconstituted milk was then sterilized at 121 ^{0}C for 5 minutes. Each flask was cooled to 35 ^{0}C and inoculated with 1% (wt/wt) commercial Hansen's yogurt culture Yo-fast 10, containing *Lactobacillus delbruckii* ssp b*ulgaricus* and *Streptococus thermophilus*, and incubated at 35 ^{0}C for 6 hours. Growth of lactic acid bacteria was determined by sampling at 1.5 hour interval and plating on de Man Rogosa, and Sharpe (MRS) agar (Difco). Plates were incubated at 35^{0}C, 48 hours under aerobic conditions. After incubation the colonies formed were counted and results expressed as CFU/ml.

5.3. Skim milk and yogurt titratable acidity (TA)

Prior to determining titratable acidity (TA) the samples were thoroughly mixed. Nine grams of each sample was placed in Erlenmeyer flask to which 4 drops of phenolphthalein indicator was added. The mixture was titrated with 0.1N NaOH to the first permanent shade of pink.

Percent TA was calculated as follows:

%TA = (ml of NaOH × N of NaOH × meq.wt lactic acid)/ wt of sample

Where ml = milliliter of sodium hydroxide used, meq = milliequivalent weight in which 1ml of 0.1N NaOH =0.009008g $C_3H_6O_5$, wt = weight of sample.

5.4. Yogurt manufacture

Four batches of yogurt were manufactured at Michigan State University Dairy Plant using the following formulation; 80.5% whole milk, 4.0% nonfat dry milk (NDM) (Michigan Milk Producers Association, Ovid, MI, USA), 0.5% stabilizer (Continental Custom Ingredients, Chicago, IL, USA) and 5.0% sucrose (Michigan Sugar Company, Saginaw, MI, USA). The whole milk was fortified with ferrous bisglycinate (63 mg/kg), ferrous sulfate microencapsulate (83 mg/kg) or ferrous lactate (79 mg/kg). Control yogurt had no iron salts added. The yogurt mix was warmed to 60^{o}C, homogenized dual stage 2000, 500 psi and pasteurized at 85^{0}C for 30 min then cooled to 43 ^{0}C. It was inoculated with 1% (wt/wt) commercial Hansen's yogurt culture Yo-fast 10, containing *Lactobacillus delbruckii* ssp b*ulgaricus* and *Streptococus thermophilus* and incubated at 43 ^{0}C until pH 4.6 was reached. Finally, 10% strawberry puree (Kraus & Co., Walled Lake, MI, USA) was added as flavorant.

Yogurt for each treatment was divided and stored into two separate containers. One part was used for sensory analysis while the other was used for chemical analysis of lipid oxidation. Yogurt for sensory analysis was separated and stored at refrigeration temperature for one week before sensory evaluation was done in order to give time for the iron to fully interact with milk component. It was feared that if the sensory analysis

was done immediately after manufacturing yogurt the sensory results would not reflect the actual sensory attributes of stored yogurt as commercial yogurt spend some time in storage or on shelves before it is bought and consumed by consumers. Yogurt for chemical analysis was also stored at refrigeration temperature and the analysis was done after every 5 days till 35 days storage period was reached. This period was chosen because it approximates the shelf life of yogurt under refrigeration.

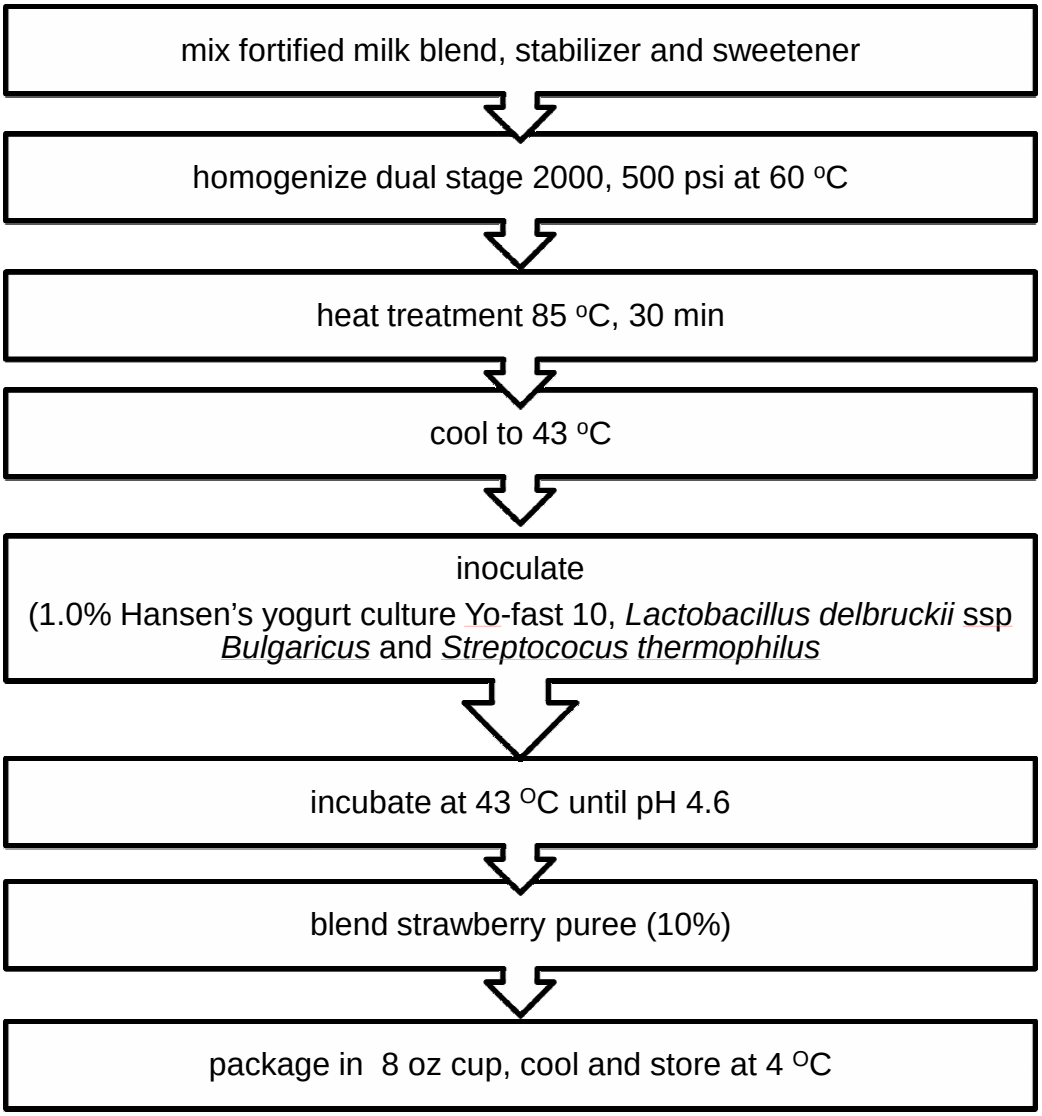

Figure 2: Flow diagram showing stages during processing of yogurt.

5.5. Processing of pasteurized milk

Whole milk (Bunda College Student Farm, Lilongwe, MW) was fortified with ferrous bisglycinate (63 mg/kg), ferrous lactate (79 mg/kg) and ferrous sulfate (83 mg/kg). No iron salts was added to control treatment. Batch pasteurization was used to pasteurize milk from each treatment separately where milk was heated in a container to $63^{O}C$ and held at this temperature for 30 minutes. Thermometer was used to detect temperature changes. While milk was being pasteurized bottles were being heated at temperature around $100^{O}C$ to reduce recontamination of pasteurized milk. When the pasteurization temperature and time was reached, the milk was then cooled and packed in bottles and stored under refrigeration temperature. Ten bottles were prepared for each treatment; therefore 40 bottles were prepared for all the four treatments. At each testing interval a single bottle from each treatment was used and the remaining contents were discarded.

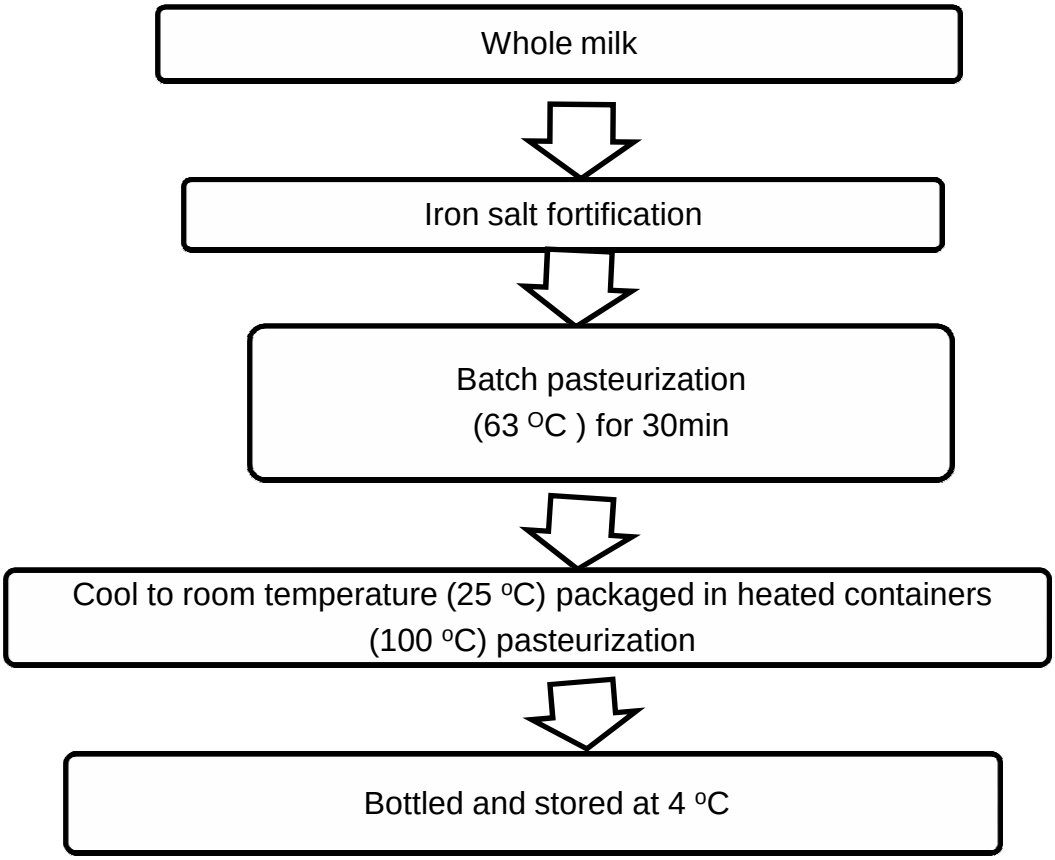

Figure 3: Flow diagram showing stages during processing of pasteurized milk

5.5. Determination of pH

The pH meter was used to measure the pH of the samples. The tip of the pH meter sits in a buffer which is standardized to a neutral pH 7. Small samples of yogurt were taken from each treatment for measurements. The electrode was inserted in yogurt for few minutes and was read when it stabilized. This was repeated for each sample and cleaning the electrode with distilled water after using on each sample and finally the tip for the electrode was dipped in a buffer solution.

5.6. Peroxide value determination

Yogurt was diluted with distilled water to 20% solution. Five grams of 20% yogurt solution was put in 250 ml Erlenmeyer flask to which 30 ml of acetic /chloroform solution was added and swirled in order to dissolve the sample. 0.5 ml of saturated KI was added by pipette and the solution was allowed to stand while occasionally shaken for 1 minute. Thirty mL of distilled water and 1 mL starch indicator (1% starch) was added. 0.01 N sodium thiosulphate solution was titrated immediately until the brown (or yellow) color disappeared representing end point. This procedure was repeated for pasteurized liquid milk where 5g sample was used in place of 20% yogurt. The determination on the blank was done on 30 mL acetic acid/chloroform solution + 0.5 mL KI solution +30 mL distilled water + 1mL starch indicator using 0.001N sodium thiosulfate as a titrant. The peroxide values were expressed as milliequivalents of peroxide per 1000g sample using.

PV was calculated as follows:

$$PV = [(S-B)\times N\times 1000] / \text{weight of samples in grams}$$

where S = mL of thiosulfate required for titration, B= mL of thiosulphate required for the blank and N= normality of thiosulphate solution.

5.7. Thiobarbituric acid (TBA) value

One gram yogurt was diluted with 100ml distilled water then 3.0 g diluted yogurt was put in Erlenmeyer flask (125-300ml) separately, and 10 ml toluene was added to samples separately. Then 10 ml of TBA reagent which was prepared by dissolving 1g

of TBA powder in 75ml of 0.1N NaOH and diluted to 100ml with distilled water according to Sinnhuber and Yu, (1977), was pipetted (using a pipette bulb) into the flask. The flasks were swirled and shake frequently for four minutes. The entire content was transferred into 250 ml separatory funnel. The layers was allowed to separate and the lower layer was collected in a screw cap test tube (18 ×145 mm or 25 ×200mm). The tube was heated in boiling water for 30 minute. The test tube was cooled under running water and transferred the portion of the sample to the Spectronic 20 cuvette. The absorbance of the sample at 530nm was read using distilled water as the blank. Average absorbance ×100 as the TBA values is reported with the result normalized per g of sample.

TBA was calculated as follows:

TBA = Absorbance × 100)/g of sample

5.8. Sensory evaluation

5.8.1. Yogurt

Consumer acceptance sensory test was carried out in which four samples of yogurt fortified with ferrous bisglycinate (63mg/kg), ferrous lactate (79mg/kg), ferrous sulfate microencapsulated (83 mg/kg) and a control treatment were presented to 100 untrained panelists upon approval by University Committee on Research Involving Human Subject (UCRIHS). The panelists were recruited by posting flyers around Michigan State University (MSU) campus and by sending emails containing the flyer to different departments at MSU. Ninety eight panelists consisting mainly graduate students, undergraduate students and faculty at MSU. Sensory evaluation was

conducted in individual booths in the sensory laboratory in the Department of Food and Human Nutrition at MSU. Upon arrival at the sensory laboratory each subject read an explanation of the study and gave their informed consent. Yogurt samples were put into 2 oz plastic cups labeled with randomly selected three digit numbers and refrigerated till time of evaluation. The samples were presented in a randomized manner across subjects to ensure that the order did not introduce bias into the results. Subjects were asked to taste and evaluate all four samples and indicated their degree of liking on a nine point hedonic scale from 1=dislike extremely to 9=like extremely and 5=neither like nor dislike. The panelist evaluated each sample for likeability of the appearance, body texture, flavor and overall acceptance. Panelists were provided with purified water at room temperature for rinsing between samples.

5.8.2. Pasteurized Milk

Pasteurized milk was manufactured at Bunda College Food Laboratory in the Department of Home Economics and Human Nutrition. The milk was stored for 2 days at refrigeration temperature before consumer acceptance sensory test was done. The milk was evaluated using consumer panel. The panelists were recruited by flyer pasted on different notice boards around Bunda College campus. One hundred panelists consisting primarily of undergraduate students, graduate students and staff members participated in the sensory evaluation. Upon arrival at the food laboratory each subject read an explanation of the study and gave their informed consent. There was not a separate approval for this evaluation because the UCRIHS approval was for both yogurt and pasteurized milk. Fifty millimeters of each sample were put in a hundred millimeters cup labeled with randomly selected three digit numbers. The order of presentation was randomized. Subjects were asked to taste all the four samples and indicate their degree

of liking on a nine point hedonic scale from 1=dislike extremely to 9= like extremely and 5=neither like nor dislike. The panelists evaluated the samples based on appearance, mouth feel, taste, flavor and overall acceptance. The panelists were provided with water for rinsing between samples

5.9 Statistical analysis

Statistical parameters that were generated were mean, standard deviation and one way analysis of variance (ANOVA) for sensory, TA, PV and TBA data. Statistical software IBM SPSS Statistics 20 was used for statistical analysis of the results. For yogurt sensory analysis SIMS 2000 for windows was used to generate means and statistical differences. Least Significant Difference (LSD) at $p<0.05$ was used to determine significant differences between means from all the treatments. All experiments were replicated two times except for starter culture growth and activity experiment which was done in one replicate with two analyses.

6.0 RESULTS AND DISCUSSIONS

6.1. Effect of iron salts on starter culture growth and activity

Figure 4 shows that the growth of commercial Hansen's yogurt culture Yo-fast 10 containing *Lactobacillus delbruckii* ssp *Bulgalicus* and *Streptococus thermophilus* were different from each other amongst treatments. Though at time 0 hr the bacterial count was almost the same for all treatments there was steady increase in counts as time progressed. After 6 hours both ferrous bisglycinate and ferrous sulfate had the highest bacterial population indicating that they supported bacterial growth the best. None of the iron salts were inhibitory to the growth of the yogurt culture.

However, the growth of yogurt starter culture was affected differently. In the control treatment the growth was slower but after three hours the growth rate increased significantly which shows that there was rapid increase in number of bacteria. Growth of yogurt starter culture in the presence of ferrous lactate was rapid in the first one and a half hours and later the graph flattens before rapid increase after three hours. This shows that in the first 1.5 hours ferrous lactate enhanced bacterial growth more than any other treatment. At the same time this behavior as compared to a control treatment shows that ferrous lactate was not as supportive of yogurt starter culture growth as the rest of the treatments. There was more growth in ferrous bisglycinate which is indicative of a more supportive effect of ferrous bisglycinate. The general picture from **Figure 4** is that the three iron salts, ferrous bisglycinate, ferrous lactate and ferrous sulfate microencapsulate were more favorable to the growth of yogurt culture growth as shown by having shortest lag phase.

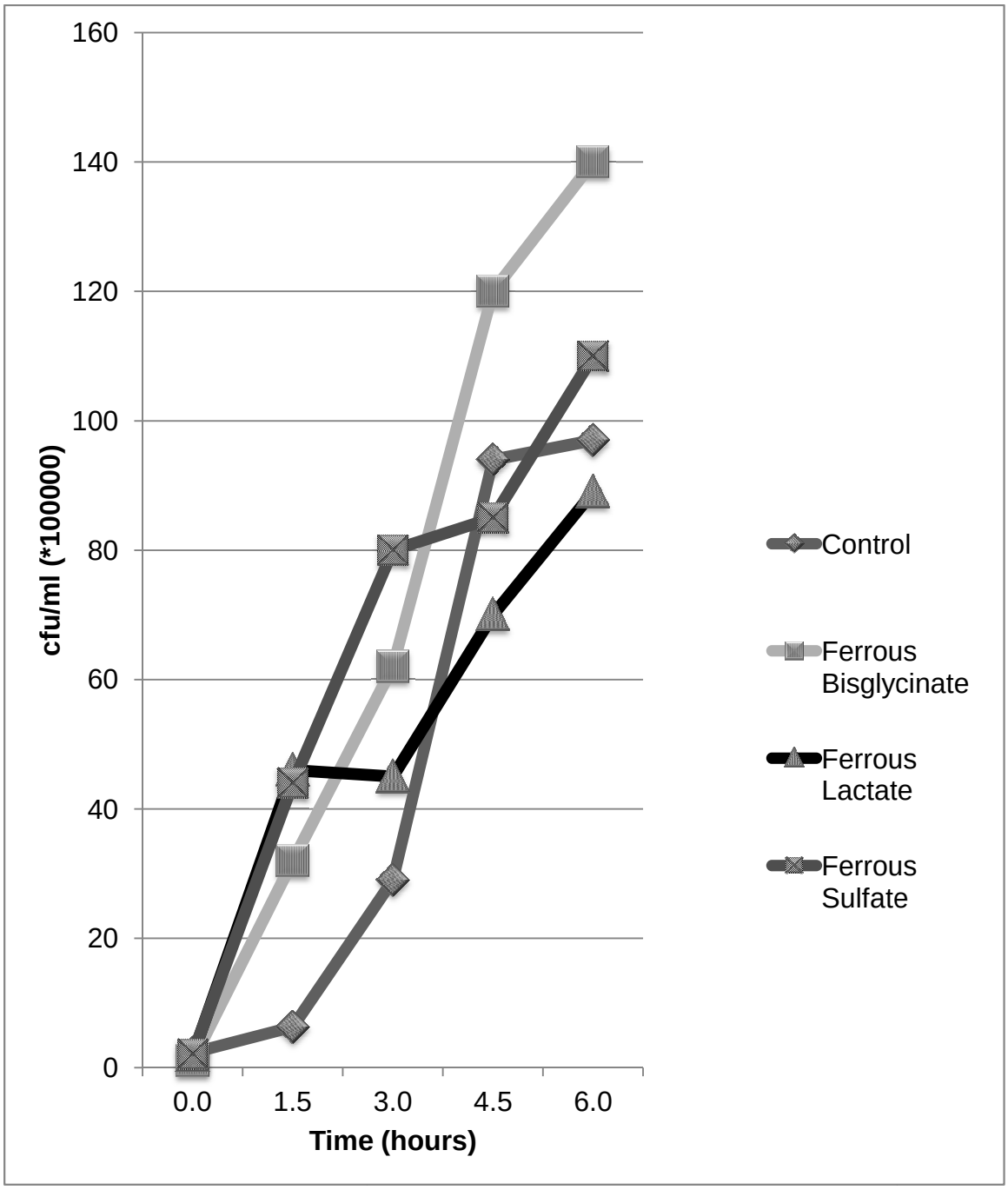

Figure 4: Effects of iron salts on growth and activities of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococuss thermophilus* in iron fortified reconstituted skim milk incubated at 35 ^{O}C for 6 hours

The control treatment had the longest lag phase. This shows that fortifying skim milk with iron enhanced bacterial growth to a larger extent in ferrous bisglycinate and ferrous sulfate microencapsulated than in ferrous lactate enriched yogurt. This contradicts what Hekmat and MacMahon (1997) reported. They reported that counts of *L. delbrueckii* ssp *bulgaricus* and *S. thermophilus* after one day of storage in iron fortified skim yogurt were not significantly different from counts in unfortified yogurt. It was therefore concluded that starter culture growth was independent of whether or not the milk had been fortified with iron. However this conclusion was based on casein-chelated iron and whey protein-chelated iron compounds which are different from those used in the present study.

6.2. Titratable acidity and pH for skim milk

Results presented in Figure 6 show that there was a steady increase in TA in all the treatments as time progressed though TA increases were not significantly different among treatments at each testing interval. This means that there was increase in microbial activities and microbial growth which resulted in more lactic acid being produced by lactic acid bacteria. This is especially true of *Lactobacillus delbruckii* ssp *bulgalicus* and *Streptococus thermophilus.* Hatkins and Nannen (1993) observed that during growth and fermentation the pH of the medium decreases due to accumulation of organic acid primarily lactic acid. This therefore increased TA in the sample.

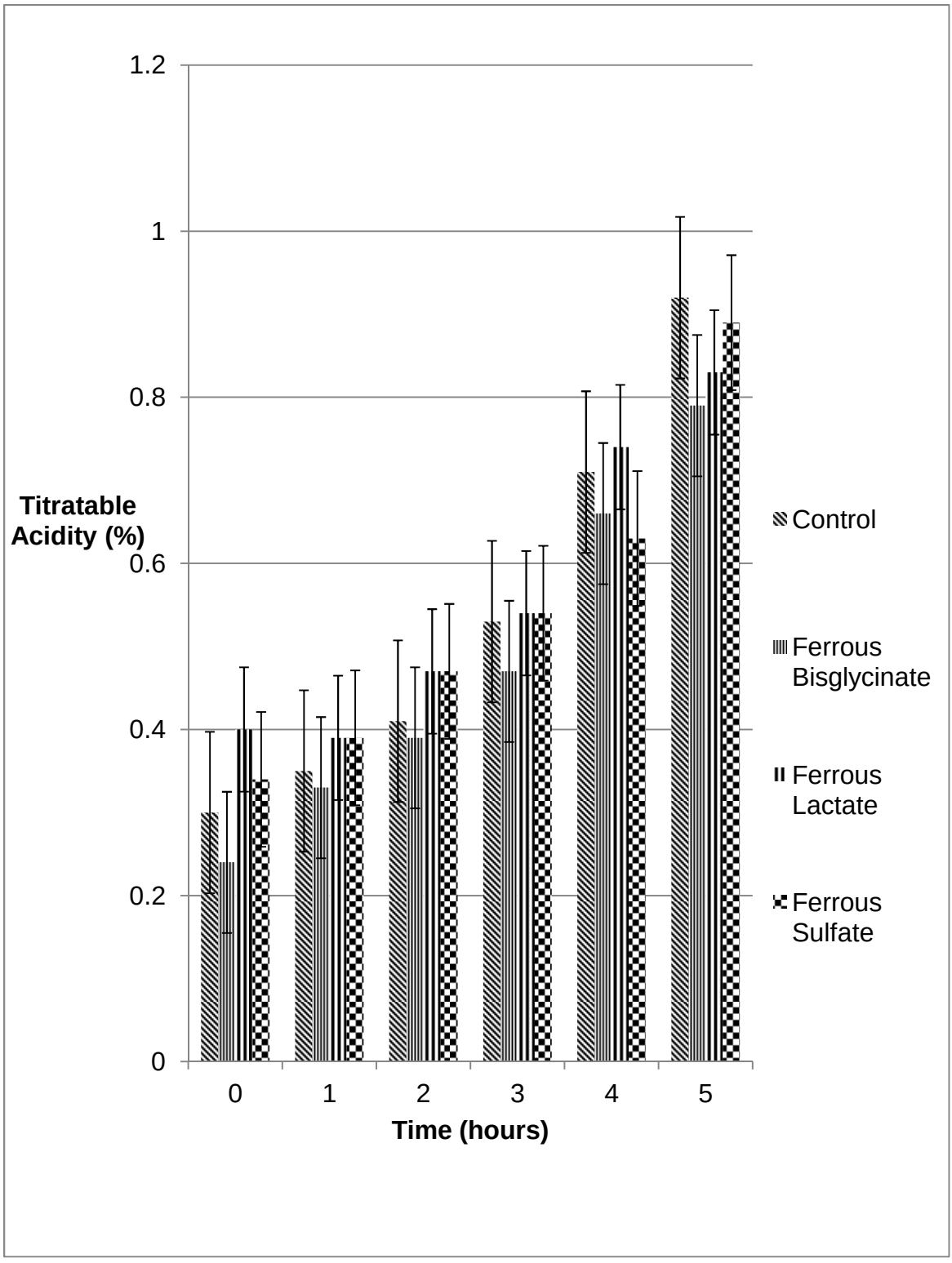

Figure 5: Changes of Titratable Acidity in skim milk fortified with different iron salts and incubated at 35 ^{O}C for 6 hours

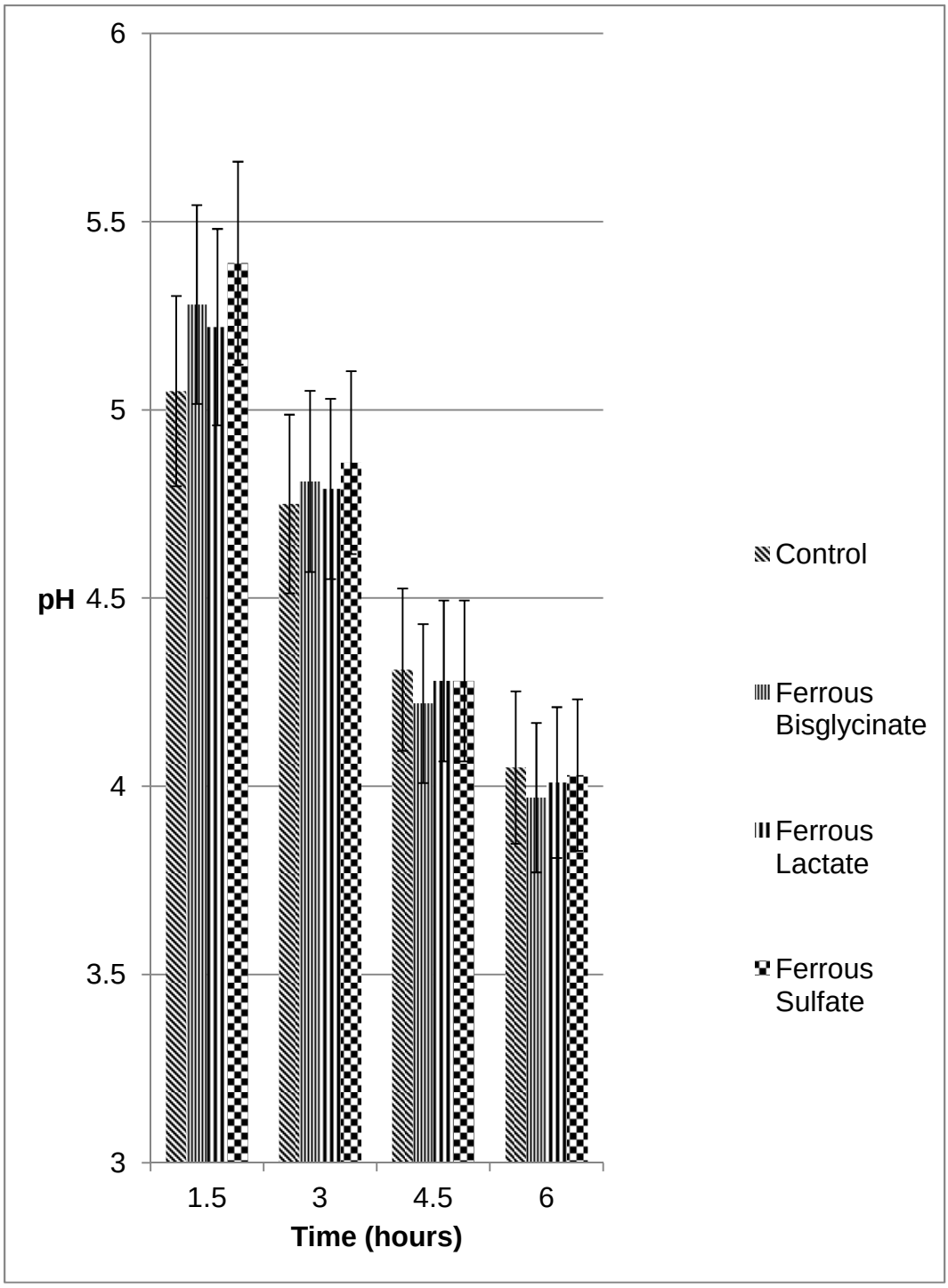

Figure 6: Changes of pH in skim milk fortified with different iron salts and incubated at 35 ^{O}C for 6 hours.

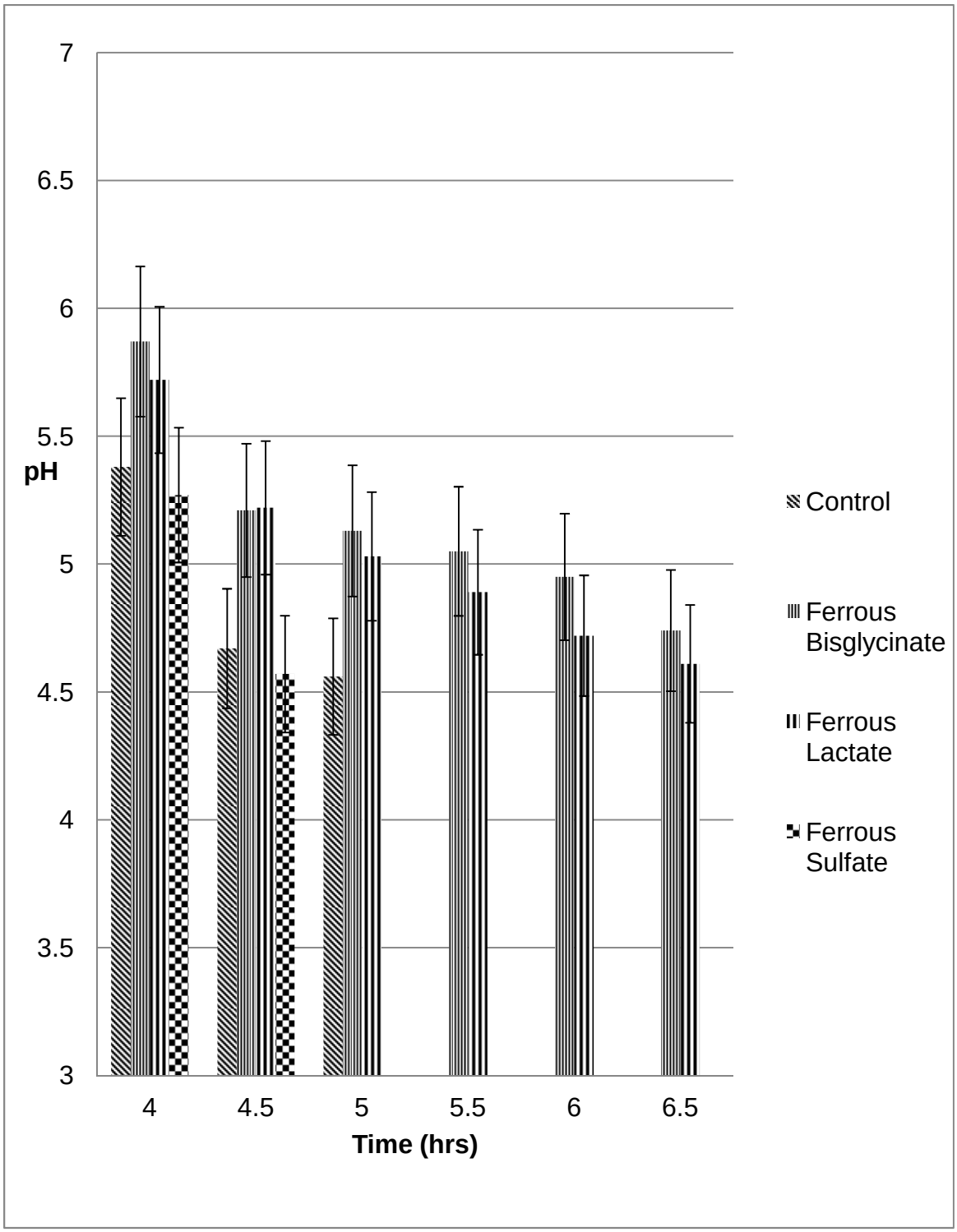

Figure 7: Changes in pH for yogurt fortified with different iron salts during incubation at 43 ^{O}C till pH 4.6 was reached

When yogurt batches were put in the incubator there were noticeable differences in reaching the desirable pH of 4.6 which is the isoelectric point of caseins. There were significant differences in decrease of pH amongst treatments. **Figure 7** shows that the treatment fortified with ferrous sulfate was the first to reach the desirable pH, after 4.5 hours. This agrees well with the results in **Figure 4** where ferrous sulfate microencapsulates fortified yogurt shows rapid microbial growth and therefore rapid production of lactic acid by LAB which lowers the pH. This shows that ferrous sulfate microencapsulate was more supportive of yogurt culture growth. Ferrous lactate was the last to reach pH 4.6 due to low lactic production. Likewise in **Figure 4** ferrous lactate showed less supportiveness to yogurt culture growth than any other iron salts. Control reached pH 4.6 after 5 hours. Ferrous bisglycinate and ferrous lactate took more than 6.5 hours to reach desirable pH. These differences in reaching the desirable pH were due to the difference in production of lactic acid by Hansen's yogurt culture Yo-fast 10, *Lactobacillus delbruckii* ssp b*ulgaricus* and *Streptococus thermophilus* in the presence of different iron salts. This disagrees with what Hekmet and MacMahon (1997) and El-nagar and Shenana (1998) found. They reported that iron fortification had no effect on the incubation time required for yogurt mixes. Hekmet and MacMahon (1997) used casein-chelated iron and whey protein-chalated iron to fortify yogurt and determined the effects of iron on bacterial and sensory qualities of yogurt. It was found that all batches reached pH 4.3 after 5 hours. The pH values of control and fortified samples reached pH 4.2 after 1 day. In this present study ferrous bisglycinate, ferrous lactate and ferrous sulfate microencapsulated were used. All treatments reached pH 4.2 more than 5 hours which also contrast their findings. Unlike Hekmet and MacMahon (1997) study whose

desired pH was 4.3 the present study's desired pH was the isoelectric point of caseins (pH 4.6). Yogurt fortified with ferrous bisglycinate and ferrous lactate took a longer time to reach a desirable pH due to slow production of lactic acid by lactic acid bacteria while the control treatment and yogurt fortified with ferrous sulfate reached pH 4.6 earlier. Based on these results the incubation period were different for each treatment which clearly indicates that iron compounds affected the time yogurt reached desired pH during incubation. Another observation on the results in this present study revealed that pH change was more rapid in skim milk than in yogurt when incubated for the same length of time. For example pH after 4.5 hours for iron fortified skim milk are as follows; control 4.31, ferrous bisglycinate 4.22, ferrous lactate 4.28 and ferrous sulfate microencapsulated 4.28. After the same time in yogurt the pHs were; control 4.67, ferrous bisglycinate 5.21, ferrous lactate 5.22 and ferrous sulfate microencapsulated 4.57. The reason for this behavior was well explained by King and others (1959) who reported that all the added iron is associated with skim milk. When Iron is added it binds to the colloidal phase of caseins at about 80-90% and hence reduces the pH of skim milk upon addition. This decrease in pH is related to the acidities of iron solutions and to exchanges between iron ions and micellar bound H^+ (Gaucheron 2000). Therefore the combined effect of exchanges between iron ion and micellar bound H^+ and LAB activities resulted in rapid decrease in pH for skim milk.

Results in **Figure 8** shows that there was a general trend in all the treatments in regard to the TA in stored yogurt. There was an increase in lactic acid expressed as %TA up to first 15 days. Thereafter there was a decrease in TA. During growth and fermentation, the pH of the medium decreases because of the accumulation of organic

acids, primarily lactic acid **(see Figure 9**). Growth of lactic acid bacteria continues as long as there are enough growth nutrients, no toxic or inhibiting compounds and the hydrogen ion concentration is maintained above the level that specific strain can tolerate (Hutkins and Nannen, 1993). The lowest pH was at day 15 which was due to high lactic acid accumulation (high TA). This high lactic acid became the growth limiting factor of bacteria hence bacterial growth was retarded and eventually the production of lactic acid decreased. Consequently the pH started increasing at day 20, see **Figure 9.**

Not only do most lactic acid bacteria grow more slowly at low pH, but acid damage and loss of cell viability may also occur in cells held at low pH. Moreover, inhibition of the starter culture by lactic acid and low pH acts to prevent, in part, over-acidification of yogurt (Hutkins and Nannen, 1993). This decrease in pH in fermented food is advantageous in the sense that organic acids produced during yogurt fermentation can potentially enhance iron and zinc absorption via the formation of soluble ligands (Gibson and others, 2006). Especially in weaning foods, such physicochemical properties of fermented foods is highly desirable, for the fact that children are most of the time highly vulnerable for food pathogens due to their physiological conditions (Jay, 2000; Wambugu and others, 2002). According to Elyas and others (2002), the increased acidity and low pH as a result of fermentation enhances the keeping quality of fermented foods, by inhibiting microbial growth and also contributing to the flavor of the processed food.

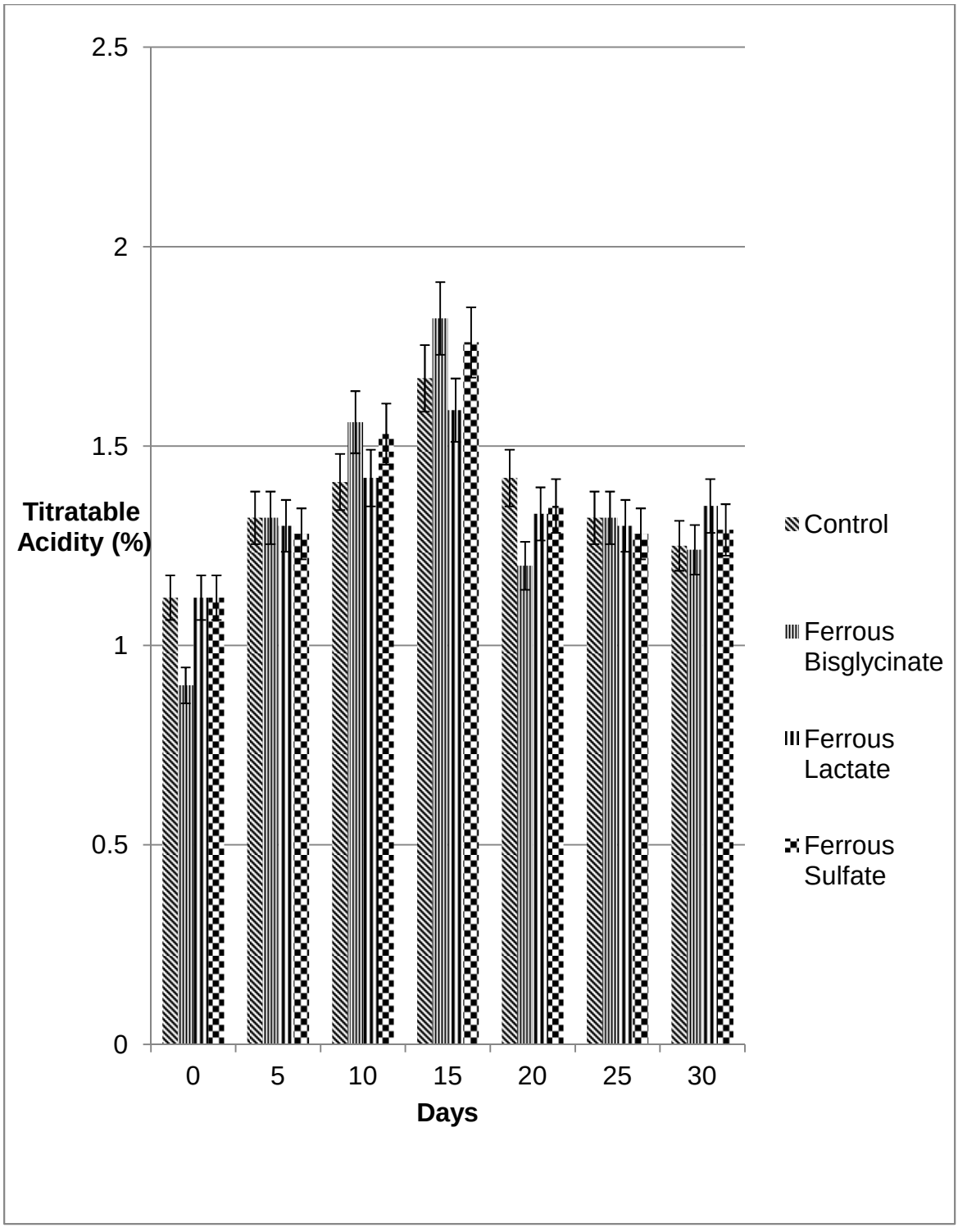

Figure 8: Changes in Titratable Acidity for yogurt fortified with different iron salts and stored at 4 ^{O}c for 30 days

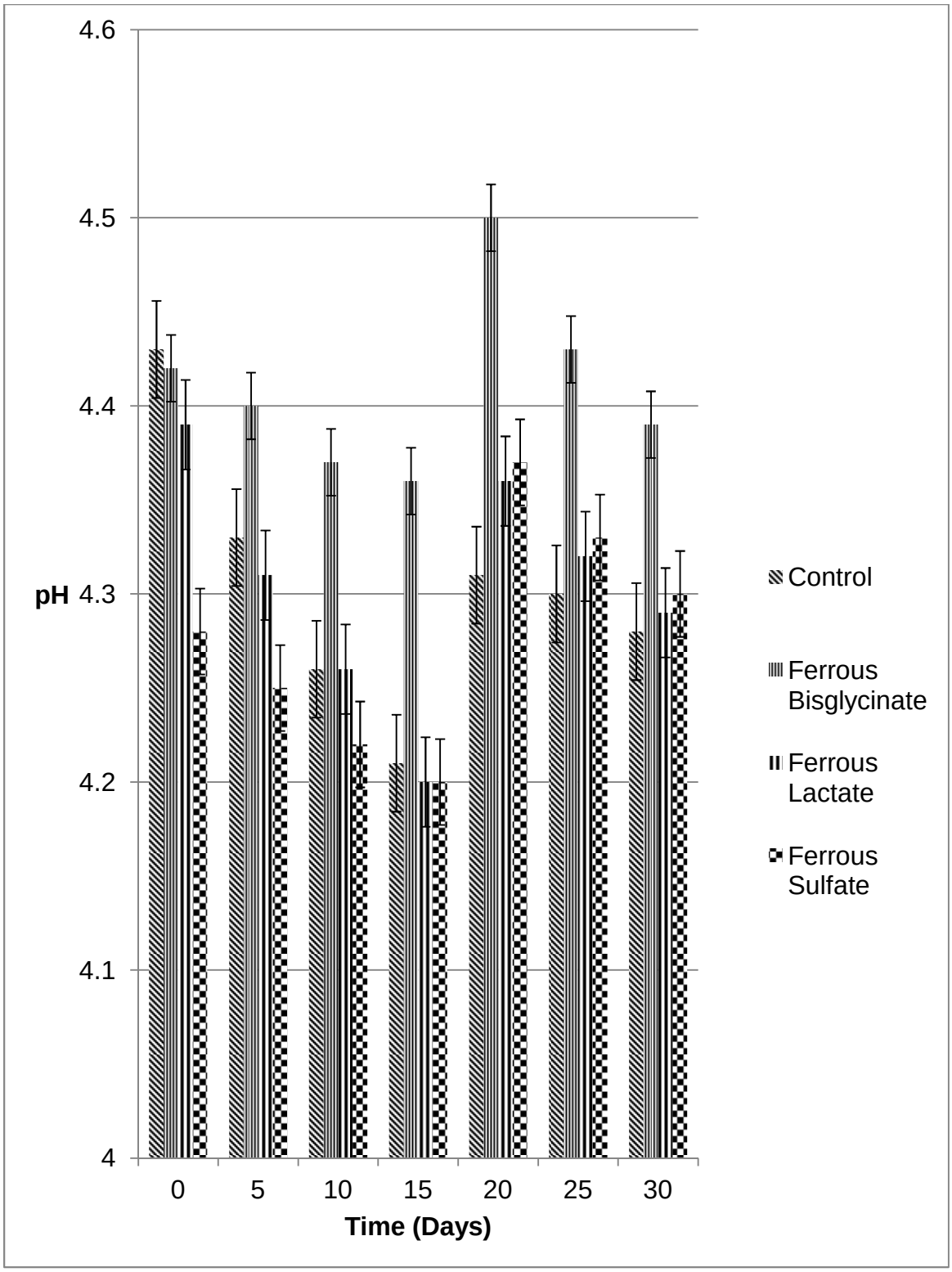

Figure 9: Effects of different iron salts on pH for yogurt stored at 4^{O}C for 30 days

The pH was lowest in yogurt fortified with ferrous sulfate microencapsulated. This was due to high lactic acid produced indicative of highest culture activity. This disagrees with what El-Kholy and others (2011) found. They reported that fortification of yogurt with different iron salts has no effect on the total lactic acid bacteria when fresh and during cold storage. The differences in pH for different iron salts suggest that bacterial activities in stored yogurt were different in each of the treatments. In all cases the pH for yogurt fortified with ferrous bisglycinate was highest except on day zero. The highest pH meant that yogurt culture growth was still more active as low pH was not yet a limiting factor. No wonder TA values were also high **(see Figure 8**).

6.3. Sensory Analysis

6.3.1. Sensory Evaluation of Yogurt.

Data in Table **2** shows that there were significant differences in sensory attributes between samples. There was no significant differences between the control yogurt and yogurt fortified with ferrous sulfate microencapsulated in all the attributes studied ($p<0.05$, $n =98$). This shows that fortifying yogurt with ferrous sulfate microencapsulate does not affect the consumer acceptability of the sensory properties of yogurt. This is a welcome idea considering that the average relative bioavailability of ferrous sulfate when used as a fortificant is 100% (Hurrell 1997). Both ferrous bisglycinate and ferrous lactate were significantly lower than control treatment in appearance, body texture, flavor and overall acceptance ($p<0.05$, $n=98$). This means that they both altered consumer acceptability of all sensory properties of yogurt evaluated. . However the effects on yogurt were not significantly different from each other. The difference in

appearance could be overcome by using flavor or coloring like chocolate that will mask any change in color due to iron salt. Lack of significant differences between control yogurt and yogurt fortified with ferrous sulfate is due to microencapsulation of ferrous sulfate. Microencapsulation keeps the iron from coming in contact with the food vehicle thereby preventing undesirable interaction that happen when conventional ferrous sulfate is used (Boccio and others, 1997).

Table 3: Sensory mean scores of yogurt fortified with different iron salt stored at 4 ^{O}C for 7 days

Attribute	Control	Ferrous sulfate	Ferrous bisglycinate	Ferrous lactate	P-Value	Sig
Appearance	6.92^{a}	6.72^{a}	5.03^{b}	5.24^{b}	0.0001	***
Body texture	6.95^{a}	7.04^{a}	6.33^{b}	6.46^{b}	0.0001	***
Flavor	6.67^{a}	6.66^{a}	5.63^{b}	6.00^{b}	0.0001	***
Overall acceptance	6.58^{a}	6.68^{a}	5.54^{b}	5.84^{b}	0.0001	***

$^{a-b}$Means with different superscript within a row are significantly different at $p<0.05$, n = 98

Yogurt fortified with ferrous bisglycinate had the lowest flavor and appearance acceptability scores because ferrous bisglycinate easily oxidizes to ferric form [Fe^{3+}]

which then cause off-color development and fat oxidation (Haile, 2006). Yogurt fortified with ferrous bisglycinate and ferrous lactate scored significantly lower than control yogurt and yogurt fortified with ferrous sulfate because panelists said the former were grayish in color which was unpleasant to the panelists. The grayish appearance was due to the gray to dark color of ferrous bisglycinate. While the color of ferrous lactate is white the brown appearance of ferrous lactate fortified yogurt maybe due to other reactions between ferrous lactate and milk components. Ferrous sulfate microencapsulate is brownish in color but due to its encapsulation there were no significant differences in appearance with control treatment. Use of strawberry puree lessened the degree of browning in all yogurt but it was not enough to mask everything as color of ferrous bisglycinate and ferrous lactate were not fully masked. Encouragingly, all yogurts were rated above average sensory mean score of 5. As a result it inferred that lipid oxidation that was detected by PV and TBA resulting from iron fortification had a negligible effect on how well the yogurts were liked. This was also observed by Hekmat and MacMahon (1997) where casein-chelated and whey protein-chelated iron fortified yogurts were all scored above average.

Yogurt fortified with ferrous bisglycinate had significantly higher pH at each testing interval. Coincidentally yogurt fortified with ferrous bisglycinate had the lowest sensory mean scores in all the sensory attributes that were evaluated suggesting a relationship existed between pH and sensory acceptability of yogurt. However PV showed that there were no significant differences amongst treatments at day 7 when sensory evaluation was done on yogurt. Results in Figure 8 and Figure 9 suggest that pH did not affect PV.

6.3.2. Sensory evaluation of pasteurized milk.

Sensory data in Table 4 shows no significant differences in color among samples at $p<0.05$. This disagrees with the finding from sensory evaluation of iron fortified yogurt above **(see Table 3)** where appearance acceptability were not significantly different in control yogurt and yogurt fortified with ferrous sulfate microencapsulate but were scored significantly lower in yogurt fortified with ferrous bisglycinate and yogurt fortified with ferrous lactate. The same was the case with body texture, flavor and overall preference. There were no significant differences in flavor in pasteurized liquid milk across all treatments. Milk fortified with ferrous sulfate microencapsulate had significantly lower sensory mean score on taste than any other treatment ($p<0.05$). There were significant differences in overall preference between milk fortified with ferrous sulfate microencapsulate, which had the lowest sensory mean score, and the rests of the treatments.

Data from **Figure 11** shows that milk fortified with ferrous sulfate microencapsulate had significantly higher PV than milk fortified with ferrous bisglycinate and control milk at the time sensory evaluation was done (after day 2). However PV for milk fortified with ferrous sulfate was not significantly different from milk fortified with ferrous lactate at the time of sensory evaluation. As expected, sensory mean score for milk fortified with ferrous sulfate shows that it was least preferred. This, therefore, suggests that acceptability of pasteurized milk fortified with ferrous sulfate was based also on effects of lipid oxidation in the milk among other factors that influenced scores for sensory attributes that were evaluated.

Table 4: Sensory mean scores of pasteurized milk fortified with different iron salt stored at 4 ^{O}C for 7 days.

Attributes	Control	Ferrous Bisglycinate	Ferrous Lactate	Ferrous Sulfate
Appearance	7.48 ± 1.4^{a}	7.49 ± 1.42^{a}	7.20 ±1.73^{a}	7.30 ± 1.83^{a}
Flavor	6.74 ± 1.78^{a}	6.58 ± 1.65^{a}	6.67 ± 1.76^{a}	6.52 ± 1.89^{a}
Taste	6.88 ±1.74^{a}	7.08 ± 1.63^{a}	6.92 ±1.59^{a}	6.29 ± 2.25^{b}
Texture	6.52 ± 1.88^{a}	7.01 ±1.72^{b}	6.82 ± 1.78^{b}	6.59 ± 2.0^{a}
Overall preference	6.92 ± 1.48^{a}	6.99 ± 1.51^{a}	6.84 ± 1.51^{a}	6.45 ± 2.12^{b}

$^{a-b}$Means with different superscript in a row are significantly different to each other ($p<0.05$), n = 100

Overall milk fortified with ferrous sulfate microencapsulate was least preferred especially due to its taste. The panelists were unable to detect significant differences in color and flavor. This is an interesting observation since ferrous bisglycinate is dark in color and it was expected to impact color changes in milk fortified with it. However results indicates that this was not the case as it did not significantly impact on color of the fortified milk ($p<0.05$). All milk were rated from like slightly to like moderately in all sensory attributes under study on the hedonic scale.

6.4. Chemical analysis of lipid oxidation

6.4.1. PV test for yogurt during storage.

There is a general trend of increasing the PV as storage time increases as shown in **Figure 10**. But after 10 days of storage PV increased rapidly in yogurt fortified with ferrous sulfate microencapsulated an indication that there was high lipid oxidation. However there was a sharp decrease after 20 days of storage. This decrease is due to decomposition of hydroperoxides into secondary products of lipid oxidation like aldehyde, ketones and alcohol. These changes coincide with an increase in TBA **(Figure 10)** that measures MDA, secondary product of lipid oxidation. Yogurt fortified with ferrous lactate had the least lipid oxidation. After 23-25 days the control had the highest PV. This may be due to the delay in decomposition of hydroperoxides into secondary products of oxidation as there was no iron to catalyze oxidation process. This may also be due to relatively slow peroxidation in the early days and this had an effect on the time hydroperoxide disintegrated into secondary products.

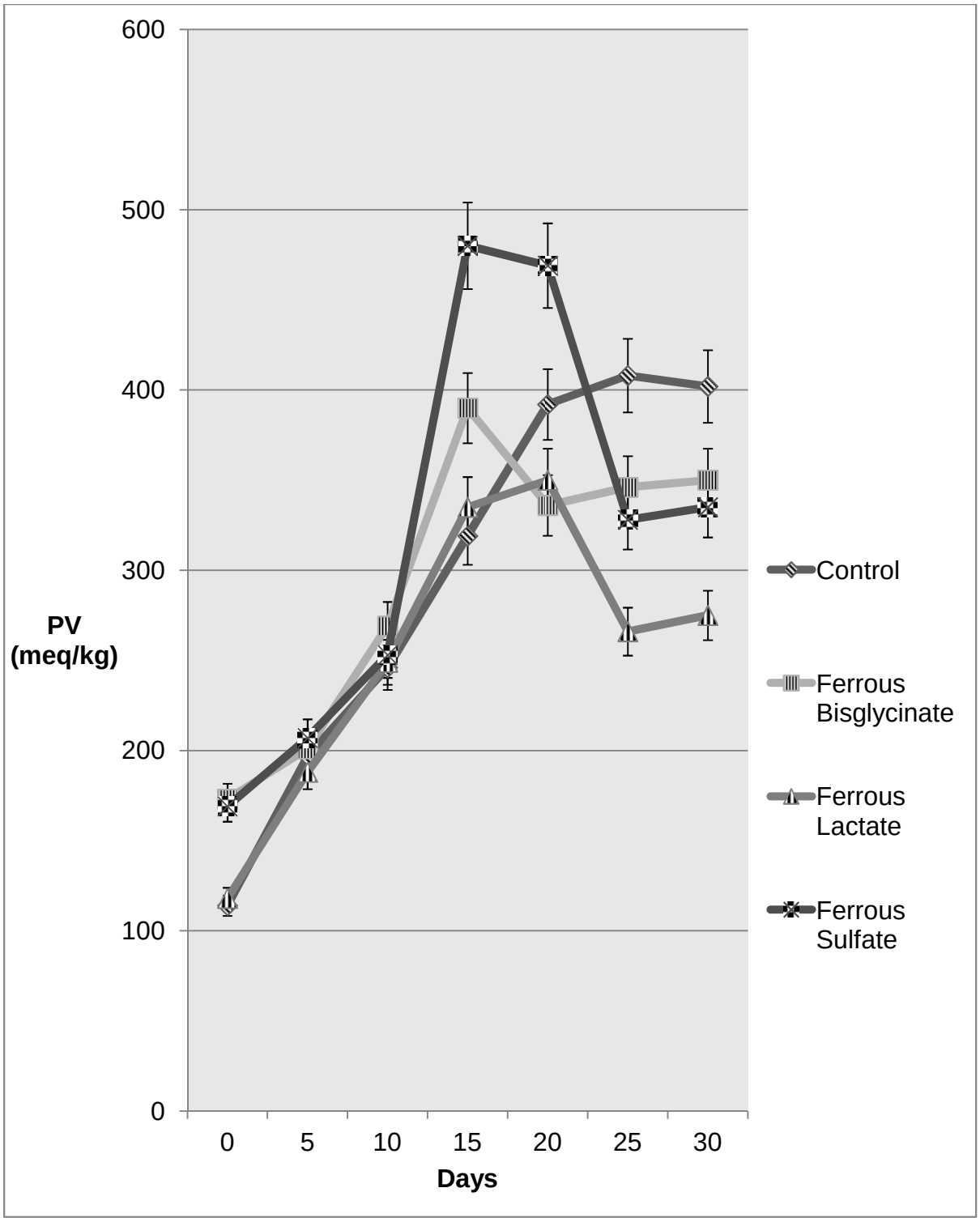

Figure 10: Changes in PV in yogurt fortified with different iron salts and stored at 4^{o}C for 30 days

4.6.2. PV test for pasteurized milk in storage.

PV results for pasteurized milk shows that there were significant differences ($p<0.05$) between treatments even after just 2 days. Control has the lowest PV values throughout the testing period seconded by milk fortified with ferrous sulfate microencapsulate. Milk fortified with ferrous bisglycinate had high PV values after 12 days. Overall there were no significant differences in mean PV between milk fortified with ferrous bisglycinate and milk fortified with ferrous lactate. This trend differs with results obtained on yogurt PV values within the same storage time **(see figure 10**) where there were no significant differences till after 10 days.

After 10 days of storage time yogurt fortified with ferrous sulfate microencapsulate gave the highest PV values. However the trend remains the same that after some time PV values in all treatments start to decrease due to degradation of hydroperoxide into secondary products. There was rapid degradation of hydroperoxides in milk fortified with ferrous bisglycinate and milk fortified with ferrous lactate than in control and milk fortified with ferrous sulfate microencapsulate. Control had a least PV values throughout the testing period. This does not agree with the finding in yogurt where there was no difference in PV between control yogurt and yogurt fortified with ferrous lactate, see **Figure 10**. The prooxidant effects of ferrous bisglycinate and ferrous lactate were more pronounced throughout the testing period. This present study clearly indicates that iron fortification significantly reduces shelf life of pasteurized milk and if pasteurized liquid milk is to be fortified there is a need to encapsulate iron so that interaction between iron and milk lipids is significantly reduced.

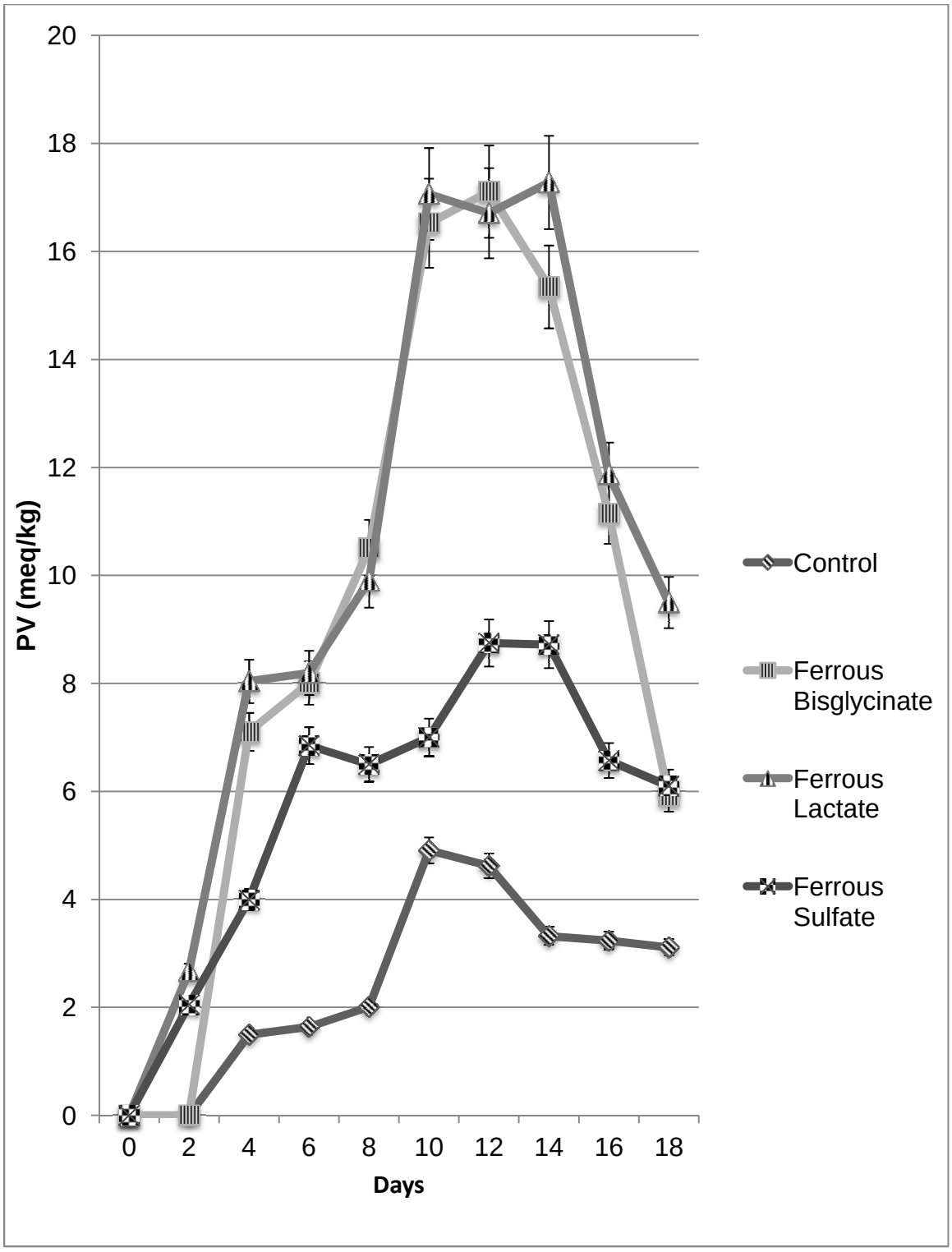

Figure 11: Effects of iron fortification on lipid oxidation in pasteurized milk stored at 4 ^{O}C for 18 days as determined by PV

This finding differs with the result obtained in yogurt where highest PV values were observed in yogurt fortified with ferrous sulfate microencapsulate. The major disadvantage of the PV method is that iodine can be absorbed by unsaturated double bonds of fatty acids, and also oxygen present in potassium thiosulfate solution may also liberate iodine. This may result into higher PV.

Another important observation is that in both cases, PV and TBA tests, ferrous sulfate microencapsulate shows that it enhances lipid oxidation more than other iron salts. The reason behind this behavior is not clear from present study. However it may be concluded that microencapsulation did not provide enough barriers between the iron and milk lipids such that oxidation occurred. This may be due to loss of microencapsulation during homogenization, incubation and microbial growth and activities. This is not conclusive and more studies have to be done on this. This behavior was observed in yogurt and not in pasteurized milk**.**

6.4.3. TBA test for yogurt in storage.

Data in **Figure 12** show that control yogurt had the lowest TBA values seconded by yogurt fortified with ferrous lactate. There was steady increase in TBA values for all the treatments except in the control. Yogurt fortified with ferrous sulfate microencapsulates and yogurt fortified with ferrous bisglycinate had the highest TBA values. TBA reagent when reacted with MDA, a secondary product of lipid oxidation resulting from degradation of hydroperoxide, forms a pink color complex called chromagen which has absorbance at 530nm. As more of MDA are produced as oxidation continues more chromagen are formed and higher TBA numbers are obtained

at absorbance 530nm. This also shows that different iron salts had different effects on formation of chromagen reflected by different TBA numbers.

Low TBA in control may be due to low hydroperoxide formation in the early stages of lipid oxidation which consequently resulted into low MDA produced and the subsequent low TBA numbers. If there is high rate of oxidation the MDA produced will be high and more chromagen is formed. This will increase absorbance and subsequently results into high TBA numbers. In yogurt fortified with ferrous bisglycinate, ferrous lactate and ferrous sulfate microencapsulate the steady increase in TBA may be due to increased breakdown of hydro peroxides to MDA which resulted into increased TBA values when reacted with TBA reagent. Since control had the lowest TBA therefore it can be concluded that all the iron salts under study enhanced lipid oxidation in yogurt. However there are other TBA reactive substances (TBARS) that affect the results of TBA test. Fernandez and others 1997 noted that iron salts affect TBA values because it catalyzes the breakdown of hydroperoxides to MDA and catalyzes degradation of amino acids to sugars (deoxyribose, hexoses, pentoses) in the presence of air to yield MDA. This will definitely give high TBA which may be misinterpreted as resulting from oxidation.

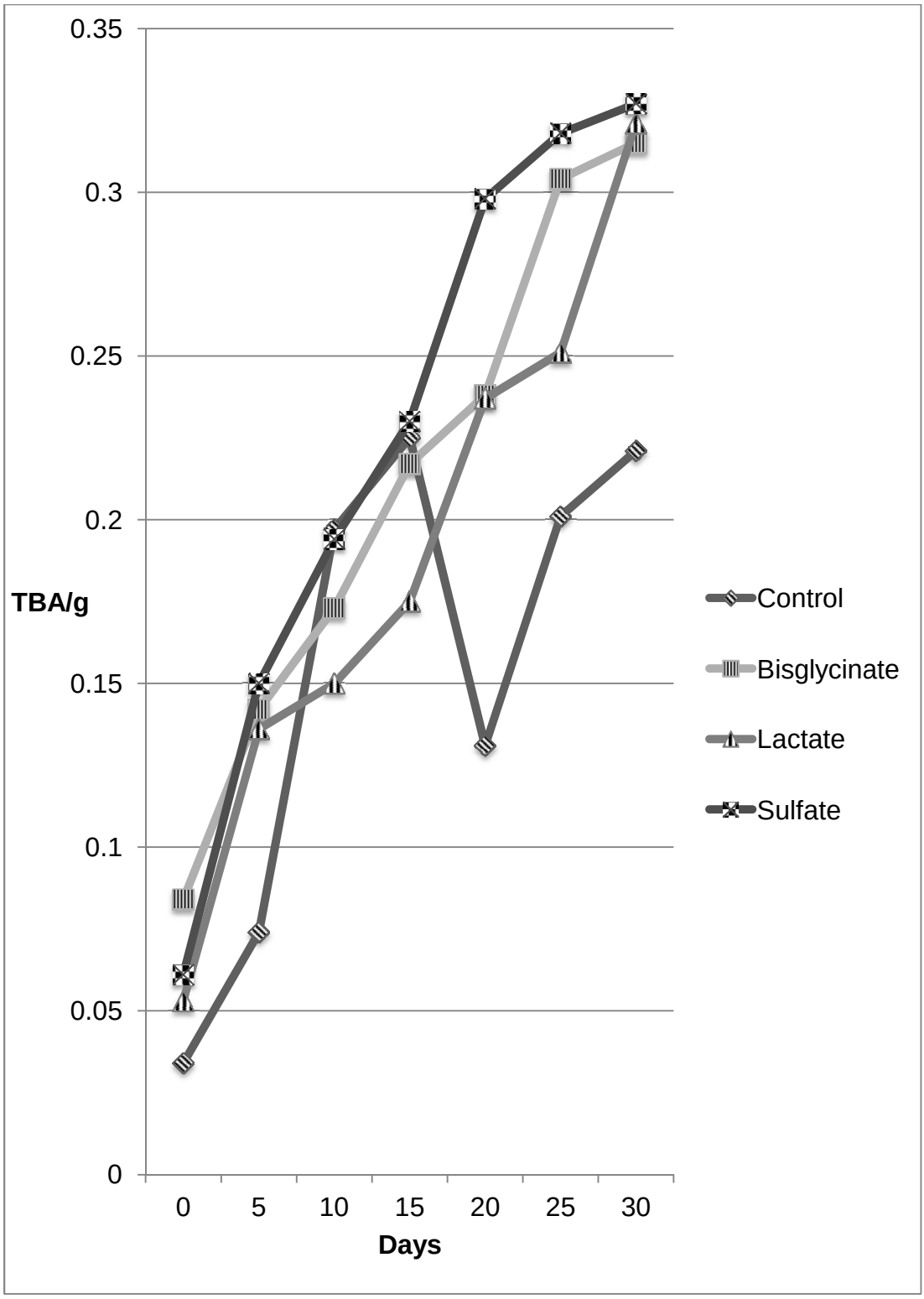

Figure 12: Effect of iron fortification on lipid oxidation in yogurt stored at 4 ^{O}C for 30 days as determined by TBA test

7.0. CONCLUSION AND RECOMMENDATIONS

All the iron salts studied supported the growth and activities of yogurt starter culture studied though their effects were different for each iron salt. Ferrous bisglycinate supported culture growth the best after 6 hours of incubation. More lactic acid was produced as incubation time increased to 6 hours for iron fortified reconstituted skim milk as shown by an increase in TA. In yogurt there was an increase in TA during early days of storage and a decrease in TA towards the end of 30 days storage time.

Consumer acceptance sensory analysis indicated that yogurt fortified with ferrous sulfate microencapsulated were not statistically different from unfortified yogurt in all sensory attributes under study. However, it was statistically higher in sensory acceptability than yogurt fortified with ferrous bisglycinate and ferrous lactate as both gave a darker color which could have been masked by using a more dark food coloring like chocolate.

Pasteurized milk showed statistically insignificant differences in color for all the treatments an indication that the panelist were unable to detect color change as was the case with yogurt. Of all the attributes under study in pasteurized milk, taste was scored the lowest in ferrous sulfate microencapsulated which presumably led to it being preferred the least by panelist.

In both yogurt and pasteurized liquid milk lipid oxidation increased as storage time increased as measured by PV and TBA. Ferrous sulfate fortified yogurt was similar in consumer acceptability to unfortified yogurt in all sensory attributes. However, TBA and PV tests showed higher values in yogurt fortified with ferrous sulfate

microencapsulate than in yogurt fortified with ferrous bisglycinate and yogurt fortified with ferrous lactate. This may be attributed to increased lipid oxidation as microencapsulation was done using vegetable oil which may also have been oxidized leading to high PV and TBA numbers. Or microencapsulation was destroyed during yogurt mixes homogenization and incubation such that there was free interaction between ferrous sulfate and milk fats leading to high PV and TBA. Therefore, there is a need to establish a reason why ferrous sulfate microencapsulated gave higher PV and TBA (an indication of oxidation) and higher mean sensory score (an indication better sensory quality) because, as would be expected, higher PV and TBA was supposed to go with lower sensory means scores. This study has shown that microencapsulation does not reduce oxidation in yogurt especially when fortification was done before homogenization, pasteurization and incubation but it does reduce oxidation in pasteurized liquid milk.

In this present study bioavailability of fortified iron in yogurt when consumed has not been examined. Therefore another study, *in-vitro* or *in-vivo*, would be important to help find out the bioavailability of iron in the body after consuming iron fortified yogurt and milk using animal subjects.

8.0 APPENDICES

8.1. Milk flyer

Do you like milk?

Welcome to Sensory Evaluation of **Iron Fortified Pasteurized Liquid Milk**

It will not take you much time! Just 5 – 10 minutes!!!

Venue: HE/HN Food Laboratory

Date: Friday, December 7, 2012

Time: 9:00 am till 100 panelists have participated

Everybody is welcome!!!

Incentives shall be provided to participants as soon s/he finishes the Sensory Test.

First Year Students are also encouraged to participate

For more information call 0999 746 608/0882 943 912

See you there everybody!!!!!!!

8.2. Yogurt flyer

DO YOU LIKE STRAWBERRY YOGURT?

PLEASE JOIN US FOR A SENSORY STUDY FOR STRAWBERRY YOGURT!!!!!

TIME: 10:00 AM – 1:00 PM or Until 75 panelists have participated

Date: Tuesday July 3rd, 2013

Where: Sensory Lab (Room 102 G. Malcolm Trout Building). Corner of Wilson and Farm Lane

The test will take approximately 10-15 min.
You will receive a FREE MSU Dairy Store Coupon (2 scoops)

8.3. Questionnaire for pasteurized milk

You will be provided with **4 samples**. Please evaluate each sample in the order presented. Remember to rinse your mouth with water provided when moving to the next sample.

1. **Sample 537**
   Take the sample and sensory evaluate it. Put the number that corresponds to your degree of liking (in the first column) below the attribute being evaluated.

	appearance	**flavor**	**taste**	**mouth feel**	**Overall preference**
9. Like extremely					
8. like very much					
7. like moderately					
6. like slightly					
5. neither like or dislike					
4. dislike slightly					
3. dislike moderately					
2. dislike very much					
1. dislike extremely					

2. **Sample 674**

Rinse you mouth with water and evaluate the sample as in 1 above

	Appearance	flavor	taste	mouth feel	Overall preference
9. Like extremely					
8. like very much					
7. like moderately					
6. like slightly					
5. neither like or dislike					
4. dislike slightly					
3. dislike moderately					
2. dislike very much					
1. dislike extremely					

3. **Sample 317**

Rinse your mouth and evaluate the sample as in 1 above

	Appearance	flavor	taste	mouth feel	Overall preference
9. Like extremely					
8. like very much					
7. like moderately					
6. like slightly					
5. neither like or dislike					
4. dislike slightly					
3. dislike moderately					
2. dislike very much					
1. dislike extremely					

4. **Sample 413**

Rinse your mouth and evaluate the sample as in 1 above

	appearance	**flavor**	**taste**	**mouth feel**	**Overall preference**
9. Like extremely					
8. like very much					
7. like moderately					
6. like slightly					
5. neither like or dislike					
4. dislike slightly					
3. dislike moderately					
2. dislike very much					
1. dislike extremely					

How many times do you drink milk a month? _____

Is milk always available? _____

Thank you for your participation!

8.4 Questionnaire for Yogurt

5. **Sample 537**

   Take the sample and sensory evaluate it. Put the number that corresponds to your degree of liking (in the first column) below the attribute being evaluated.

	appearance	Body texture	flavor	Overall preference
9. Like extremely				
8. like very much				
7. like moderately				
6. like slightly				
5. neither like or dislike				
4. dislike slightly				
3. dislike moderately				
2. dislike very much				
1. dislike extremely				

6. **Sample 674**

   Rinse you mouth with water and evaluate the sample as in 1 above

	appearance	Body texture	flavor	Overall preference
9. Like extremely				
8. like very much				
7. like moderately				
6. like slightly				
5. neither like or dislike				
4. dislike slightly				
3. dislike moderately				
2. dislike very much				
1. dislike extremely				

7. **Sample 317**

Rinse your mouth and evaluate the sample as in 1 above

	appearance	**Body texture**	**flavor**	**Overall preference**
9. Like extremely				
8. like very much				
7. like moderately				
6. like slightly				
5. neither like or dislike				
4. dislike slightly				
3. dislike moderately				
2. dislike very much				
1. dislike extremely				

8. **Sample 413**

Rinse your mouth and evaluate the sample as in 1 above

	appearance	**Body texture**	**flavor**	**Overall preference**
9. Like extremely				
8. like very much				
7. like moderately				
6. like slightly				
5. neither like or dislike				
4. dislike slightly				
3. dislike moderately				
2. dislike very much				
1. dislike extremely				

8.5. Consent Form for Research Involving Human Subjects

IRON FORTIFICATION OF PASTEURIZED MILK AND YOGURT

Invitation to participate

You are invited to participate in a research study, which compares sensory properties of UHT milk and yogurt fortified with different iron salts

Purpose of the study

To evaluate sensory attributes and overall acceptability of iron fortified UHT milk and yogurt

Basis for subject selection

They will be selected based on their ability to detect differences in sensory attributes. Those with cold or allergies to a specific ingredient will not be asked to participate. Participants must be at least 18 year old.

Potential risks

The UHT milk to be evaluated will be sterilized milk with no any other ingredients added other than the iron salts (ferrous sulfate microencapsulated, ferrous Bisglycinate or ferrous lactate). All these salts are USDA/FDA approved for use in foods intended for human consumption at approved levels. Yogurt will contain the following ingredients in addition to iron; milk, culture, sugar, Non-fat dry matter, stabilizer, cream, water. All these are FDA approved for yogurt production. These products sample pose no adverse health risk upon ingestion, provided the subject has not been identified as being susceptible to allergic reaction. If you believe there is a potential of an allergic reaction upon ingesting the test product or you believe that participating will violate your religious or cultural belief, notify the on-site sensory evaluation coordinator or principal investigator immediately. You will be released from participating in the study.

Potential benefit

There are no direct benefits gained from participating in this study. However your participation provides valuable data for the development of iron fortified milk and yogurt. Information obtained from this study will be published in appropriate scientific journals to expand our current knowledge in enhancing the health value of fortification in dairy products

Explanation of procedure

You will be provided with four coded samples and a questionnaire. You will be provided with water for rinsing your mouth between samples. The testing excersize will take a maximum of 25 minutes of your time depending upon your speed of testing

Assurance of confidentiality

Any information obtained in connection with this study that will be identified with you will be kept confidential by ensuring that all consent forms are securely stored and your privacy will be protected to the maximum extent allowable by law. All data analyzed will be reported in an aggregate format that will not permit associating with specific responses or findings.

Withdrawal from this study

Participating from this study is voluntary. Your decision to refuse participation will not affect your present or future relationship with the principal investigator or MSU. You are also free to withdraw or stop participating at any time you feel it is necessary to do so.

Compensation for participation

After you have completed your sensory testing session and turned in your sensory ballot, you will be offered an ice cream coupon for your time and effort.

Offer to answer questions

If you have any questions, please do not hesitate to contact the onsite evaluation leader or principal investigator. You are voluntarily making a decision to participate in this study today. Your signature certifies that you have decided to participate after having read the information provided above and that you had an adequate opportunity to discuss this study with principal investigator and have had your questions answered to your satisfaction. You will be given a copy of this consent form to keep upon request. In case you have question you may email Smith Nkhata on nkhatasm@msu.edu.

SIGNATURE OF SUBJECT: _____ **DATE:** _____

In my judgment the subject is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this research study.

SIGNATURE OF INVESTIGATOR: _____ **DATE**: _____

8.6. Application For Initial Review

APPROVAL OF A PROJECT INVOLVING HUMAN SUBJECTS

Biomedical, Health Sciences Institutional Review Board (BIRB)
Social Science, Behavioral, Education Institutional Review Board (SIRB)
207 Olds Hall, Michigan State University
East Lansing, MI 48824-1047
Phone: (517) 355-2180
Fax: (517) 432-4503
E-mail: irb@msu.edu

Office Hours: M-F (8:00 A.M.-5:00 P.M.)

IRB#: x12-614
APPLICATION ID#: i041351

Title of Project: Iron Fortification of UHT milk and yogurt

Table 5: Principal Investigator's (PI) details

Responsible Project Investigator:	**Zeynep Ustunol**	Mailing Address:	**2105 S. Anthony Hall MSU**
Identification Number:	**XXX-XX-1560**	Phone:	**5-7713 EXT. 184**
Department:	**Food Science & Human Nutrition**	Fax:	**517-353-1676**
College:	**AGRICULTURE AND NATURAL RESOURCES**	Email:	**ustunol@msu.edu**
Academic Rank:	**Professor**		

The Human Research Protection Program (HRPP) has deemed this project as exempt, in accord with federal regulations for projects exempt from Institutional Review Board (IRB) review. As an exempt protocol, the appropriate IRB will not be further involved with the review or continued review of the project, as long as the project maintains the properties that make it exempt.

- Since the HRPP is no longer involved in the review and continued review of this project, it is the Principal Investigator who assumes the responsibilities for protection of human subjects in this

project and ensures that the project is performed with integrity and within accepted ethical standards, particularly as outlined by the Belmont Report (see exempt educational materials).

- The Principal Investigator assumes responsibility for ensuring that the research subjects be informed of the research through a documented or undocumented consent process, if appropriate.
- The Principal Investigator assumes the responsibility to maintain confidentiality of the subjects and the data, and maintain the privacy of the subjects and protection of the data through appropriate means. If data is anonymous, the investigators will make no attempt to identify any individuals.
- The Principal Investigator assumes the responsibility that co-investigators and other members of the research team adhere to the appropriate policies to protect human subjects, maintain confidentiality and privacy, and adhere to accepted ethical standards.
- If the Principal Investigator adds additional investigators to an exempt protocol, he/she may inform the HRPP of the additions. This may be of particular importance to graduate students if the Graduate School requires proof of IRB approval.
- Any complaints from participants regarding the risk and benefits of the project must be reported to the HRPP.
- Since the Principal Investigator and co-investigators are charged with human subject protection and adhering to ethical principles in exempt research, it is appropriate that investigators be trained in human subject principles. The Principal Investigator and all members of the research team are required to complete MSU IRB educational requirements or equivalent.
- Any change in the protocol which may raise the project from exempt to an expedited or full review category must be presented to the HRPP. If there is any question about a change in protocol the Principal Investigator should consult the Director of the HRPP. Failure to submit changes which raise the protocol out of the exempt category will be considered non-compliance and will be subject to investigation and action by the HRPP.
- I accept responsibility for conducting the proposed research in accordance with the protections of human subjects as specified by the IRB, including the supervision of faculty and student co-investigators. There will be adequate resources and facilities to carry out the research.

By signing below, the Principal Investigator assures that he/she will abide by the terms of this assurance and the HRPP exempt policy.

SIGN HERE: _____

Date: _____

9.0 REFERENCES

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