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# DEVELOPMENT AND EVALUATION OF MICROENCAPSULATED IRON FORTIFIED YOGHURT

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

**MASTER OF TECHNOLOGY**
**IN**
**DAIRY CHEMISTRY**

BY
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B.Tech. (Dairy Technology)

DAIRY CHEMISTRY DIVISION
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
KARNAL–132001 (HARYANA), INDIA
**2013**

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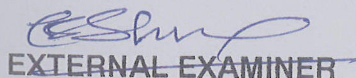
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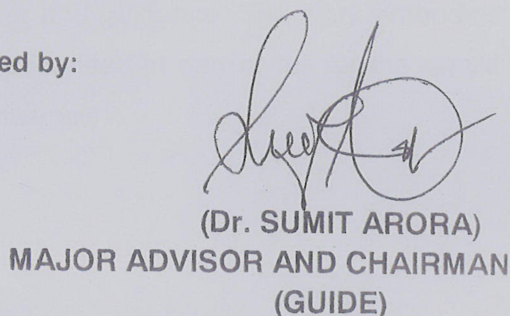
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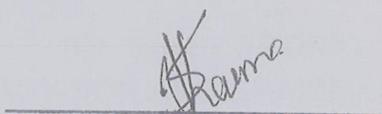

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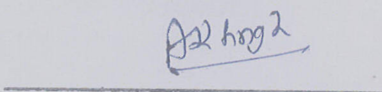

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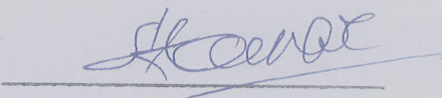

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This is to certify that the thesis entitled **"DEVELOPMENT AND EVALUATION OF MICROENCAPSULATED IRON FORTIFIED YOGHURT"** submitted by **Ms. CHITRA GUPTA** in partial fulfillment of the requirement for award of the degree of **MASTER OF TECHNOLOGY** in **DAIRY CHEMISTRY** of the **NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY), KARNAL (HARYANA)** is a bonafide research work carried out by her under my supervision and guidance. The work embodied in this thesis is original and no part has been submitted in part or full for the award of any diploma or degree of this or any other university.

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to my

reward

parents

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# List of Abbreviations

| AAS | Atomic absorption spectrophotometer |
|---|---|
| BIS | Bureau of Indian Standards |
| $CaCl_2$ | Calcium chloride |
| cm | Centimetre |
| cfu | Colony forming unit |
| CHD | Coronary heart diseases |
| D | Dalton |
| MRS | deMan-Rogosa-Sharpe |
| $^{o}C$ | Degree centigrade |
| EDTA | Ethylene diamine tetra acetic acid |
| EE | Encapsulation efficiency |
| $FeSO_4$ | Ferrous sulphate |
| GA | Gum arabic |
| g | Grams |
| hrs | Hours |
| ICMR | Indian Council of Medical Research |
| ICN | International Conference on Nutrition |
| INACG | International Nutritional Anaemia Consultative Group |
| IDD | Iodine deficiency disorder |
| IDA | Iron deficiency anaemia |
| kg | Kilograms |
| L | Litre |
| MD | Maltodextrin |
| MS | Modified starch (HiCap 100) |
| mg | Milligram |
| ml | Millilitre |
| Mm | Millimetre |
| µl | Microlitre |
| min | Minutes |
| nm | Nanometre |
| NCDC | National Collection of Dairy Cultures |
| NFHS | National Family Health Survey |
| NHRP | National Health Research Policy |
| NNMB | National Nutrition Monitoring Board |
| NNP | National Nutrition Policy |
| $HNO_3$ | Nitric acid |
| N | Newton |
| Ns | Newton second |

| | |
|---|---|
| ppm | Parts per million |
| $HClO_4$ | Per chloric acid |
| PCB | Phosphate citrate buffer |
| PGMS | Polyglycerol monostearate |
| RDA | Recommended dietary allowance |
| rpm | Revolutions per minute |
| SEM | Scanning electron microscopy |
| sec | Second |
| SA | Sodium alginate |
| NaCl | Sodium chloride |
| NaOH | Sodium hydroxide |
| $H_2SO_4$ | Sulphuric acid |
| TBA | 2-thiobarbituric acid |
| TS | Total solids |
| UV | Ultra-violet |
| USFDA | United States Food and Drug Administration |
| USA | United States of America |
| VAD | Vitamin A deficiency |
| WHO | World Health Organisation |

# ABSTRACT

Micronutrient malnutrition is a serious threat to health and productivity of billions of people worldwide. Among micronutrients, iron is an essential trace element in animal and human diets. Lack of this trace element is the most common nutritional deficiency around the world. Fortification of iron in milk and milk products results in metallic taste, unacceptable flavours and undesirable colour changes. Microencapsulation could be a suitable method for adding iron to yoghurt for preventing these negative effects. Various iron microencapsules were prepared using different coating materials i.e. phosphatidylcholine, polyglycerol monostearate (PGMS), sodium alginate (SA), blend of SA and pectin, blend of SA and modified starch HiCap 100 (MS), blend of gum arabic (GA), maltodextrin (MD) and MS and same core material as ferrous sulphate heptahydrate. Among all microencapsules, GA 500 1:10 prepared using alcohol as dehydrating media (300 mg) was selected for fortification in yoghurt on the basis of encapsulation efficiency (EE), sensory analysis, scanning electron microscopy (SEM) structure and TBA value. Optimised level for fortification of iron microencapsules in yoghurt was 30 ppm iron based on sensory evaluation. Combination of dry and wet digestion and 8 hrs ashing time were most suited analytical conditions for estimation of iron content in yoghurt and microencapsules. Yoghurt fortified with GA 500 1:10 @30 ppm iron was analysed for the effect of fortification on sensory, physicochemical, rheological and microbiological parameters of yoghurt. Sensory analysis revealed that iron microencapsules fortified yoghurt was similar ($P>0.05$) to control yoghurt upto 7 days of storage. These microencapsules release the iron slowly after fortification in yoghurt.

# सुक्ष्मसंपुटीत लोह तत्व संपूरित योगर्ट का विकास एवं आंकलन

| **शोध छात्रा** | **प्रमुख सलहाकार** | **प्रभाग** |
| --- | --- | --- |
| चित्रा गुप्ता | डॉक्टर सुमित अरोड़ा | डेरी रसायन |

## सारांश

सूक्ष्म पोषक कुपोषण दुनिया भर के करोडो लोगों के स्वास्थ्य और प्रजनन के लिए एक गंभीर खतरा है | सूक्ष्म पोषक तत्वों मे लोह तत्व पशु एवं मानव आहार में एक आवश्यक पोषक तत्व हे इस सूक्ष्म पोषक तत्व की कमी विश्व भर में मुख्य पोषक न्यनता है | लोह तत्व की दूध एवं दूध उत्पादों में सम्पुरणता करने से दूध में धातु स्वाद , अस्वीकार्य जयका और अवन्छिनये रंग जैसे परिणाम मिलते है | योगर्ट में इन नकारात्मक प्रभावों को रोकने के लिए लोह तत्व का सूक्ष्म संपुटीकरण करना एक उपयुक्त तरीका है | विभिन्न लोह तत्व (फेरस सलफेट ) सूक्ष्म संपुटी अलग अलग कोटिंग सामग्री जैसे कि लेसिथिन, पॉलीग्लिसरॉल मोनोसटीरेएट (PGMS), सोडियम ऐल्जीनेट (SA ) एवं पेक्टिन का मिश्रण, सोडियम ऐल्जीनेट एवं मॉडिफाइड स्टार्च (MS) का मिश्रण तथा गम एरेबिक, माल्टोडेक्सट्रिन एवं मॉडिफाइड स्टार्च के मिश्रण के उपयोग से बनाये गए | विभिन्न प्रकार के सूक्ष्मसंपुटी में से, सूक्ष्म संपुटी दक्षता, संवेदी विश्लेषण, स्कैनिंग इलेक्ट्रान माइक्रोस्कोपी सरंचना तथा TBA मुल्यांकन के आधार पर GA 500 1:10 सूक्ष्मसंपुटी, जो कि अल्कोहल को निर्जलीकरण माध्यम के रूप में उपयोग करके बनाये गये, योगर्ट में लोह तत्व को संपूरित करने के लिय चुने गये | सवेंदी विश्लेषण के आधार पर 30 ppm तक लोह तत्व को योगर्ट में संपूरित करने के लिय चुना गया | योगर्ट एवं सूक्ष्मसंपुटी में लोह तत्व के आंकलन के लिए सूखे एवं आद्र डाईजेसन का मिश्रण तथा आठ घंटे का भस्मावशेष समय उपयुक्त विश्लेषणात्मक परिस्थितियां थी | GA 500 1:10 (30 ppm) द्वारा संपूरित योगर्ट में लोह तत्व को संवेदी विश्लेषण, भोतिक रसायन, सूक्षमजीवी मनको का संपूरित प्रक्रिया पर प्रभाव के आधार पर विश्लेषण किया गया | संवेदी विश्लेषण के आधार पर यह भी पता चला कि सूक्ष्मसंपुटी संपूरित योगर्ट एवं असम्पुरित योगर्ट सात दिनों के भण्डारण के पश्चात् भी स्वीकार्य थे | भण्डारण के पश्चात् ये सूक्ष्म संपुटी धीरे धीरे लोह तत्व को योगर्ट में निस्तार कर देते है |

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# CHAPTER – 1

# Introduction

# INTRODUCTION

Iron is an essential trace element in animal and human diets. It is naturally present in the structure of cytochromes, enzymes, haemoglobin and myoglobin (Abbasi and Azari, 2011). Besides its important role in transport and storage of oxygen, it is also a cofactor in various biochemical processes, including DNA synthesis (Petranovic *et al.* 2008). Lack of this trace element is the most common nutritional deficiency around the world that causes iron deficiency with or without anaemia (Gaucheron 2000). It is estimated that 20-45% of productive age women and 70% of children in developing countries are anaemic (Derbyshire *et al.* 2010). Iron deficiency is usually the result of insufficient dietary iron intake, poor utilisation of iron from digested foods or a combination of the two (Xia and Xu 2005). Its deficiency causes lower growth rate, impaired cognitive score in children, poor pregnancy outcomes and lower working capacity in adults (Derbyshire *et al.* 2010). It can also reduce haem proteins, iron containing enzymes and reactions in which iron is involved as cofactor (Petranovic *et al.* 2008)

The recommended daily intake of dietary iron for normal infants is 1 mg/kg and for children, male and female adolescent it is 10, 12 and 15 mg per day respectively. For women during reproductive years it is 15 mg per day (El- Behairy and Mohamed 2010).

In general, three approaches are recommended for amending nutritional iron deficiency in an individual or combined forms (i) dietary modification and diversification to improve nutritional value and iron bioavailability, (ii) supplementation and (iii) fortification (Derbyshire *et al.* 2010).

Dairy products are recommended as suitable vehicles for iron fortification due to their high consumption by children, a high risk group with regard to iron deficiency and outstanding nutritional properties (Derbyshire *et al.* 2010). In addition, dairy products are excellent source of calcium and protein but contain very little iron (0.2-0.4 mg/kg) (Xia and Xu 2005). Therefore, dairy products are not suitable for providing dietary iron nevertheless, fortification can make these as excellent source of this trace element alongside with its high calcium content for health conscious consumers (Hekmat and

McMahon 1998). An advantage of using dairy products as the vehicle for fortifying iron is that, iron fortified dairy products have a relatively high iron bioavailability (Woestyne *et al.* 1991).

Among all the dairy products, yoghurt is preferred since it is widely consumed throughout the world for its sensory and nutritional benefits. It is obtained by the lactic acid fermentation of whole, skimmed or standardized milk by the action of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, and can be accompanied by other lactic bacteria which, for their part, contribute to the characteristics of the final product (Brasil 2007). Yoghurt is believed to be effective in both the prevention and treatment of various illnesses, viz., gastrointestinal disorders, heart diseases and tumor development in man as well as animals (Vijayendra and Gupta 2011).

Yoghurt fortification with iron is not an easy task because it may cause metallic taste and unacceptable flavour as a result of the oxidation or rancidity of fats (Mehansho 2006). It is therefore proposed that iron salts should be microencapsulated to prevent these negative effects (Xia and Xu 2005).

Microencapsulation is a technology of packaging solids, liquids or gaseous materials in miniature sealed capsules that can release their contents at controlled rates under the influence of specific conditions. In general different types of microcapsules can be produced by wide range of wall materials using various microencapsulation techniques (Champange and Fustier 2007).

Different methods are available for iron microencapsulation, i.e., using polyglycerol monostearate (PGMS) and liposome. Hence, the present study was proposed with the following objectives:

1. To prepare yoghurt with microencapsulated iron.
2. To evaluate the fortified yoghurt for sensory, physicochemical, rheological and microbiological parameters during storage.

# CHAPTER –2

## Review of Literature

## 2.1 MICRONUTRIENTS

Micronutrients are nutrients required by humans and other living creatures in small quantities to orchestrate a whole range of physiological functions, which the organisms itself cannot produce (Lieberman and Bruning 1990).

### 2.1.1 Status of micronutrient malnutrition

Micronutrient deficiencies such as iron deficiency anaemia (IDA), vitamin A deficiency (VAD) and iodine deficiency disorders (IDD) continue to be significant public health problems; affecting more than one third of the world's population (Lotfi *et al.*1996). Apart from well established deficiency cases of iron, iodine and vitamin A, emerging evidences have been reported on low plasma levels of zinc, folic acid and vitamin D as well. Sporadic deficiencies related to vitamin $B_1$, $B_2$, $B_{12}$ and evidences of increasing fracture risk in Indian population attributed to calcium and vitamin D deficiency have also been reported in recent past (NNMB 2003; Gragnolati *et al.* 2005; Marwaha *et al.* 2005; Sachan *et al.* 2005; Salvi and Damania 2005; Harinarayan *et al.* 2007; Pathak *et al.* 2007; Puri *et al.* 2008; Teotia and Teotia 2008; Yajnik *et al.* 2008; Krishnaveni *et al.* 2009; Babu and Calvo 2010; Bandgar and Shah 2010; Singh *et al.* 2010; Jain *et al.* 2011; Osei *et al.* 2010; Kapil and Jain 2011).

Iron deficiency has earned distinction as the most common nutritional deficiency in the world today. It affects more than 20% of world's population as iron imparts an important role in the formation of blood (Walter *et al.* 1998; Navarrete *et al.* 2002). Iron deficiency often leads to anaemia, defined as having a blood haemoglobin level below standard which is usually the result of insufficient dietary intake of iron, poor utilisation of iron from ingested food or a combination of the two (Gaucheron 2000). The incidence of iron deficiency anaemia (IDA) is high among infants, teenagers and women of child bearing age, in both developing and industrialised countries. About 3.5 billion persons in the developing world are affected by this nutritional disorder (ACC/SCN 2000). The prevalence of anaemia among pregnant women is 52% in the world and 76% in South Asia (WHO 2000).

Global prevalence of anaemia is 47.4% in preschool aged children, 41.8% in pregnant women and 30.2% in non-pregnant women (Badham *et al.* 2007). In India, prevalence of anaemia is 79% in children of 6-35 months age, 55% in women of 15-49 years and 24% in men of 15-49 years of age (NNP 1993; NFHS 2007).

**2.1.2 Strategies for the control of micronutrient malnutrition**

The control of micronutrient deficiencies is an essential part of the overall effort to fight hunger and malnutrition. Pharmaceutical supplementation involves the administration of supplements to individuals diagnosed with a certain micronutrient deficiency. However, it fails owing to poor compliance, poor coverage, absence of commitment at the national and community levels and poorly designed communications (Gibson *et al.* 1998). The International Conference on Nutrition (ICN) held in Rome (1992) emphasised the importance of food based activities in their plan of action geared at addressing the issue of micronutrient malnutrition (FAO/WHO 1992).

Micronutrient deficiencies can be prevented and even eliminated if optimal quantities of the micronutrients are consumed by populations on a regular basis. The 11th five year plan (2007-2012) report of the working group on integrating nutrition with health (www.motherchildnutrition.org/india/pdf/mcn-integrating-nutrition-with-health.pdf, 2012) and the Draft National Health Research Policy (NHRP 2010) identified micronutrient malnutrition as a major concern. The loss due to micronutrient deficiencies costs India 1% of its GDP which is ultimately a loss of Rs. 27,720 crores per annum in terms of productivity, illness, increased health care costs and death (Kotecha 2008). “Providing micronutrients” has been recognised as having the best cost/benefit ratio to achieve a major impact in the developing world as stated by more than fifty economists in the Copenhagen Consensus, 2008 (www.copenhagenconsensus.com, 2012). Food fortification, the addition of specific nutrients to food or water, has been shown to be an effective strategy to combat micronutrient malnutrition (Cook and Reusser 1983; Stekel *et al.* 1988).

Policy and programme responses include food-based strategies that can be used to improve micronutrient malnutrition management:

a) Supplementation with pharmaceuticals dispensed in adequate forms

b) Fortification of food or water with nutrients

c) Consumer education and dietary change through the expansion of the demand for, and supply of, nutrient rich food.

(Salgueiro *et al.* 2007)

## 2.2 Food fortification

Food fortification is the public health policy of adding micronutrients (essential trace elements and vitamins) to foodstuffs to ensure that minimum dietary requirements are met. The Codex *General Principles for the Addition of Essential Nutrients to Foods* (Codex 1987) defines "fortification" as "the addition of one or more essential nutrients to a food whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups". Food fortification differs from other programmes as it involves the addition of nutrients to foods in respect to its nutritional intervention with a specifically defined target and fortified food products are expected to become a main source of the specifically added nutrient. Consequently, food fortification is expected to help in preventing nutritional inadequacy in targeted populations in which a risk of nutrient deficiency has been identified. A realistic level of fortification is to provide the equivalent of 20-40% of the daily requirements of target nutrient through a single food item (Huma *et al.* 2007).

The public health benefits of fortification may either be demonstrable or indicatable as potential or plausible by generally accepted scientific research, and include:

- Prevention or minimisation of the risk of occurrence of micronutrient deficiency in a population or specific population groups.
- Contribution to the correction of a demonstrated micronutrient deficiency in a population or specific population groups.
- Potential for improvement in nutritional status and dietary intakes that may be, or may become, suboptimal as a result of changes in dietary habits or lifestyles.

- Plausible beneficial effects of micronutrients consistent with maintaining or improving health (e.g. there is some evidence to suggest that a diet rich in selected antioxidants might help to prevent cancer and other diseases).

**2.2.1 Types of fortification**

Food fortification can take several forms. It is possible to fortify foods that are widely consumed by the general population (mass fortification); foods designed for specific population subgroups, such as complementary foods for young children or rations for displaced populations (targeted fortification); allow food manufacturers to voluntarily fortify foods available in the market place (market-driven fortification); fortify foods at household level (household fortification) and/or fortification of the staple foods by breeding and genetic modification of the plants (biofortification). Mass fortification is nearly always mandatory, while targeted fortification can be either mandatory or voluntary depending on the public health significance of the problem it is seeking to address. Market-driven fortification is always voluntary. However, it is governed by regulatory limits (Allen *et al.* 2006). Household and biofortification are the new technologies which are gaining importance (Nestel 2003).

**2.2.1.1 Mass fortification**

Mass fortification is the term used to describe the addition of one or more micronutrients to foods commonly consumed by the general public, such as cereals, condiments and milk. It is usually instigated, mandated and regulated by the government sector. Mass fortification is generally the best option when the majority of the population has an unacceptable risk, in terms of public health, of being or becoming deficient in specific micronutrients. Mass food fortification is being promoted as the nutritional intervention to prevent the physiological and clinical consequences associated with micronutrient deficiencies and has the most favourable cost to benefit ratio (Allen *et al.* 2006). Although this statement may be true in many cases, it depends on the existence of several preconditions. These include:

- Accessibility of the food fortification vehicle by the target population.
- Adequate manufacturing and industrial settings which ensure cost effective production and supervision.

- Compatibility of fortificants (the source of micronutrients) with the nature and use of the food vehicle.
- Affordability of additional costs resulting from the fortification process for consumers and manufacturers.
- Confirmation of the quality of the product at the production site.
- Reliable enforcement actions by government authorities to assure compliance of standards and regulations.
- Legitimate and justifiable food labeling
- Nutrition and health claims that promote healthy practices.

(www.a2zproject.org/pdf/Food-Fortification-Formulator.pdf*,* 2012)

**2.2.1.2 Targeted fortification**

In targeted food fortification programmes, foods aimed at specific subgroups of the population are fortified, thereby increasing the intake of that particular group rather than that of the population as a whole. Examples include complementary foods for infants and young children, foods developed for school feeding programmes, special biscuits for children and pregnant women and rations (blended foods) for emergency feeding and displaced persons. In some cases, such foods may be required to provide a substantial proportion of daily micronutrient requirements of the target group (Allen *et al.* 2006).

**2.2.1.3 Market-driven fortification**

The term "market-driven fortification" is applied to situations whereby a food manufacturer takes a business-oriented initiative to add specific amounts of one or more micronutrients to processed foods. Although voluntary, this type of food fortification usually takes place within government-set regulatory limits (Allen *et al.* 2006).

**2.2.1.4 Household fortification**

Efforts are underway in a number of countries to develop and test practical ways of adding micronutrients to foods at the household level, in particular, to complementary foods for young children. In effect, this approach is a combination of supplementation and fortification, and has also been referred to as "complementary

food supplementation” (Nestel 2003).

#### 2.2.1.5 Bio fortification of staple foods

It involves the breeding and genetic modification of plants so as to improve their nutrient content and absorption. Certain cereals and legumes for their high iron content, various varieties of carrot and sweet potatoes for their favourable β-carotene levels, and maize for their low phytate content can be used as the vehicle for such fortification (Beyer 2002; Lucca *et al.* 2002).

### 2.2.2 History of food fortification

Food fortification has a long history of use in industrialized countries for the successful control of deficiencies of vitamins A, D, several B vitamins (thiamine, riboflavin and niacin), iodine and iron. Salt iodization was introduced in the early 1920s in both Switzerland (Burgi 1993) and the United States of America (Marine and Kimball 1920) and has since expanded progressively all over the world to the extent that iodized salt is now used in most countries. Darnton-Hill and Nalubola (2002) have identified at least 27 developing countries that could benefit from programmes to fortify one or more foods. Fortification is often more cost-effective than other strategies, especially if the technology already exists and if an appropriate food distribution system is in place (Horton 1999).

### 2.2.3 Selection of food vehicle

Selection of a suitable food vehicle and compatible fortificant are fundamental criteria for justifying intervention in the form of food fortification. In practice, selection of a food vehicle-fortificant combination is governed by various factors, both technological and regulatory. The choice of fortificant is often a compromise between reasonable cost, bioavailability from the diet, interaction with food matrix and other food components and the acceptance of any sensory changes. Safety is also an important consideration. The level of consumption that is required for fortification to be effective must be compatible with a healthy diet (www.nutritionimprovement.com, 2012).

#### 2.2.3.1 Milk and milk products as a vehicle for iron fortification

Dairy products are recommended as suitable vehicles for iron fortification due to their high consumption by children, a high risk group with regard to iron deficiency and outstanding nutritional properties (Derbyshire *et al.* 2010). In addition, dairy products are excellent source of calcium and protein but contain very little iron (0.2-0.4 mg/kg) (Xia and Xu 2005). Therefore, dairy products are not suitable for providing dietary iron nevertheless; fortification can make these as excellent sources of this trace element alongside with its high calcium content for health conscious consumers (Hekmat and McMahon 1997). An advantage of using dairy products as the vehicle for fortifying iron is that, iron fortified dairy products have a relatively high iron bioavailability (Woestyne *et al.* 1991).

Among all the dairy products, yoghurt is preferred since it is widely consumed throughout the world for its sensory and nutritional benefits. It is obtained by the lactic acid fermentation of whole, skimmed or standardized milk by the action of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, and can be accompanied by other lactic bacteria which, for their part, contribute to the characteristics of the final product (Brasil 2007). Yoghurt is believed to be effective in both the prevention and treatment of various illnesses, viz., gastrointestinal disorders, heart diseases and tumor development in man as well as animals (Vijayendra and Gupta 2011).

## 2.3 Iron fortification

Iron fortification of foods is regarded as the most cost effective method for reducing the prevalence of nutritional iron deficiency (Huma *et al.* 2007).

### 2.3.1 Physiological significance of iron

Iron is an essential trace element in animal and human diets. It is naturally present in the structure of cytochromes, enzymes, haemoglobin and myoglobin (Abbasi and Azari 2011). Besides its important role in transport and storage of oxygen, oxidative metabolism, cellular proliferation and growth it is also a cofactor in various biochemical processes, including DNA synthesis (Petranovic *et al.* 2008). It is a redox metal and participates in most of the reversible redox reactions by switching between the two oxidation states, ferrous and ferric. This redox activity of

iron can produce free radicals responsible for cell signaling processes and iron mediated toxicity. Iron is also an essential mineral for all known pathogens. In human beings, it is absorbed primarily in the duodenum, transported through the blood stream and extracellular fluid bound to transferrin and stored intracellular predominantly in the form of ferritin. Iron balance is rigorously controlled by the regulation of its absorption (Allen *et al.* 2006).

### 2.3.2 Iron absorption

Many components of diet as well as a person's nutritional status with respect to nutrients other than iron can have a significant impact on both iron absorption and internal iron metabolism. Iron is widely distributed in human food items (Bothwell *et al.* 1979). Animal tissues contain iron in the form of haem, ferritin and hemosiderin as well as iron bound to membranes and low molecular weight compounds. Iron in other animal-derived foods may also be bound to specific proteins such as lactoferrin in milk, ovotransferrin in egg white and phosvitin in egg yolk. Plant foods contain iron in the form of metalloproteins, plant ferritins, iron present in the sap and iron complexed to structural components or storage compounds predominantly as phytates (Hazell 1985). In addition, foods may contain inorganic iron salts such as ferric oxides and hydroxides as contaminants (Cook 1990) or iron compounds added during processing to fortify the diet.

### 2.3.3 Sources

Dietary sources of iron are found in two forms: haem iron and non-haem iron (Chen and Oldwage 2002). Haem sources are provided by animal tissues (meats) and are readily absorbed. Approximately 40% of iron found in meat is haem, with the best sources being liver, seafood, fish, lean meat and poultry. Non-haem iron is provided by plant sources and elemental components of animal tissues. It is less efficiently absorbed and the amount absorbed depends upon the body's needs. If there are low stores, more iron will be absorbed and vice-versa. Non-haem sources that are high in iron include cooked spinach, beans, eggs, nuts, fortified breads, cereals and flours.

### 2.3.4 Iron requirements

Basal iron loss forms the fundamental requirement for all. Actual body iron losses amount to approximately 1 mg/day in healthy adult men (Green *et al.* 1968; Hallberg *et al.* 1998) and are normally adequately covered by dietary iron absorption (Cook 1990; Hulten *et al.* 1995). However, regular blood losses cause menstruating women to face an additional loss of 0.5-1.5 mg iron per day (Hallberg and Rossander-Hulten 1991), which is more difficult to balance by dietary means. Likewise, children and adolescents require 0.5-1.5 mg in excess of their daily iron losses to satisfy the extra needs associated with growth. Finally, the total iron cost of pregnancy is estimated at 1040 mg, of which 200 mg are retained by the woman when blood volume decreases after delivery and 840 mg are permanently lost (Picciano 2003). The average daily requirements reach as high as 4-6 mg during the second and third trimester for foetal growth and blood volume expansion (Carpenter and Mahoney 1992).

**Table 2.1: Recommended dietary allowances of iron for Indians**

| Group | Recommended dietary allowances | | | |
|---|---|---|---|---|
| | **Body weight (kg)** | **Requirement (µg/kg/d)** | **Absorption assumed (%)** | **RDA (mg/d)** |
| Adult Man | 60 | 14 | 5 | 17 |
| Woman (NPNL) | 55 | 30 | 8 | 21 |
| Pregnant woman | 55[a] | 51 | 8 | 35 |
| Lactating woman (0-6 m) | 55 | 23 | 8 | 25 |
| Infants 0-6 m | 5.4 | 46 | --- | --- |
| 6-12 m | 8.4 | 87 | 15 | 5 |
| Children 1-3 y | 12.9 | 35 | 5 | 9 |
| 4-6 y | 18.0 | 35 | 5 | 13 |
| 7-9 y | 25.1 | 31 | 5 | 16 |
| Adolescents Boy 10-12 y | 34.3 | 31 | 5 | 21 |
| Girl 10-12 y | 35.0 | 38 | 5 | 27 |
| Boy 13-15 y | 47.6 | 34 | 5 | 32 |
| Girl 13-15 y | 46.6 | 29 | 5 | 27 |
| Boy 16-17 y | 55.4 | 25 | 5 | 28 |
| Girl 16-17 y | 52.1 | 25 | 5 | 26 |

[a]Pre-pregnancy weight

(Source: ICMR 2009)

Net iron losses from the body through the skin, urine and blood are about 2 mg/day for women, 1 mg/day for men and 0.8 mg/day for children, reflecting that net iron requirements are indeed very low. Recommended dietary allowance (RDA) of iron is much higher than net iron requirement, taking into account the poor bioavailability of dietary iron (Herbert 1987). Recommended dietary allowances of iron for Indians have been shown in table 2.1.

### 2.3.5 Iron deficiency

The main risk factors for iron deficiency in body are low intake of haem iron and vitamin C (ascorbic acid), poor absorption of iron from foods high in phytates, periods of life when iron requirements are especially high (i.e. growth and pregnancy) and heavy blood losses in menstruation or parasite infections. Iron deficiency induces anaemia which affects more than 30% of the world's population, especially infants, women and teenagers. Around 60% women of reproductive age and 40-50% teenagers are affected by iron deficiency. Fortification of milk with iron and vitamin C in Chile produced a rapid reduction in the prevalence of iron deficiency in infants and young children (Stekel *et al.*1988; Hertrampf 2002).

### 2.3.6 Safety issues

Higher levels of iron intake and elevated body stores are potential risk factors for both coronary heart disease (CHD) and cancer. Inflammatory response is a risk factor for CHD and also increases serum ferritin, which might explain why an association between the risk of CHD and increased serum ferritin is sometimes observed (Danesh and Appleby 1999). It has been hypothesised that the presence of unabsorbed fortified iron in the body, much of which reaches the colon, leads to free radical generation that damages the colon mucosa. However, there is no evidence that the free radicals survive long enough to cause tissue damage (Lund *et al.* 1999).

### 2.3.7 Choice of iron fortificant

Technically, iron is the most challenging micronutrient to be added to foods. Iron compounds that have the best bioavailability tend to be those which interact most strongly with food constituents to produce undesirable organoleptic changes. The ideal product for food fortification is one that supplies highly bioavailable iron,

does not diminish the nutritional value of the food vehicle through nutrient oxidation, does not alter its sensory properties, can be used to fortify solid and liquid foods, is resistant to food technology processes and is low in cost so that it can be accessible to the whole population (Boccio *et al.* 1996).

The selection of iron compound for fortification is important in order to avoid interactions of iron with food vehicle or the total meal because a minor change in organoleptic characteristics of the food will result in consumer rejection. When the iron compound is added, it is necessary to evaluate possible changes in food colour, taste or appearance with time and storage under adverse temperature and humidity conditions. Solubility, chemical reactivity, bioavailability and cost are other important issues when selecting an iron compound. For instance, ferrous sulphate is a highly bioavailable and relatively inexpensive compound, however, because of its high reactivity, it produces undesirable changes in some fortified foods. On the other hand, elemental iron (reduced, electrolytic or carbonyl) is also inexpensive, however, it has been reported to have a low bioavailability depending on particle size and the food vehicle to be fortified (Hallberg 1981; Cook 1983; Hallberg *et al.* 1986).

**2.3.7.1 Types of iron fortificants**

A wide variety of iron compounds are currently being used as food fortificants (table 2.2). These can be broadly divided into four categories:

**2.3.7.1.1 Water soluble compounds**

Being highly soluble in gastric juices, the water soluble iron compounds have the highest relative bioavailability amongst all the iron fortificants. However, these compounds are also most likely to have adverse effects on the organoleptic properties of foods, in particular, on the colour and flavour. During prolonged storage, the presence of fortified iron in certain foods can cause rancidity and subsequent off-flavours. Moreover, in the case of multiple fortification, free iron, produced from the degradation of iron compounds present in the food, can oxidise some of the vitamins supplied in the same fortificant mixture. The water-soluble forms of iron are especially suited to fortify cereal flours. Water soluble iron compounds are also useful for dry foods, such as pasta and milk powder, as well as dried milk-based infant formulas (Allen *et al.* 2006).

Among the various iron fortificants, ferrous sulphate is a water soluble compound that has the highest relative bioavailability among conventional iron compounds (Hurrell and Cook 1990). However, it is relatively unstable and may reduce vehicle-food quality and shelf life due to potential oxidised off-flavours, colour changes and metallic flavours (Jackson and Lee 1992a).

**2.3.7.1.2 Iron compounds that are poorly soluble in water but soluble in dilute acids**

This type of iron fortificants are also reasonably well absorbed from food, as they are soluble in the gastric acids produced in the stomach of normal healthy adults and adolescents. In most people, iron absorption from these compounds is likely to be similar to that from water soluble iron compounds. Poorly water soluble iron compounds, such as ferrous fumarate, have the advantage of causing fewer sensory problems in foods than the water soluble compounds, and are generally next in line for consideration, especially if more water soluble forms cause unacceptable organoleptic changes in the chosen food vehicle. Ferrous fumarate and ferric saccharate are the most commonly used iron compounds in this group and as bioavailable as ferrous sulfate. The former is frequently used to fortify infant cereals and the latter to fortify chocolate drink powders (Allen *et al.* 2006)

**2.3.7.1.3 Iron compounds that are insoluble in water and poorly soluble in dilute acids**

Relative to ferrous sulfate, the absorption of iron from water insoluble compounds ranges from approximately 20 to 75%. However, these have far less effects on the sensory properties of food. They are generally regarded as the last resort option, especially in settings where the diet of the target population is high in iron absorption inhibitors. Within this category of iron fortificants, the ferric phosphate compounds i.e. ferric orthophosphate and ferric pyrophosphate are used to fortify rice and some infant cereals and chocolate containing foods. The relative bioavailability of ferric pyrophosphate is reported to be 21–74% and that of ferric orthophosphate 25-32% (Theuer *et al.* 1973; Hurrell *et al.* 1991).

Elemental iron is half bioavailable and more stable as compared to ferrous sulphate (Hallberg *et al.* 1986). If elemental iron is used as the fortificant, higher

doses (two or three times more than those required for ferrous sulphate) may be needed to compensate for the lower bioavailability. This makes the cost of fortification with elemental iron higher as compared with ferrous sulphate. Elemental iron in wheat flour fortified at a higher rate also had an adverse effect on the chapatti quality, however, there is contradiction among different studies, as the preparation and baking method are different (Hinnai *et al.* 2000; Rehman *et al.* 2003).

**2.3.7.1.4 Encapsulated or protected forms**

Novel microencapsulation technologies render iron fortificants that are more resistant to interaction with other components in the food vehicles, thus minimising organoleptic changes, increasing shelf life and maximising consumer acceptance. Most of the iron compounds can be microencapsulated, however, the most available products are the microencapsulated form of both ferrous sulphate and ferrous fumurate (Boccio *et al.* 1997). The coatings are usually a mixture of phospholipids, polysaccharides, protein or partially hydrogenated oil. Microencapsulation has little influence on relative bioavailability and the main advantages are fewer organoleptic changes and a prolonged shelf life of fortified foods. This is a result of the bilayer coating, protecting against the interaction between iron and absorption factors in the fortified foods.

Encapsulation increases cost 3 to 5 folds, which when expressed in terms of iron amounts, is equivalent to a 10-folds increase in cost relative to the use of dried ferrous sulfate. The main purpose of encapsulation is to separate iron from the other food components, thereby mitigating sensory changes (Hurrell 1999, 2002; Swain *et al.* 2003). When developing encapsulated iron fortificants, it is important to select a coating material which provides an adequate balance between stability and bioavailability. Iron compounds are usually encapsulated with hydrogenated vegetable oils, however, mono- and diglycerides, maltodextrins and ethyl cellulose, have also been used (Allen *et al.* 2006).

Iron compounds listed as generally recognised as safe (GRAS) by United States Food and Drug Administration (USFDA) include elemental iron, ferrous ascorbate, ferrous carbonate, ferrous citrate, ferrous fumarate, ferrous gluconate,

ferrous lactate, ferrous sulphate, ferric ammonium citrate, ferric chloride, ferric citrate, ferric pyrophosphate and ferric sulphate (Whittaker 1998).

### 2.3.8 Novel iron fortificants

**Table 2.2: Key characteristics of iron compounds used for food fortification purpose: solubility, bioavailability and cost**

| Compound | Iron content (%) | Relative bioavailability[a] | Relative cost[b] (per mg iron) |
|---|---|---|---|
| 1. **Water soluble** | | | |
| Ferrous sulphate. 7 $H_2O$ | 20 | 100 | 1.0 |
| Ferrous sulphate, dried | 33 | 100 | 1.0 |
| Ferrous gluconate | 12 | 89 | 6.7 |
| Ferrous lactate | 19 | 67 | 7.5 |
| Ferrous bisglycinate | 20 | >100[c] | 17.6 |
| Ferric ammonium citrate | 17 | 51 | 4.4 |
| Monosodium ferric EDTA | 13 | >100[c] | 16.7 |
| **2. Poorly water soluble, soluble in dilute acids** | | | |
| Ferrous fumarate | 33 | 100 | 2.2 |
| Ferrous succinate | 33 | 92 | 9.7 |
| Ferric saccharate | 10 | 74 | 8.1 |
| **3. Water insoluble, poorly soluble in dilute acids** | | | |
| Ferric orthophosphate | 29 | 25-32 | 4.0 |
| Ferric pyrophosphate | 25 | 21-74 | 4.7 |
| Elemental iron | | | |
| H-reduced | 96 | 13-148[d] | 0.5 |
| Atomised | 96 | (24) | 0.4 |
| CO-reduced | 97 | (12-32) | <1.0 |
| Electrolytic | 97 | 75 | 0.8 |
| Carbonyl | 99 | 5-20 | 2.2 |
| **4. Encapsulated forms** | | | |
| Ferrous sulphate | 16 | 100 | 10.8 |
| Ferrous fumarate | 16 | 100 | 17.4 |

EDTA: ethylenediaminetetraacetate; H-reduced: hydrogen reduced; CO-reduced: carbon monoxide reduced.

a. Relative to hydrated ferrous sulphate ($FeSO_4$. $7H_2O$), in adult humans. Values in parenthesis are derived from studies in rats.

b. Relative to dried ferrous sulphate per mg of iron, the cost of hydrated and dry ferrous sulphate in similar.

c. Absorption is two-three times better than from ferrous sulphate if the phytate content of food vehicle is high.

d. The high value refers to a very small particle size which has only been used in experimental studies.

**Sources:** Hurrell 1999, 2002; Swain *et al.* 2003

In recent years, considerable efforts have been devoted to the development and testing of alternative iron fortificants, in particular, fortificants that provide better protection against iron absorption inhibitors than those currently available. Among those at an experimental stage are monosodium ferric EDTA (NaFeEDTA), ferrous bisglycinate and various encapsulated and micronised iron compounds. In high-phytate foods, the absorption of iron from NaFeEDTA is 2–3 times greater than that from either ferrous sulfate or ferrous fumarate. In foods with low phytate content, however, iron absorption is similar (INACG 1993 and Hurrell *et al.* 2000). The Joint FAO/WHO Expert Committee on Food Additives has approved the use of NaFeEDTA at 0.2 mg iron/kg body weight per day (FAO/WHO 1999).

### 2.3.9 Methods used to increase the amount of iron absorbed from fortificants

The bioavailability of iron from fortificants depends not only on the solubility of the fortificant, but also on the composition of the diet, in particular, on the proportion of inhibitors of iron absorption in the diet, notably iron-binding phytates and certain phenolic compounds. The addition of ascorbic acid (vitamin C) or sodium ethylenediaminetetraacetic acid ($Na_2EDTA$) and the removal of phytates, all of which reduce the effect of the inhibitors, can be effective ways of increasing the total amount of iron absorbed from iron fortified foods.

### 2.3.10 Enhancers of non-haem iron absorption

#### 2.3.10.1 Ascorbic acid

Ascorbic acid enhances iron absorption, if eaten in diet together with iron. The absorption enhancing effect is more pronounced in inhibitory meals and meals which contain two main inhibitors of iron absorption viz. phytates and polyphenols (Cook and Monsen 1977; Siegenberg *et al.* 1991). Ascorbic acid acts by maintaining iron in a soluble bioavailable form as the luminal pH rises once the gastric contents enter the duodenum, since ferric form of iron is soluble only at acidic pH, however, the complex of iron and ascorbic acid is soluble over a wide pH range (Conrad and Schade 1968).

#### 2.3.10.2 Organic acids

Gillooly *et al.* (1983) measured the iron absorption associated with 17 vegetable meals. All the vegetables associated with good iron bioavailability

contained appreciable amounts of one or more of the organic acids (citric, malic or ascorbic acids). The addition of citric, malic or tartaric acid to a rice-based meal improved iron absorption 2- to 4-folds (Ballot *et al.* 1987)

#### 2.3.10.3 Animal tissues

Several animal tissues, including beef, chicken, fish, lamb, liver and pork, improve iron status both by supplying highly available haem iron and by improving absorption from non-haem iron pool (Cook and Monsen 1976; Lynch *et al.* 1989). Although the factors in animal tissue responsible for these beneficial properties remain poorly characterised, it has been suggested that peptides released during proteolytic digestion by pepsin in the stomach may increase the solubility of inorganic iron (Kane and Miller 1984; Hurrell *et al.* 1988; Slatkavitz and Clydesdale 1988).

### 2.3.11 Inhibitors of non-haem iron absorption

#### 2.3.11.1 Phytates

A large number of studies have demonstrated that phytates are the major iron absorption inhibitors in cereal foods such as wheat, oats, sorghum, unpolished rice and beans (Gillooly *et al.* 1983, 1984; Rossander-Hulthen *et al.* 1990; Hurrell *et al.* 1992). Monoferric phytate, which constitutes only a small proportion of the phytate in bran, is not inhibitory (Simpson *et al.* 1981), however, the formation of diferric and tetraferric phytate complexes in the gastrointestinal tract may render iron unavailable for absorption (Morris and Ellis 1982). Dephytinisation of food products seems to be a possible method to improve iron absorption (Hurrell 1992).

#### 2.3.11.2 Polyphenols

Disler *et al.* (1975a) reported that tea is a powerful inhibitor of iron absorption; this is primarily the result of its tannin content (Disler *et al.* 1975b). Polyphenols are thought to act through the formation of complexes between the hydroxyl groups of the phenolic compounds and iron molecules, rendering the iron unavailable for absorption.

#### 2.3.11.3 Calcium

The addition of calcium in the form of milk or an inorganic salt to a meal reduces percentage non-haem iron absorption in human beings. The addition of

calcium and phosphate diminished percentage iron absorption while the addition of calcium alone had no effect. Hallberg *et al.* (1991) demonstrated that doses of calcium chloride (between 40 to 600 mg calcium) caused a dose-related reduction (upto 300 mg calcium) in non-haem iron absorption from a meal of wheat rolls containing 10 mg native calcium and 3.8 mg iron.

**2.3.11.4 Fiber**

Components of fiber bind iron *in vitro*. However, there is little current evidence to suggest an important role for fiber in human studies. Enzymatic dephytinisation of bran almost completely removes the inhibitory effect (Hallberg 1987). Several purified fiber components have been tested for their effect on human iron absorption. Cellulose and pectins are not inhibitory. Ispagula and psyllium cause a mild reduction in absorption (Rossander 1987).

**2.3.12 Food vehicles for iron fortification**

**2.3.12.1 Cereal products**

Cereal flours are currently the most frequently used vehicles for iron fortification that reach the entire population. The amount of iron added is usually relatively low because it is added only to restore the iron level in milled flour to that of whole grain. With true fortification, a higher amount than is usually present would be added. The contribution of fortified iron to iron intake is highest in the United States, where it accounts for 20-25% of total iron intake. The contribution of fortified iron to iron intake in the United Kingdom is much lower i.e. around 6% (Hurrell 1997).

Wheat flour enrichment is mandatory in many countries. Recently, it has been proposed that wheat flour in Central America to be fortified with 60 mg/kg of flour with reduced electrolytic iron of particle size less than 40 μm. Technology also exists for fortifying whole grains such as rice. This can be done by coating, infusing or by using extruded grain analogues. The fortified grains are then mixed 1:100 or 1:200 with the normal grains. Hunnell *et al.* (1985) described a sophisticated method of preparing fortified rice grains by first infusing B vitamins and then adding iron, calcium and vitamin E in separate layers of coating material. There are two major disadvantages in using cereal products as vehicles for iron fortification. First, they

contain high levels of phytic acid, a potential inhibitor of iron absorption upto 1% in whole grains and about 100 mg/100 g in high-extraction flours (Davidsson 2003). Second, these are extremely sensitive to fat oxidation during storage when highly bioavailable iron compounds such as ferrous sulphate are added (Hurrell *et al.* 1989).

For organoleptic reasons, cereal flours such as wheat and maize are usually fortified with poorly absorbed elemental iron powders and rice with ferric orthophosphate or ferric pyrophosphate (Hunnell *et al.* 1985). Only bread and wheat flour stored for less than 3 months and pasta products, because of their low moisture content, can be fortified with highly bioavailable ferrous sulphate (Barret and Ranum 1985). However, even in these foods, iron absorption will be inhibited by the presence of phytic acid, unless an absorption enhancer is present (Hurrell 1997).

In India, it recommended that fortified atta and maida contain either one or more of these materials i.e. calcium, carbonate, iron, thiamine, riboflavin and niacin (FSSAI 2011).

**2.3.12.2 Salt**

Iodine fortified salt has successfully eradicated iodine deficiency in many countries (Nadiger *et al.* 1980), therefore, salt would also seem a highly suitable vehicle for iron fortification. However, iron fortification of salt poses many technical problems, and for developing countries, an efficient production and distribution system must also exist. Almost all of the developmental work for the fortification of salt with iron has been conducted in India. Colour changes during storage have been the main problem, because salt in India is relatively crude and contains upto 4% moisture. All soluble iron compounds and vitamin C caused unacceptable colour changes (Hurrell 1997). Fortification was possible only with insoluble iron compounds and ferric orthophosphate was recommended at a level of 1 mg per g salt so as to provide about 15 mg extra iron per day. When $NaHSO_4$ (sodium hydrogen sulphate) was added as an absorption promoter, absorption was reported to be 80% to that of ferrous sulphate.

A small scale fortification trial in which the fortified salt was included in a school feeding program, demonstrated an improvement in iron status in India

(Andersson *et al.* 2008). Salt that contains fewer impurities would undoubtedly be easier to fortify. However, the extra cost to the consumer is always a major consideration in developing countries. In addition, there is always the possibility that the iron fortified salt will cause unacceptable colour reactions if added to vegetables in a meal. This was one of the explanations offered for the failure of a fortification program in the Seychelles and Mauritius in the early 1960s (Foy 1976). The other reasons were the relatively poor bioavailability of the ferric pyrophosphate used and the fact that it separated from the salt and sank to bottom of salt barrels. Overcoming all the difficulties, the scientists at National Institution of Nutrition (NIN), Hyderabad, India, have developed a technology for double fortification of salt with iodine and iron (www.fnbnew.com, 2012).

In India, iron fortified common salt should contain around 850-1100 ppm iron salt and permitted anti caking agent like calcium, sodium and potassium ferrocyanide at the level not exceeding 10 mg/kg singly or in combination expressed as ferrocyanide (FSSAI 2011).

**2.3.12.3 Sugar**

Sugar is an alternative vehicle for iron fortification in the regions of the world where it is produced, such as the Carribean and Central America, however, in other developing countries; refined sugar consumption is more common in middle and upper socio-economic segments of the population (Lynch *et al.* 1993). Iron from fortified sugar would be expected to be well absorbed if consumed with citrus drinks but poorly absorbed from coffee and tea owing to phenolic compounds or, if added to cereal products, owing to phytates. As with salt, the main technical problem is to select a bioavailable iron compound that does not cause unwanted colour changes in less pure sugar products.

In Guatemala, this was overcome by adding monosodium ferric EDTA (NaFeEDTA). Commercially, white cane sugar would appear easier to fortify. Disler *et al.* (1975a) reported the successful addition of different ferric and ferrous compounds (100-200 mg iron/kg) together with vitamin C to white cane sugar. There were, however, unacceptable colour reactions when added to coffee and tea or to certain maize products. The use of sugar fortified with iron tris-glycinate chelate in

preschool children of Brazil was reported to be effective in the prevention of iron deficiency anaemia (Paula and Fisberg 2001).

In India, it is recommended that plantation white sugar, refined sugar, khandsari sugar, bura sugar and misri should be free from any iron filings (FSSAI 2011).

**2.3.12.4 Condiments**

Condiments that are traditionally used in developing countries, such as monosodium glutamate, fish sauce, curry powder and bouillon cubes, could be useful fortification vehicles (Lynch *et al.* 1993). Monosodium glutamate (MSG) is widely used as a flavour enhancer in Asia and has been successfully fortified with ferric orthophosphate and ferrous sulphate encapsulated in zinc stearate. Pilot fortification trials with fish sauce or curry powder, both fortified with monosodium ferric EDTA, resulted in significant improvement in iron status in the population consuming the fortified products (Ballot *et al.* 1989; Garby and Areekul 1974). The success of fortified condiments presumably depends on the absence of adverse colour reactions and on the addition of an absorption enhancer, such as EDTA.

**2.3.12.5 Coffee**

Coffee is widely consumed by adults as well as some children, and it is technically and economically feasible to fortify coffee with iron. Johnson and Evans (1977) reported the use of ferrous fumarate in roasted and ground coffee, in which one cup (200 ml) provided 1 mg added iron. The addition of iron to soluble coffee is also relatively easy as reported by Klug *et al.* (1973), who found that addition of a range of soluble ferrous and ferric compounds was possible. Flavour and colour changes are the potential problems for forfication of coffee with iron compounds. Coffee and coffee like products (tea and cocoa) contain phenolic compounds that strongly inhibit iron absorption.

**2.3.13 Applications of iron fortification in the dairy industry**

**2.3.13.1 Cheddar cheese**

Cheddar cheese was manufactured from iron supplemented milk (Zhang and Mahoney 1988, 1989, 1990, 1991). Different sources and levels of iron were used. Iron recoveries in the cheeses were 71-81% for ferric chloride, 52-53% for ferric

citrate, 55-75% for iron-casein complex and 70-75% for ferripolyphosphate–whey protein complex. TBA numbers increased slightly in iron fortified cheeses but were within the range reported by others for unfortified cheeses. Aging cheeses up to three months did not change the TBA assay or oxidised off-flavour and cheese flavour scores. Cheddar cheese fortification (40-50 mg/kg) with ferripolyphosphate-whey protein complex, ferric-casein complex and ferric chloride was possible without affecting its quality.

**2.3.13.2 Mozzarella cheese**

Mozzarella cheese containing 25 and 50 mg iron/kg cheese was manufactured from milk fortified with casein- or whey protein-chelated iron or ferric chloride. Addition of 25 mg iron/kg of cheese had no effect on the physical properties of cheese. Apparent viscosity of cheese fortified with 50 mg of iron/kg of cheese tended to be slightly higher than the control cheese, although this difference was not significant at all storage times. No increase in chemical oxidation was observed between the control and iron fortified cheeses. Slight increase in metallic and oxidised flavours was observed in the iron fortified cheese. Sensory scores for iron fortified cheese made using casein-chelated iron or whey protein-chelated iron were not significantly different from those of cheese made using ferric chloride (Rice and McMahon 1998).

**2.3.13.3 White soft cheese**

A white soft cheese was produced from pre-cheese retentate fortified with electrolytic iron, ferric chloride and ferrous sulphate at a level of 80 mg iron/kg. White soft cheese fortified with electrolytic iron or $FeCl_3$ was acceptable as compare to others (El-Samragy *et al.* 1995).

**2.3.13.4 Baker's and cottage cheese**

Skim milk fortified with ferric ammonium citrate was used for the manufacture of Baker's and cottage cheese (Sadler *et al.* 1973). Approximately 14% of the iron was retained in Baker's cheese and 58% in the cottage cheese. Added iron had no effect on starter activity or on the initial flavour of the product. During storage for 2 months, no accelerated development of off-flavours was observed in the iron fortified cheese.

### 2.3.13.5 Infant foods

Infant formula is an excellent vehicle for iron fortification as it is consumed by the non-breastfed infants in large quantities. Further, the commercially available infant formulas, which are either milk based or soy based products, can be fortified with readily bioavailable ferrous sulphate without causing discolouration or fat oxidation. Ziegler and Fomon (1996) reported that infant formulas always contain ascorbic acid (minimum levels approximately 50 mg ascorbic acid per litre formula) which would further increase the amount of available iron. Presently, formulas (for healthy term infants) in the United States are commonly fortified with 12 mg iron/litre, in agreement with the recommendation of the American Academy of Pediatrics' Committee on Nutrition (1999). Dried whole milk powders and dried or ready-to-feed milk-based infant formulas can be successfully fortified with ferrous sulfate (together with ascorbic acid to enhance absorption). In Chile, ascorbic acid (700 mg/kg) and iron (100 mg as ferrous sulfate/kg) are routinely added to dried milk powders consumed by infants.

In Europe, infant formula should contain between 3 and 10 mg iron/litre, although formulas with no added iron are permitted providing a statement is made to the effect that, when the product is given to infants over the age of 4 months, their total iron requirements must be met from other additional sources (Moy 2000). Ziegler and Fomon (1996) reported that in the United States, "unfortified" or low-iron infant formulas contain small amount of added iron (1.5-4.0 mg/L). It is however, discussed whether the iron fortification levels, especially those in the United States, are too high (Dallman 1989; Moy 2000). This is based on the findings showing that iron absorption increases with decreasing iron content (Fomon *et al.* 1997). Further, long term evaluations (0-9, 1.5-6 or 6-12 months of age) have shown that infant formulas with low iron content can be just as effective in preventing iron deficiency as those with high iron content (Lonnerdal and Hernell 1994; Walter *et al.* 1998).

Infant cereals are commonly fortified with iron. Hurrell *et al.* (1989) and Theuer (2002) have suggested that less soluble iron compounds are used to avoid organoleptic problems since ferrous sulphate provokes off-colours as well as fat oxidation. These include elemental iron powders in United States and ferric

pyrophosphate and ferric orthophosphate in Europe (Hurrell 1989). It is difficult to fortify infant cereals with readily available iron compounds, since these have the further disadvantage of containing phytic acid. However, the inhibitory effect of phytic acid can be overcome by the addition of ascorbic acid (Davidsson *et al.* 1997).

In India, it is mandatory to fortify infant milk food, infant formula, follow up formula and weaning food (milk/processed cereal based complementary food) with iron at a level not less than 5mg/100g. For infant milk food, infant formula and follow up formula recommended salts for fortification are ferrous sulphate, ferrous lactate, ferrous citrate, and ferric pyrophosphate and for weaning food (milk/processed cereal based complementary food) it is hydrogen reduced iron and electrolytic iron (FSSAI 2011).

**2.3.13.6 Yoghurt**

Yoghurts (non-fat and low fat) were manufactured and fortified with 10, 20 and 40 mg of iron/kg of yoghurt (Hekmat and McMahon 1997) using whey protein and casein chelated iron. Counts of *Lactobacillus delbruckii* ssp. *bulgaricus* and *Streptococcus thermophilus* after 1 day of storage in iron fortified skim yoghurt was $7.0 \times 10^8$ cfu/ml which were similar to numbers in unfortified yoghurts. No increase in chemical oxidation was detected. However, iron fortified yoghurts had slightly higher oxidised flavour scores than the control yoghurt. There was no increase in metallic, bitter or other off-flavours. The consumer panel did not detect any differences in the appearance, mouthfeel, flavour or overall quality between fortified and unfortified flavoured yoghurts.

Achanta *et al.* (2007) fortified fat free plain set yoghurt with various minerals and observed better water holding capacity of the yoghurt in case of iron fortification. El-Kholy *et al.* (2011) fortified yoghurt with five different iron salts (20mg iron/Kg milk) viz. ammonium ferric sulphate, ammonium ferrous sulphate, Fe-casein complex, Fe-whey protein complex and ferrous lactate. Fortification of yoghurt with ammonium ferric sulphate and ammonium ferrous sulphate resulted in significant increase in oxidation (as measured by TBA test) as compare to unfortified yoghurt. All samples were acceptable on the sensory basis.

Bulgarian yoghurt was also fortified with 8, 15 and 27mg of iron/kg of yoghurt using ferrous lactate hydrate and growth was monitored for the starter culture bacteria during milk fermentation and over 15 days of storage at $4^0$C.Fortification of milk with iron did not affect the growth of starter culture during manufacture and storage of yoghurt. Counts of yoghurt bacteria at the end of fermentation of iron fortified milk was between $2.1 \times 10^{10}$ and $4.6 \times 10^{10}$cfu/ml, which were significantly not different from numbers in unfortified yoghurts. It was also found that iron fortified yoghurt did not differ significantly in their sensory, chemical and microbiological parameters with unfortified yoghurt. It suggested that yoghurt is a suitable vehicle for iron fortification and ferrous lactate is an appropriate iron source for yoghurt fortification (Simova *et al.* 2008).

**2.3.13.7 Iron fortified chocolate milk**

Chocolate milks, fortified with iron compounds were evaluated for changes in colour and flavour. Sodium ferric pyrophosphate, ferripolyphosphate and ferripolyphosphate–whey protein complex produced little or no off-colour change in the products initially and after 2 weeks of storage. All other added compounds resulted in initial and persistent off-colours. Flavour evaluation showed that ferric compounds produced little or no off-flavours in chocolate milks initially or after holding at 4ºC for 7 and 14 days. Ferrous compounds produced off-flavours initially, however, flavour scores improved after milks were held at 4ºC for 14 days (Douglas *et al.* 1981). Iron fortification of milk has always been difficult because of production of undesirable colour and flavour changes due to iron stimulated autoxidation of milk fat. Kinder *et al.* (1942) proposed that cocoa and chocolate contain natural antioxidants which would prevent development of oxidative rancidity in chocolate milk.

**2.3.13.8 Iron fortified milk**

Ferrous sulfate and many other soluble iron compounds cannot be used to fortify liquid whole milk and other dairy products since they cause rancidity and off-flavours. Iron fortificants are best added after the milk has been homogenised and the fat internalised in micelles, so as to help in protecting the fat against oxidation. Ferrous bisglycinate is widely used to fortify whole milk and dairy products in Brazil

and Italy. Micronised ferric pyrophosphate is added to dairy products in Japan. Ferric ammonium citrate, ferrous bisglycinate and micronised ferric pyrophosphate have been suggested for fluid milk fortification and ferrous sulphate plus ascorbic acid for dry milk (Allen *et al.* 2006).

Wang and King (1973) have reported that ferrous sulphate, ferric ammonium sulphate and citrate caused TBA values of 0.092, 0.046 and 0.023 at concentrations of iron 10 ppm only, thereby proving that ferric ammonium citrate was the best compound to be used for fortification in milk. Kurtz *et al.* (1973) reported that ferric ammonium citrate and ferric chloride @20 ppm iron could be used for fortification of milk without causing adverse flavour effects. Olivares *et al.* (1997) suggested ferrous bisglycinate for fortification of fluid whole milk and other high fat vehicles. The good bioavailability of ferrous bisglycinate makes this compound a suitable alternative to be considered for iron fortification programme.

Ravikiran *et al.* (1977) showed that no appreciable flavour was detected after fortification of milk with ferric lactose at 4ºC upto 24 hrs. The combination of ascorbic acid and iron in the doses studied (50 mg ferric lactose + 50 mg ascorbic acid per 100 ml of milk) could provide an effective antioxidant system without much loss of ascorbic acid. Ferric lactose alone caused oxidation, resulting in a higher TBA value as compared to that with control (unfortified milk). Ascorbic acid not only protected the milk from fat oxidation, but it also completely suppressed oxidation of milk fat caused by ferric lactose (50 mg/100 ml). No appreciable detrimental effect could be observed on pH, flavour (organoleptic) and rancidity (lipolytic) or other physico-chemical characteristics.

Baldwin *et al.* (1982) reported that fortification of milk with reduced iron, complexed with citric acid and phosphoric acids, lowered the intensity of cooked flavour and exerted little influence on oxidised flavour in milk pasteurised at 80ºC for 25 seconds. They also concluded that the likelihood of oxidised off-flavours would be greater if the iron sources were added after pasteurisation at 72ºC for 15 seconds.

### 2.3.14 Microencapsulation

Microencapsulation is a technology of packaging solids, liquids or gaseous materials in miniature sealed capsules that can release their contents at controlled

rates under the influence of specific conditions (Champange and Fustier 2007). Direct addition of iron to milk and milk products might have detrimental effects on the quality and acceptability due to the development of oxidised off-flavour, colour changes and metallic flavours (Wang and King 1973). Microencapsulation has been used for many years to protect sensitive food components, preserve desirable flavours and aroma, inhibit nutritional loss and mask undesirable flavours (Dziezak 1988).

**2.3.14 1 Microencapsulated iron for milk fortification**

Boccio *et al.* (1996) used a compound called SFE-171 (commercial name Biofer™), a ferrous sulfate microencapsulated with lecithin and claimed that this product had the same bioavailability as ferrous sulfate when fortified in milk and milk products. However, it had the advantage of being coated with a phospholipid membrane. This kept the iron from coming in contact with the food vehicle, prevented the undesirable interactions that happened when conventional ferrous sulfate is used.

Kwak *et al.* (2003a) developed a microencapsulated iron that could be used to fortify milk and determined the sensory properties of milk fortified with microencapsulated iron. Wall material was polyglycerol monostearate (PGMS) and selected core material was ferric ammonium sulfate. The highest efficiency of microencapsulation was 75% with 5:1:30 ratio (w/w/v) as coating to core materials to distilled water. Iron release was 12% when stored at 4°C for 3 days. TBA value was lowest when 100 ppm of encapsulated iron was added to milk and was significantly lower in encapsulated groups compared with that in unencapsulated groups. In a sensory analysis, most aspects except for metallic taste and colour were not significantly different between control and encapsulated iron fortified milk at 3 days of storage. However, encapsulated and unencapsulated groups differ significantly in their sensory attributes. Xia and Xu (2005) prepared iron containing liposomes (15 ppm iron as $FeSO_4$) using egg phosphatidylcholine, cholesterol and Tween 80 by reverse-phase evaporation method. The encapsulation efficiency (EE) was 67% and milk fortified with ferrous sulfate liposomes was stable to heat sterilisation (100ºC/30 minutes) and storage at 4ºC for one week.

Abbasi and Azari (2011) prepared the liposomes by reverse phase evaporation method and PGMS iron microencapsules by fatty acid ester (FAE) method. Liposomes were evaluated for the effect of iron/lipid ratio, tween 80 concentration and PGMS iron microencapsules for PGMS/iron salt ratio. Highest encapsulation efficiency (85.5%) of liposomes were obtained at iron/lipid ratio and tween 80 concentration of 0.04 and 5%, respectively whereas highest EE (81.8%) of PGMS iron microencapsules were obtained at PGMS/iron salt ratio 15:1. These microencapsules were fortified in pasteurised milk and evaluated for the stability, colour and sensory characteristics during storage. It was reported that the chemical lipid oxidation proceeded more rapidly in milk fortified with plain iron salt than in milk fortified with microencapsulated iron. In terms of sensory scores there was no significant difference in astringency, bitterness and colour score but significant difference in smell and metallic taste between iron fortified and unfortified samples. It was observed that FAE microencapsule showed similar results to control particularly at low concentration (7mg/l).

**2.3.14.2 Microencapsulated iron for cheese fortification**

Jackson and Lee (1992b) fortified the Harvati cheese with stearine coated microcapsules containing iron as $FeSO_4$, $FeSO_4$ with ascorbic acid and $FeCl_3$. Encapsulated $FeSO_4$ with ascorbic acid was fortified in cheese and obtained identical sensory scores as control cheese but other fortified cheeses showed lower sensory scores and undesirable oxidized flavour. Kwak *et al.* (2003b) encapsulated the ferric ammonium sulphate and L-ascorbic acid with PGMS and observed highest encapsulation efficiency of iron and L-ascorbic acid, 72 and 94%, respectively. TBA absorbance was significantly lower in microencapsulated treatments than unencapsulated treatment during ripening of the cheddar cheese.

**2.3.14.3 Microencapsulated iron for yoghurt fortification**

Kim *et al.* (2003) encapsulated the ferric ammonium sulphate and L-ascorbic acid with PGMS and observed highest encapsulation efficiency of iron and L-ascorbic acid as 73 and 95%, respectively. TBA absorbance was significantly lower in microencapsulated treatments than unencapsulated treatment during storage of the drinking yoghurt. Jayalalitha *et al.* (2012) fortified the probiotic yoghurt with

ferrous sulphate as encapsulated form. Encapsulated form was prepared by coating the iron whey protein chelate with sodium alginate. It was observed that iron fortification does not affect the viability of probiotic bacteria but showed highly significant difference in TBA value of control and iron fortified yoghurt.

**2.3.15 Different coating materials for microencapsulation**

**2.3.15.1 Sodium alginate, pectin and modified starch (HiCap 100) (MS)**

SA is water soluble and widely used for food, cosmetic and pharmaceuticals because it is biodegradable and biocompatible without any immunogenic effect. Mandal (2006) encapsulated *Lactobacillus casei* NCDC-298 with alginate and carrageenan and observed that survival of the probiotic bacteria improved at low pH, high bile salt conc. and during heat treatments. Kailasapathy (2006) encapsulated probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium lactis*) using alginate starch as coating material and observed that survival of the probiotic bacteria improved during storage. Homayouni (2007) microencapsulated *Lactobacillus casei* in the form of calcium alginate beads and evaluated it for the effect of lecithin and calcium chloride solution on the microencapsulation process yield. It was observed that the yield in microencapsulation process increased with increasing the volume of calcium chloride solution and decreased with increasing the amount of lecithin. Suthar (2008) microencapsulated *Enterococcus faecium* 59 and *Lactobacillus acidophilus* NCDC 15 using sodium alginate, corn starch and different levels of inulin and maximum survivability was observed at 1.5% level of inulin. Khosroyar *et al.* (2012) encapsulated ferric saccharate with calcium alginate coating by emulsification and produced fine iron capsules which resulted in better contact and enhanced bioavailability.

Jayalalitha *et al.* (2012) microencapsulated whey protein chelated ferrous sulphate using SA as coating material and reported that highly significant difference was observed in TBA value of control, microencapsulated iron and iron salt fortified yoghurt. Calleros *et al.* (2007) used SA blended with pectin or modified starch for microencapsulation of *Lactobacillus casei*. Extrusion technique was used for the microencapsulation.

Jankowski *et al.* (1997) encapsulated lactic acid bacteria in SA starch complex and reported the preparation of immobilized lactic acid bacteria using this coating material. Kim *et al.* (2008) microencapsulated *Lactobacillus acidophilus* using SA as wall material by dropping method and reported that microencapsulation was effective in protecting the microorganisms from heat and acid treatment and also in delivering the viable cells to intestine without any significant adverse effect on their functionalities.

Lee and Heo (2000) microencapsulated *Bifidobacterium longum* in the form of calcium alginate beads and evaluated the effect of sodium alginate and bead size on survival of bacteria and reported that viability of bacteria increased with an increase in sodium alginate concentration and bead size.

Gbassi *et al.* (2009) microencapsulated *Lactobacillus plantarum* using SA as wall material coated with whey protein and reported that only whey protein coated bacteria can survive stimulated intestinal fluid media conditions. Aral and Akbuga (1998) prepared chitosan beads using SA and pectin and observed EE of these beads as 13-90%. Madziva *et al.* (2005) prepared folic acid microencapsules using SA and pectin as coating material and reported that use of SA in combination with pectin produced more robust capsules and contributed to greater encapsulation efficiency.

Kailaspathy (2009) microencapsulated the probiotic bacteria using SA and Hi Maize starch as coating material and observed that survival of probiotic bacteria was improved by encapsulation and it did not affect the sensory properties of yoghurt.

**2.3.15.2 Phosphatidylcholine**

Boccio *et al.* (1996); Xia and Xu (2005); Abbasi and Azari (2011) prepared iron microencapsules coated with phosphatidyl choline and evaluated for the stability in milk and milk products.

**.3.15.3 Polyglycerol monostearate**

It was used as a coating material for microencapsulation of ferric ammonium sulphate and fortified it in milk, drinking yoghurt and cheddar cheese (Kwak *et al*. 2001; Kwak *et al*. 2003b; Kim *et al*. 2003).

### 2.3.15.4 Gum arabic, maltodextrin and modified starch (HiCap 100)

Zilberboim *et al.* (1986a) encapsulated paprika oleoresin and aromatic esters by using gum arabic as a coating material and absolute alcohol as a dehydrating medium. It was observed that high retention values were achieved by low core to shell material ratio, high solid concentration, high viscosity of emulsion continous phase, absolute alcohol as dessicant, short contact times between dessicant and capsules and low air pressure in the formation of microencapsules. Trindade and Grosso (2000) encapsulated the ascorbic acid with gum arabic and rice starch by spray drying method. It was observed that the ascorbic acid microencapsule coated with gum arabic was more stable as compare to microencapsule coated with rice starch.

Krishnan *et al.* (2005) encapsulated cardamom oleoresin in the blend of gum arabic, maltodextrin and modified starch (HiCap 100) by spray drying method. Different combinations of blend were evaluated for the encapsulation efficiency and stability. It was observed that the 4/6, 1/6, 1/6 blend of gum arabic, maltodextrin and modified starch (HiCap 100) offered better protection than gum arabic alone. Kanakdande *et al.* (2007) also encapsulated cumin oleoresin in the blend of gum arabic, maltodextrin and modified starch (HiCap 100) by spray drying method and evaluated for encapsulation efficiency and stability. This study was in agreement with the study of Krishnan *et al.* (2005).

Encapsulation of α-tocopherol was done with gum arabic and spruce galactomannan by Laine *et al.* (2008). Encapsules were prepared with gum arabic, galactomannan and combination of gum arabic and galactomannan. Gum arabic showed maximum encapsulation yield and smallest droplet size of the microencapsules. Yu *et al.* (2012) prepared the microencapsules by complex coacervation method followed by spray drying. Gelatin and gum arabic was used as wall material and olive oil as core material. Optimum process parameters were, dosage of the crosslinker 3ml, concentration of the wall materials 3%, pH value 4.0 and ratio of core to wall material were 1:1. Olive oil microencapsules prepared with these optimal process conditions had good dispersion effect and high encapsulation efficiency.

# CHAPTER –3

# Materials and Methods

The present study was carried out for development and evaluation of yoghurt fortified with microencapsulated iron. Iron microencapsules were prepared using different coating materials and evaluated for their encapsulation efficiency. Most suitable iron microencapsules was selected on the basis of encapsulation efficiency and sensory analysis of the fortified yoghurt. Yoghurt was fortified with selected iron microencapsules and evaluated for its physicochemical, rheological, microbial and sensory properties during storage. Atomic absorption spectrophotometer (AAS) method was also standardized for the determination of iron content in the microencapsules and yoghurt.

This chapter deals with the materials and methods used for the preparation of fortified yoghurt. The physicochemical and analytical methods used for product analysis are also discussed.

### 3.1 Chemical reagents

All the reagents used were of "AR" grade unless otherwise specified.

#### 3.1.1 Chemical and reagents used for iron microencapsules preparation

Ferrous sulphate heptahydrate which has the GRAS status (part 184, title 21 of Code of Federal Regulations 1984) was used for iron fortification and was procured from Sigma Aldrich, St. Louis, MO, USA. Ferrous sulphate is the most frequently used water soluble iron fortificant, because it is the cheapest and also has high iron bioavailability. Ascorbic acid was purchased from Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai, India which was used to prevent the oxidation of ferrous sulphate during preparation of iron microencapsules

Polyglycerol monostearate was obtained from two sources i.e. Zhengzhou honest food co., Ltd. and oleon, Ertvelde- Belgium. Egg phosphotidyl choline, gum arabic and maltodextrin were purchased from Sigma Aldrich, St. Louis, MO, USA. Sodium alginate was purchased from Thomas baker (chemicals) Ltd., Mumbai, India. Modified starch was obtained from National starch, CP ingredients India (Pvt.) Ltd., Navi Mumbai, India. Soy phosphotidylcholine was obtained from Srinidhi Enterprises, Indore, India.

### 3.1.2 Chemicals

Disodium hydrogen phosphate, calcium chloride, sodium chloride, sodium hydroxide, potassium dichromate, M17 broth, MRS broth, oxalic acid and dialysis membrane (molecular weight cut off 12000 D), dialysis membrane clips, potato dextrose agar, violet red bile agar, M17 agar, β-glycerophosphate and tween 80 were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Chloroform, and perchloric acid, from Rankem, RFCL Ltd., New Delhi, India; Tween 60, Tween 40, 2-thiobarbituric acid (TBA), cyclohexanone, pectin and MRS agar, from Sigma Aldrich, St. Louis, MO, USA; phenolphthalein and isoamyl alcohol, from Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai, India; starch, hydrochloric acid, sulphuric acid, nitric acid, ethylene diamine tetra acetic acid (EDTA), cholesterol, diethyl ether and sodium citrate from Thermo Fisher Scientific India Pvt. Ltd., Delhi, India; ethyl alcohol from Jiangsu Huaxi International Trade Company Ltd., Jiangsu, China; skim milk powder from Modern Dairies Ltd., Karnal, Haryana, India; ammonium sulphate, sodium thiosulphate and potassium iodide from Glaxo Laboratories, Mumbai, India; citric acid and gluteraldehyde from sd fine chemicals Ltd., Mumbai, India; Megazyme acetaldehyde assay kit from Prolab Marketing Pvt. Ltd., New Delhi, India; yoghurt starter culture NCDC 074 (*Streptococcus thermophilus*) and NCDC 009 (*Lactobacillus delbrueckii* subsp. *bulgaricus*) were obtained from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal, Haryana, India.

## 3.2 Apparatus and glassware

All volumetric flasks, pipettes and burettes were class "A". Burette (50 ml), funnels (small and large), measuring cylinder (10, 50, 100, 250, 500 and 1000 ml), beaker (50, 100, 250, 500, 1000 and 2000 ml), silica crucible, kjeldahl flask (100 ml), volumetric flasks (5, 10, 50, 100, 250, 500 and 1000 ml, low actinic and/or clear), milk butyrometer, Pipettes (1, 2, 5, 10 and 10.75 ml) and glass petridishes (200 mm x 20 mm and 100 mm x 17 mm) were purchased from The Laboratory Glass Co. (LABCO), Ambala, India. Sample containers (50 and 100 g) from Abdos labtech private Ltd., New Delhi, India; petridishes and centrifuge tubes were procured from

Tarsons Products Pvt. Ltd., Kolkata, India; parafilm from Sigma Aldrich, St. Louis, MO, USA; glass test tubes from Borosil India Ltd., Mumbai, India and filter papers (Whatman filter paper No. 1, 4, 41 and 42) from Whatman International Ltd., Kent, England.

## 3.3 Equipments

1. Autopipettes (10-100 µl and 100-1000 µl): Tarsons Products Pvt. Ltd., Kolkata, India
2. Cream separator: Kamdhenu, KD-60E, New Delhi, India
3. pH meter: PHAN, Labindia Instruments Pvt. Ltd., Maharashtra, India
4. High precision water bath: PolyScience, Illinois, USA
5. Laboratory shaker: Spinix, Vortex shaker, Tarsons Products Pvt. Ltd., Kolkata, India
6. Magnetic stirrer: Spinot, Tarsons Products Pvt. Ltd., Kolkata, India
7. Vacuum filtration assembly (1000 ml): Schott Duran, Riviera, Wertheim, Germany
8. Vacuum pressure pump: Millipore (India) Pvt. Ltd., Bengaluru, India
9. Air compressor: ELGI equipments Ltd., Coimbatore, India
10. Ultrasonicator: SONICS, Vibra Cell, Model VCx750, Sonics and Materials Inc., New Town, USA
11. Hot plate: Advanced Technocracy Inc., Ambala, India
12. Hot air oven: Tempo Instruments and Equipments (I) P. Ltd., Mumbai, India
13. Muffle furnace: Narang Scientific Works Pvt. Limited, Delhi, India
14. BOD Incubator: Narang Scientific Works Pvt. Limited, Delhi, India
15. Airless paint sprayer: Wagema Professional Quality, Pretoria, South Africa
16. Hunter Lab Colorflex Colorimeter: Hunter Associates Laboratory Inc., Reston, USA
17. Refrigerated centrifuge: Kubota Corporation, Gyeonggi-do, South Korea
18. UV-VIS Spectrophotometer: double beam, SPEORD 200 Analytik Jena, Jena, Germany
19. Rotary vacuum evaporator, IKA RV 10, Thermo Fisher Scientific India Pvt. Ltd., Delhi, India

20. Freeze dryer: Daihan Lab Tech India Pvt. Ltd., Hyderabad, India
21. Inverted light microscope: Nikon Eclipse Ti, Tokyo, Japan with a fitted digital camera (Nikon Digital Sight, Japan). Nikon Basic Research Imaging Software (v 3.1) for size measurement.
22. Atomic absorption spectrophotometer (AAS): AA-7000, Shimadzu, Tokyo, Japan, flame mode, air-acetylene flame (temperature 2300ºC with flow rate 1.5 L/min)
23. Rancimat: Metrohm Rancimat Model 743, Herisau, Switzerland
24. Texture analyser: TA-XT2, Stable Micro Systems Ltd., Godalming, UK
25. Digital colony counter: Optics Technology, Delhi, India
26. Rheometer: Anton paar, Modular compact rheometer, MCR 52, India
27. Homogeniser : Crepaco, AVP Company, Chicago, USA
28. Scanning electron microscopy: Carl Zeizz EV018, $18^{th}$ edition, UK
29. Ion coater: Eiko $IB_3$, Japan

### 3.4 Preparation of iron microencapsules

#### 3.4.1 Microencapsulation of iron by formation of liposomes

Method of Abbasi and Azari (2011) was followed for preparation of ferrous sulphate liposomes.

**Preparation of phosphate citrate buffer (PCB)**

105 mg citric acid and 71 mg di-sodium hydrogen phosphate were dissolved in 50 ml of water and pH of buffer was adjusted to 6.8 with 0.1 N NaOH.

**Procedure**

For iron microencapsulation, 1.14 g lecithin (soy/egg lecithin) and 0.06 g cholesterol were dissolved in 30 ml diethyl ether (organic phase). The organic phase was then mixed with 10 ml of PCB solution (aqueous phase; pH 6.8) containing 0.438 g ferrous sulphate heptahydrate and 0.03 g ascorbic acid. The mixture of aqueous and organic phase was placed in ice bath and sonicated using a probe sonicator at 5ºC with 5.0 sec pulse rate until a stable emulsion was formed. The organic solvent was then evaporated using rotary evaporator (absolute pressure 300 mbar, temperature 60ºC) and a gel was formed. The gel was broken after persistent evaporation in rotary evaporator. The aqueous phase (20 ml) containing tween 80

(5% v/v) was added to broken gel and evaporation was continued for another 30 min. Liposomes thus formed were then freeze dried (-50ºC, 50mTorr) and finally stored at -20ºC.

### 3.4.2 Preparation of iron microcapsules by spray cooling method using polyglycerol monostearate (PGMS) as wall material

Iron microencapsules with PGMS was prepared by the method of Kwak *et al.* (2003a). They were prepared by taking the ratio of coating material to core material as 5:1 and dissolving in 50 ml distilled water. Ferrous sulphate heptahydrate with ascorbic acid was used as a core material. Ascorbic acid was used as an antioxidant to prevent the oxidation of ferrous to ferric form. The spray solution was heated at 55ºC for 20 min, and stirred on magnetic stirrer for 1 min at 800 rpm. An airless paint sprayer nebulized the coating material-iron mixture at 45ºC into a cylinder containing a 0.05% polyethylene sorbitan monostearate 60 (Tween 60) solution at 5ºC. The chilled fluid was centrifuged at 5300 rpm for 10 min to separate unwashed microcapsule suspension. Microcapsules were formed as lipid solidified in the chilled fluid. Pellet collected after centrifugation was subjected to lyophilisation.

### 3.4.3 Preparation of iron microcapsules using sodium alginate (SA), blend of SA and pectin and blend of SA and modified starch (HiCap 100) (MS) as wall material

Iron microencapsules were formed by spray cooling and deemulsification method using SA, blend of SA and pectin and blend of SA and MS as wall material.

#### 3.4.3.1 Preparation of iron microcapsules by spray cooling method using SA, blend of SA and pectin and blend of SA and MS as wall material

Three samples were prepared by dissolving 2 g SA (I), 1 g SA and 1 g pectin (II) and 1.8 g SA and 1.0 g MS (III) in 100 ml deionised water. Content was mixed on magnetic stirrer for 30 min at 500 rpm. It was then degassed for 15 min using sonicator. Iron salt and ascorbic acid were added to it and then stirred using the magnetic stirrer at 500 rpm for 30 min. Solution was then sprayed in different concentration of chilled $CaCl_2$ solution. Microencapsules were allowed to remain in the chilled $CaCl_2$ solution for 3-4 hrs for solidification. Content was filtered for removal of $CaCl_2$ through vacuum filtration assembly using Whatman No. 1 filter

paper. It was then frozen at -20ºC and freeze dried using lyophiliser to obtain a free flowing powder.

**3.4.3.2 Preparation of iron microcapsules by deemulsification method using SA as wall material**

Microencapsules were prepared by the method described by Khosroyar *et al.* (2012). 1.5 g of SA and 0.798 mg ferrous sulphate heptahydrate were dissolved in 100 ml water. Sunflower oil containing tween 80 in the ratio of 100:0.5 (oil:tween) was separately prepared using a magnetic stirrer at 900 rpm for 20 min. The mixture of SA and ferrous sulphate heptahydrate was added dropwise to 500 ml of oil solution (aqueous mixture to oil ratio of 1:5) and stirred at 900 rpm/30 min for emulsion formation. 500 ml of 0.1 M $CaCl_2$ solution was then added to the emulsion and stirred for another 20 min. Mixture was then left undisturbed for 30 min allowing phase separation of oil-water and microencapsules.

Oil-water phase was then discarded and capsules were washed thrice with distilled water. It was then centrifuged twice at 3000 rpm for 15 min, finally the capsules were collected and freeze dried using lyophiliser.

In this process, the calcium chloride solution was added both slowly and rapidly for disruption of emulsion and effect of addition process on EE was elucidated.

**3.4.4. Preparation of iron microcapsules by using blend of gum arabic (GA), maltodextrin (MD) and MS**

Two methods were used for the preparation of iron microencapsules, using a blend of GA, MD and MS. In first method, tween (0.05%) was used as a chilling media and in second, alcohol was used as a dehydrating media.

**3.4.4.1 Preparation of iron microcapsules by spray cooling method using the blend of GA, MD and MS**

Microencapsules were prepared by dissolving GA, MD and MS in deionised water/PCB at 60ºC and kept it for rehydration under refrigerated condition (4-7ºC) for 12 hrs. Iron salt (ferrous sulphate heptahydrate) and ascorbic acid (used as antioxidant) were dissolved in deionised water/PCB with or without stabilizer (tween) @0.1% of mixture. Solution of core and coating material was mixed and kept in the

ice bath. This mixture was sonicated using a probe sonicator at 5ºC with 5.0 sec pulse rate for 15 min. This emulsion was sprayed either directly in a petridish or in the solution of 0.05% tween using an airless paint sprayer at 1.5 $kg/cm^2$. It was then frozen at -20ºC and freeze dried using lyophiliser to obtain a free flowing powder.

#### 3.4.4.2 Preparation of iron microcapsules using alcohol as dehydrating media with the blend of GA, MD and MS

Microencapsules were prepared by dissolving GA, MD and MS in deionised water at 60ºC and kept it for rehydration under refrigerated condition for 12 hrs. Iron salt (ferrous sulphate heptahydrate) and ascorbic acid (used as antioxidant) were dissolved in deionised water. Solution of core and coating material were mixed and kept in the ice bath. This mixture was sonicated using a probe sonicator at 5ºC with 5.0 sec pulse rate for 15 min. This mixture was then sprayed in different concentration of alcohol (80%, 90% and absolute alcohol) and different ratio of mixture to absolute alcohol (1:5, 1:7.5 and 1:10) for standardisation of the process parameters. Alcohol solution was kept on magnetic stirrer and stirred at 500 rpm. Airless paint sprayer was used for spraying of the mixture in chilled alcohol which was operated at 1.5 $kg/cm^2$. After spraying, mixture was left undisturbed for 5 min. Finally, filteration was carried out under vacuum by Whatman No. 1 filter paper using vacuum filtration assembly. The retentate was then spread in petridish and stored at 4-7ºC/12-14 hrs for complete removal of alcohol.

## 3.5 Estimation of iron content

### 3.5.1 Analysis of iron content

Iron content in microencapsules was analysed using AAS by AOAC (2005) at $\lambda_{max}$ 248.3 nm.

**Cleaning of glass ware**

All glasswares used for AAS analysis were washed properly and soaked in potassium dichromate solution for 24 hrs, rinsed with deionised water, dipped in 3% EDTA solution for 24 hrs and finally rinsed with deionised water again before use.

**Preparation of standard curve for iron estimation**

4.98 mg ferrous sulphate heptahydrate (99% pure) was accurately weighed and transferred to 100 ml volumetric flask to prepare a stock solution of iron. 5 ml of

concentrated nitric acid was added to it and dissolved properly, volume was made up to the mark with double distilled water. This stock solution (100 ppm iron) was diluted to prepare standards of desired concentration: 0.25, 0.5, 1.0, 1.5 and 2.0 ppm. The samples were analysed using AAS and standard curve was prepared accordingly (Figure 3.1).

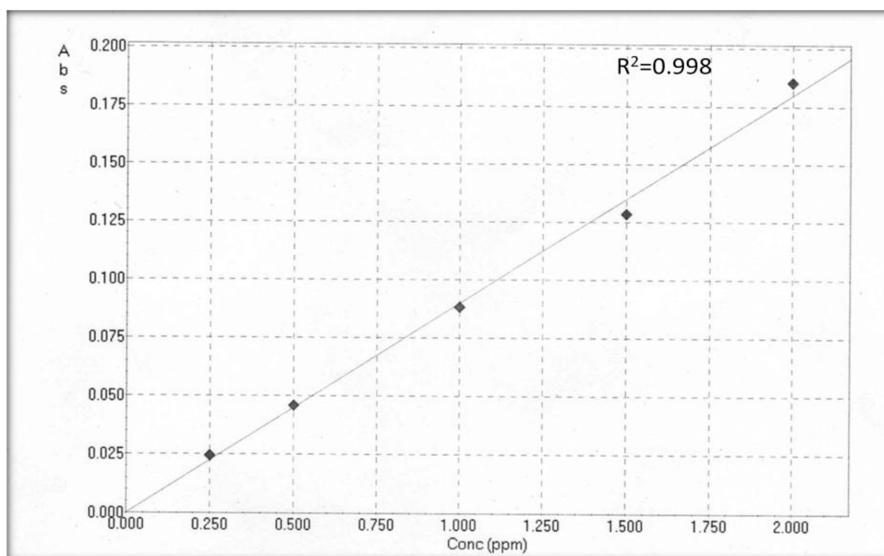



**Figure 3.1: Standard curve for estimation of iron content**

### 3.5.2 Calibration of Instrument

The instrument was calibrated daily before analysis and the standard solutions were prepared just when analysis was made. A blank sample and standard solutions of at least 5 concentrations (0.25 ppm, 0.50 ppm, 1 ppm, 1.5 ppm and 2 ppm) were measured for absorbance. For blank and standards, concentration of acid and other additives were kept same as used for samples. The difference between the concentrations of the standard solution was re-determined based on the created calibration curve (TV: self-determined true value) and the prepared concentrations were within 5% range.

### 3.5.3 Initial calibration verification

For initial calibration verification, concentrations different from the standard concentrations were used and their concentrations were within the concentration range for calibration curve. Three concentrations (0.75 ppm, 1.25 ppm and 1.75 ppm) were used for initial calibration verification. The acceptance criteria required that the measured value fall within a range between 90 to 110% of the prepared

concentration. However, if the result exceeded this range the measurement was stopped.

### 3.5.4 Duplicate sample analysis

In duplicate sample analysis, same microencapsules sample were pretreated separately and their iron content was measured and compared with each other to verify the reproducibility including the pretreatment. The samples were prepared using three different digestion methods as discussed below. The relative percent difference was calculated using following equation

$$\mathrm{RPD} = \frac{[\mathrm{S}-\mathrm{D}]}{[\mathrm{S}+\mathrm{D}]/2} \times 100$$

(Instruction manual, Analytical and measuring instruments division, Shimadzu corporation, Kyoto, Japan)

Where,

S = First sample value

D = Second sample value

### 3.5.5 Sample preparation

Three different methods of sample digestion were used for iron analysis. 100-500 mg of iron microencapsules were weighed for iron estimation.

#### 3.5.5.1 Dry digestion (AOAC 2005)

The iron microencapsules samples were weighed in silica crucible and dried for one hour at 100ºC in a forced air oven. Samples were charred on hot plate and then ashed in muffle furnace for minimum 7-8 hrs at 550ºC. Ash was dissolved in 5 ml concentrated $HNO_3$ and samples were diluted with deionised water. Dissolved ash was filtered with Whatman No. 41 filter paper if any particles were present in it. Final dilution of the sample contained iron content in the range of conc. used in standard curve.

#### 3.5.5.2 Wet digestion on hot plate using tri acid mixture ($HNO_3$: $HClO_4$: $H_2SO_4$ in 3: 2:1 ratio)

In wet digestion, samples were weighed in 100 ml kjeldahl flask. Samples were charred on hot plate. When fuming stopped, 5 ml tri acid mixture ($HNO_3$: $HClO_4$: $H_2SO_4$ in 3: 2: 1 ratio) was poured in kjeldahl flasks. Kept it on a hot plate

and digestion was continued until solutions became transparent. The samples were diluted with deionised water. Final dilution of the sample contained iron content in the range of concentration used in standard curve.

#### 3.5.5.3 Combination of wet and dry digestion

The samples were weighed in silica crucible and dried for one hour at 100ºC in a forced air oven. Samples were charred on hot plate and then ashed in muffle furnace for minimum 7-8 hrs at 550ºC. Ash was dissolved in 5 ml tri acid mixture and contents were transferred to a kjeldahl flask. Kept it on a hot plate and digestion was continued until solutions became transparent. The samples were diluted with deionised water. Final dilution of the sample contained iron content in the range of conc. used in standard curve.

### 3.5.6 Analysis procedure

All the samples were diluted to suitable dilution. Iron content was then analysed by AAS at $\lambda_{max}$ 248.3 nm.

## 3.6 Encapsulation efficiency (EE)

EE of iron microencapsules was determined by the methods described by Abbasi and Azari (2011)

**Method 1:** For calculation of total iron in microencapsules, they were ashed (at 550ºC for 7-8 hrs), solubilised in tri acid mixture and heated for the complete dissolution; and then diluted in 100 ml volumetric flask using deionised water. Iron content was then measured at 248.3 nm using AAS.

To measure the microencapsulated iron content in microencapsules, they were filled in cellulose membrane bag (MW cut off 12000 Da) and dialysed against deionised water for 24 hrs at 4ºC, where non encapsulated iron was removed. Microencapsules which remained inside the cellulose membrane bags were then treated in the way as described in the above paragraph.

$$\text{Encapsulation efficiency} = \frac{\text{Bound iron}}{\text{Total iron}} * 100$$

**Method 2:** Iron microencapsules were separated from rest of the mixture by centrifugation/filteration during preparation of iron microcapsules using PGMS, SA, blend of SA and pectin and blend of SA and MS as wall material. It was assumed

that iron in the pellet/retentate was in bound form. Non encapsulated iron was present in centrifuged supernatant/filterate. Non encapsulated iron in centrifuged supernatant/filterate was estimated by subjecting it to dry ashing, solubilised in tri acid mixture and heated for the complete dissolution; and then diluted in 100 ml volumetric flask using deionised water. Iron content was then measured at 248.3 nm using AAS. Encapsulated/bound iron was calculated by subtracting the non-encapsulated iron from total iron. Iron content which was initially added for the preparation of iron microencapsules was the total iron content. Encapsulation efficiency was calculated as follows:

$$\text{Encapsulation efficiency} = \frac{\text{Bound iron}}{\text{Total iron}} * 100$$

**Method 3:** Iron microencapsules were separated by filtration from rest of the mixture during preparation of iron microcapsules using alcohol as dehydrating media with the blend of GA, MD and MS. It was assumed that iron in the retentate was in encapsulated/bound form. Non encapsulated iron was present in the filterate. Retentate was dried in refrigerated conditions (4-7$^{o}$C) for 12-14 hrs. Encapsulated iron in retentate was estimated by subjecting it to dry ashing, solubilised in tri acid mixture and heated for the complete dissolution; and then diluted in 100 ml volumetric flask using deionised water. Iron content was then measured at 248.3 nm using AAS. Iron content which was initially added for the preparation of iron microencapsules was the total iron content. In this method bound iron was estimated in the retentate, whereas, in method 2 free iron was estimated in the centrifuged supernatant/filterate. In this method filterate contained majorly alcohol which was volatile and difficult to measure volumetrically and gravimetrically, therefore bound iron was measured in the retentate. Encapsulation efficiency was calculated as follows:

$$\text{Encapsulation efficiency} = \frac{\text{Bound iron}}{\text{Total iron}} * 100$$

EE of liposomes and iron microencapsules prepared using SA were estimated by method 1. EE of iron microencapsules prepared using PGMS, SA, blend of SA and pectin and blend of SA and MS were estimated by method 1 and 2.

EE of iron microencapsules prepared using blend of GA, MD and MS were estimated by method 1 and 3.

**3.7 Particle size analysis of iron microencapsules**

The microencapsules were diluted with distilled water (1:5). The homogeneous samples were then spread on glass slides and observed (magnification 400-fold) under an inverted light microscope and they were photographed using a fitted digital camera. The size of the microcapsules visible in the photographs was measured with the help of inbuilt software with microscope.

**3.8 Scanning electron microscopy of iron microencapsules**

Outer structure of microencapsules was studied by attaching the specimens/microencapsules on the specimen holder by double coated adhesive tape. Mounted sample was then coated with gold (20 nm thickness) on Eiko $IB_3$ ion coater at 0.05-0.07 torr for 4 min maintaining the ion current at 6mA. Specimen was finally examined under Carl zeizz EV018 scanning electron microscope at an acceleration voltage of 15 KV and micrographs were recorded on ORWO 35 mm panchromatic film. (Tomar and Prasad, 1987)

**3.9 Preparation of yoghurt**

Control (unfortified), iron salt fortified and iron microencapsules fortified yoghurt was prepared by the method described by Jayalalitha *et al.* (2012). The key steps were standardisation of mix, heat treatment, homogenisation, pasteurisation, cooling to incubation temperature, inoculation with yogurt cultures, incubation, cooling and packaging (figure 3.2). Fresh buffalo milk was standardised to 4% fat and 14% TS. Skim milk powder was added to adjust the solid content. It was then preheated to 55-65ºC and homogenised. Iron salts and iron microencapsules were added to milk and milk was then heated to 85ºC for 30 min for pasteurisation. Milk was cooled to 42ºC and starter culture was added @1.25% by weight *Streptococcus thermophilus* and 1.25% by weight *Lactobacillus bulgaricus*, finally samples were incubated at 42ºC for 5-6 hrs. After the formation of a firm coagulum, yoghurt was cooled to 4-7ºC and stored under refrigerated conditions (4-7ºC). The flow diagram of the steps involved is given below:

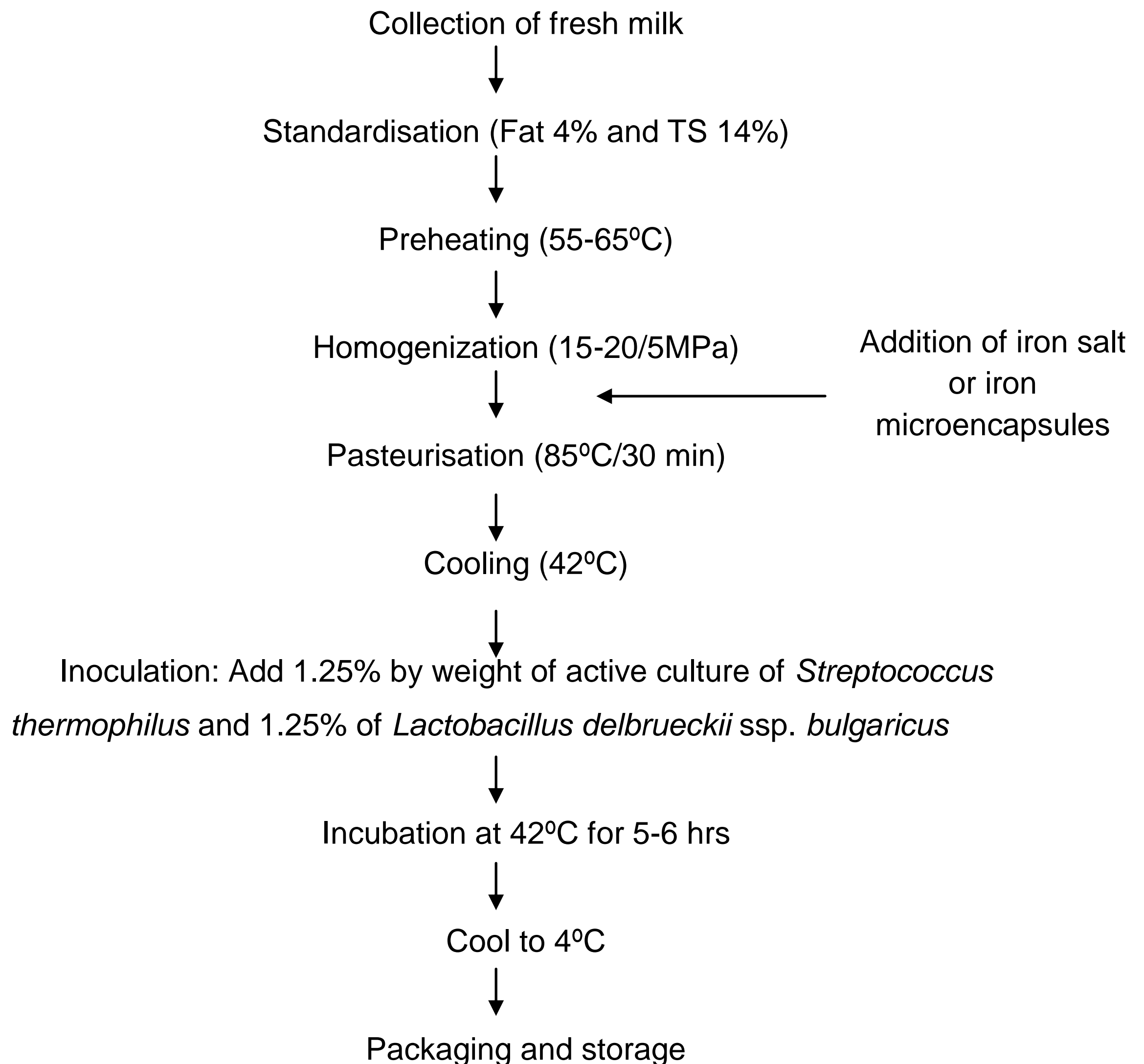



**Figure 3.2: Flow chart for manufacture of yoghurt**

### 3.10 Collection of milk samples

Fresh buffalo milk was collected from the herd of buffaloes maintained in the cattle yard of National Dairy Research Institute, Karnal, Haryana, India after the morning and evening milking. Cream was then separated with the help of cream separator. Fat and TS of milk were adjusted to 4% and 14% respectively with cream, skim milk and SMP using Pearson's square method.

## 3.11 Analysis of milk

### 3.11.1 Fat

The fat content of milk was determined by the Gerber method as detailed in IS: 1224: 18 (Part 1), 1977. 10 ml Gerber sulphuric acid was transferred into a Gerber butyrometer using an automatic measure. 10.75 ml of well mixed milk sample was pipette slowly into the butyrometer along the sides. 1 ml of iso amyl alcohol was then added with an automatic measure. The butyrometer was closed firmly with the stopper and shaken carefully to mix the contents and was kept in a waterbath at 65°C for 5 min after centrifuging for 4 min at 1100 rpm. The difference between the scale readings corresponding to the lowest point of fat meniscus and the surface of separation of fat and acid gave the fat content in% in milk.

### 3.11.2 Solid-not-fat (SNF)

The SNF content in milk was determined by the method as described in IS 10083, 1992. Lactometer reading was recorded at 27°C

$$\text{SNF (\% weight)} = 0.25\ (\text{CLR} + \text{F}) + 0.44$$

Where,

CLR= Corrected Lactometer reading (or Lactometer reading at 27°C)

F= Fat% determined by Gerber method

## 3.12 Addition of iron salt or iron microencapsules

### 3.12.1 Addition of iron salt

Amount of ferrous sulphate heptahydrate to be added for the fortification of yoghurt sample to the required level was calculated by the following formulae

$$\text{Amount of iron salt (g)} = \frac{\text{Molecular weight of iron salt}}{\text{Molecular weight of iron}} * \text{Level required}$$

### 3.12.2 Addition of iron microencapsules

Iron content in the microencapsules was estimated by the standardized AAS method for microencapsules. Amount of iron microencapsules to be added for the fortification of yoghurt sample to the required level was calculated by the following formulae.

$$\text{Amount of iron encapsules (g)} = \frac{1000}{\text{Iron content in microencapsules (ppm)}} * \text{Level required}$$

**3.13 Screening of the suitable iron microencapsules for the fortification in yoghurt**

Screening of the microencapsules was done on the basis of:

**3.13.1 Encapsulation efficiency**

Encapsulation efficiency represented the percentage of iron which was in the bound form. Higher amount of bound iron showed less oxidative changes, whereas, higher amount of free iron resulted in higher oxidative changes. Therefore, iron microencapsules which showed very less ability for binding iron were rejected.

**3.13.2 Sensory evaluation of fortified yoghurt**

Microencapsules which were selected on the basis of encapsulation efficiency were used for the fortified yoghurt preparation. Prepared yoghurt samples were evaluated by the sensory panelists. Sensory panel of ten trained judges were asked to grade all microencapsules fortified yoghurt samples for any change in flavour, body and texture, acidity, colour and appearance, container and closure and over all acceptability. Composite score card for sensory analysis of yoghurt as approved by BIS (IS: 7768 1975) was used, with slight modifications. In flavour characteristics, the main focus was on metallic, rancid, oxidised flavour and other off flavours. Other mentioned flavour characteristics were excluded. The composite sensory score card is attached as Annexure I. All sensory assessments took place in Dairy Chemistry Division of National Dairy Research Institute, Karnal, Haryana, India. The sensory booth environment was held at a constant temperature (20°C), red lighting was used to obscure any colour differences between the samples and a positive airflow removed any odours from the testing area. Saline water (0.89% sodium chloride solution) (at room temperature) was provided as palate cleanser for rinsing mouth and cleaning the tongue before sensory evaluation of each sample.

**3.14 Standardisation of AAS method for estimation of iron content in yoghurt**

**Sample preparation**

Yoghurt sample was prepared and three methods were used for the digestion of the yoghurt sample which has been described in section 3.5.5.

**Procedure**

AAS method for estimation of iron content in yoghurt sample was standardized by following the same steps which has been described in section 3.5.

### 3.14.1 Recovery (%) of iron by different digestion methods

Recovery (%) of all these methods were calculated by excluding the iron content in the control/unfortified yoghurt. Yoghurt samples were fortified with iron microencapsules @ 30 ppm iron. %Recovery of all the methods were calculated by the following formulae.

$$\%\text{Recovery} = \frac{(\text{Iron output} - \text{Iron in control yoghurt})}{\text{Iron input}} \times 100$$

## 3.15 Effect of fortification on sensory, physicochemical, rheological and microbiological properties of yoghurt during storage

### 3.15.1 Sensory evaluation

Sensory evaluation was done by a panel of ten trained judges who graded control, iron salt fortified and iron microencapsules fortified yoghurt for any change in flavour, body and texture, acidity, colour and appearance, container and closure and over all acceptability. Composite score card was used for sensory analysis of yoghurt. Sensory analysis was carried out as discussed earlier in section 3.13.2.

### 3.15.2 Physicochemical properties

Yoghurt samples were evaluated for the following physicochemical properties.

#### 3.15.2.1 pH

pH of yoghurt sample was determined electrometrically with mains operated pH meter by the method as described in IS: SP:18, part XI (1981). The pH meter was first calibrated using standard buffers of pH 4.0 and 9.2 and standardised using pH buffer of 7.0 at 20.0±0.1ºC.

#### 3.15.2.2 Setting time

The setting time of sample (in hrs) was recorded from the time of inoculation to the time when coagulum was just formed.

#### 3.15.2.3 Titratable acidity

**Phenolphthalein indicator solution**

1 g of phenolphthalein was weighed and transferred to a 100 ml volumetric flask containing about 50 ml of 95% ethanol. The flask was stoppered and shaken vigorously for few minutes. 20 ml ethanol was then further added and shaken until a clear solution was obtained and volume was finally made to 100 ml with 95% ethanol.

**Standard aqueous sodium hydroxide solution (0.1N)**

0.1 N aqueous NaOH solution was prepared and standardized against 0.1 N oxalic acid (primary standard). It was stored in an amber coloured glass bottle.

Normality of NaOH ($N_2$) = $N_1 * V_1 / V_2$

Where,

$N_1$ = Normality of oxalic acid (0.1N)

$V_1$ = Volume of oxalic acid

$N_2$ = Normality of NaOH

$V_2$ = Volume of NaOH used

**Procedure**

Titratable acidity of the yoghurt samples was estimated according to the standard AOAC procedure (AOAC 2005). Approximately, 20 g *yoghurt* sample was weighed in a conical flask and diluted with twice its volume with double distilled water. 2 ml of phenolphthalein indicator was added and the contents were titrated with 0.1 N NaOH to a persistent pink colour. Acidity was recorded as % lactic acid by weight.

$$\text{Acidity (\% lactic acid)} = \frac{9 * N * A}{W}$$

Where,

N = Normality of 0.1 N NaOH

A = Volume of 0.1 N NaOH used

W = Weight of the yoghurt sample

**3.15.2.4 Spontaneous syneresis in undisturbed set yoghurt**

Spontaneous syneresis of undisturbed set yoghurt was determined using a siphon method developed by Amatayakul *et al.* (2006). In this study, a cup of set yoghurt was taken from refrigerator kept in refrigerated conditions for 2 hrs, weighed and kept at an angle of approximately 45º to allow the whey on the surface to collect on the side of the cup. A needle connected to a syringe was used to siphon the liquid whey from the surface of the sample and the cup of yoghurt was then re-weighed. The siphon was carried out within 10 sec to prevent further leakage of whey from the curd. The syneresis was expressed as the percentage weight of the whey over the initial weight of the yoghurt sample.

#### 3.15.2.5 Water holding capacity

Water holding capacity (WHC) of yoghurt samples was analysed by the method described by Remeuf *et al*. (2003). For this, 20 g of yoghurt sample (A) was taken in a graduated centrifuge tube and the contents were centrifuged for 20 min at 2800 rpm and 20ºC. The whey expelled (B) was removed and weighed. The WHC was expressed as:

$$\text{WHC (\%)} = \frac{\text{A} - \text{B}}{\text{B}} * 100$$

#### 3.15.2.6 Colour estimation

Hunter colorimeter was used to measure the degree of change in colour produced by addition of iron salt and iron microencapsules to yoghurt as compare to control yoghurt. The colour coordinates of this meter were L=whiteness; a=redness to greenness and b=yellowness to blueness. The instrument was standardised with standard reference tile, coordinates for the tile were L=50.83 to 93.00; a= 0.92 to -26.27 and b= 1.70 to 12.12. Yoghurt samples were prepared as described above and evaluated for change in colour on $0^{th}$, $3^{rd}$, $7^{th}$, $10^{th}$ and $15^{th}$ days.

#### 3.15.2.7 Oxidative stability

Iron salt and iron microencapsules were used in the preparation of fortified yoghurt. Fat was extracted from each sample on 0, $3^{rd}$, $7^{th}$, $10^{th}$ and $15^{th}$ day by following the mojonnier method described in IS: SP:18, part XI (1981). These extracted fat samples were used to determine the oxidative stability of control

(unfortified), iron salt and iron microencapsules fortified yoghurt by determination of following parameters:

**3.15.2.7.1 Induction period (oil stability index)**

The Rancimat apparatus was used to measure the induction period of fat extracted from control, iron salt and iron microencapsules fortified yoghurt samples to evaluate the effect of iron fortification on oxidative stability of fat under accelerated oxidative conditions.

**Procedure**

Prior to the use of the Rancimat, all glasswares were thoroughly cleaned with double distilled water and alcohol. A 3.0 g sample of completely melted fat was weighed accurately into each of the reaction vessels. The vessels were then placed in the heating block of the Rancimat apparatus. Reaction vessels were then connected to the measuring vessels via connecting tube. 60 ml of deionised water was measured into each of the measuring vessels, containing the electrodes. The measuring vessels were also placed in the Rancimat apparatus. All parts were connected to the apparatus as per the operating instructions and the test was carried out until the endpoints of all the samples were reached, with a maximum allowable limit of 48 hrs. Temperature of heating blocks was set at 120ºC and a constant air flow rate (20 L/hr) was maintained throughout the run.

**3.15.2.7.2 Thiobarbituric acid (TBA) value**

TBA value of yoghurt samples were evaluated by the method described by Hegenauer *et al.* (1979). Secondary oxidation products were analyzed spectrophotometrically using this test. TBA reagent was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA (neutralized with NaOH) and 2 M $H_3PO_4$/ 2 M citric acid. Reaction was terminated by weighing 5.0 g of yogurt sample into a glass centrifuge tubes and mixed thoroughly with 2.5 ml TBA reagent. The mixture was heated immediately in boiling water bath for exactly 10 min, and then cooled on ice. Then, 5 ml cyclohexanone and 1 ml of 4M ammonium sulfate were added and centrifuged at 8200rpm for 5 min at room temperature. The orange-red cyclohexanone supernatant was decanted and its

absorbance at 532 nm was measured spectrophotometically in a 1 cm light path. All measurements were run in triplicate.

### 3.16 Rheological properties

#### 3.16.1 Texture profile analysis

The texture profile of yoghurt samples were determined using texture analyser fitted with 5 kg load cell. For texture analysis, yoghurt samples were prepared in 100 ml sterilized sample containers, the set samples were kept in immersion chamber maintained at 25ºC before the analysis. The samples were subjected to mono-axial compression of 20 mm distance on the texture analyser by the crosshead speed of 2 mm/sec. The test conditions maintained were as under:

Measured force in compression

Probe P25 cylindrical (diameter 25 mm)

Distance = 20 mm

Load cell = 25 kg

Pre-test speed = 2 mm/sec

Test speed = 1 mm/sec

Post-test speed = 2mm/sec

Compression = 50% of distance

Single TPA (texture profile analysis) i.e. single time penetration

After the completion of analysis, a graph was obtained with force experienced by probe on Y-axis and time on X-axis. The firmness of yoghurt sample was estimated as the height of positive peak force upto rupture point. A representative graph obtained for yoghurt samples is shown in figure 3.3.

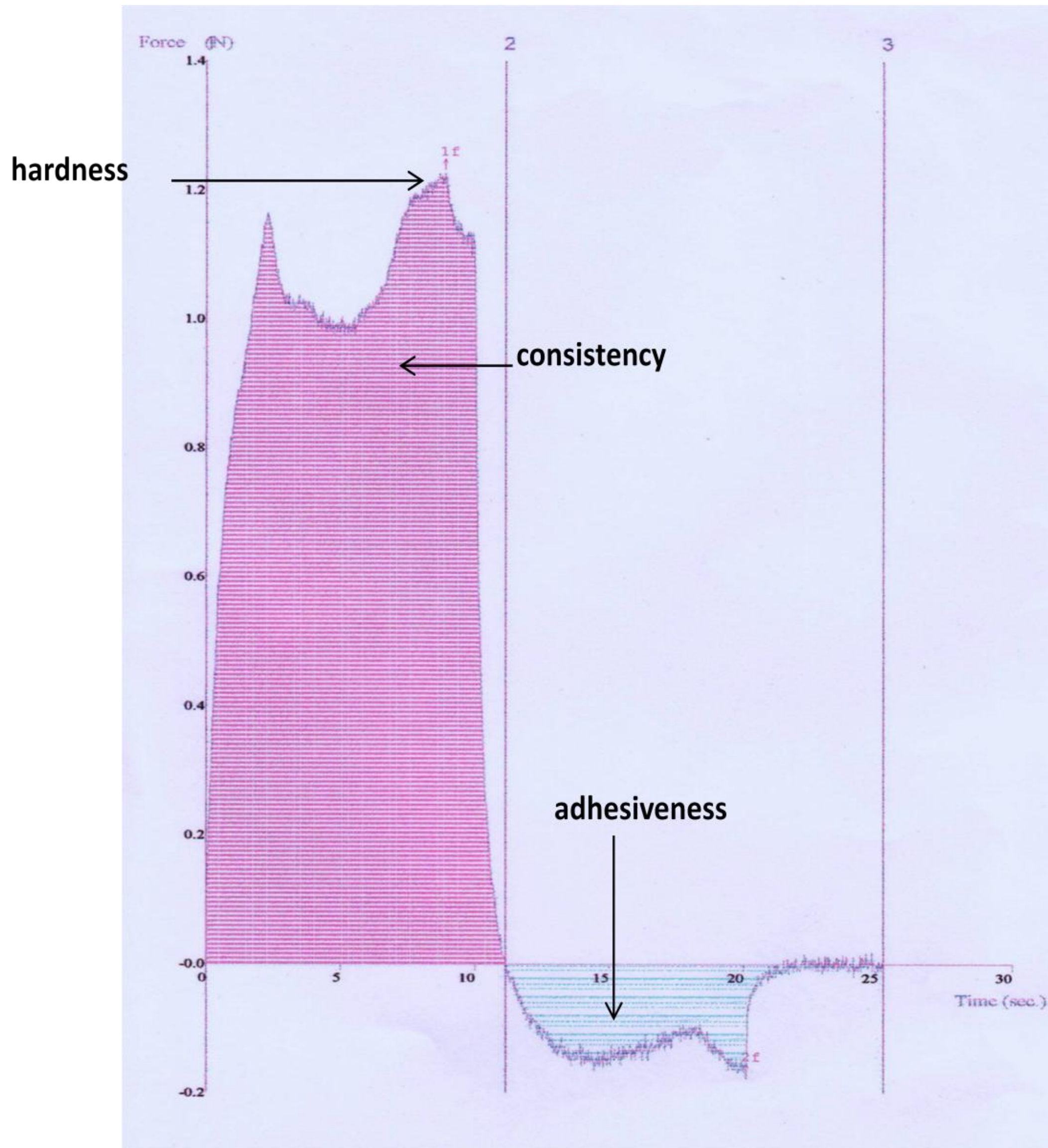



**Figure 3.3: Graphic representation of measurements using texture analyser TA.XT2i**

Consistency (N s) – the area of the resistance peak; maximal peak force-hardness (N)– the maximum resistance to the penetration peak (height of peak 2); adhesiveness (N s)– the area of the adhesion peak.

### 3.16.2 Flow behaviour

Flow behaviour property of yoghurt samples were analysed by rheometer (Modular compact rheometer, MCR 52) using a cone and plate geometry (CP 75) with 0 mm gap setting and at 25ºC constant temperature using variable shear rate (0-100 $s^{-1}$).

### 3.17 Microbiological parameters

Yoghurt sample was evaluated for Coliform, Yeast and mold (MIF, 1959), *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (IDF, 2003) count.

#### Preparation of dilution blanks

The dilution blanks consisted of 99 ml sodium citrate (2%) solution and 9 ml sodium chloride (0.9%) in screw capped dilution bottles and culture tubes, respectively. These were autoclaved at 121°C for 20 min. The dilution blanks were warmed to 45°C before use for preparation of samples.

#### Sampling of yoghurt

The external surface was aseptically cleaned with a 70% (v/v) solution of ethanol prior to opening the yoghurt sample. The sample was opened and the contents were mixed carefully with a sterile spatula. 11.0±0.1 g of sample was weighed into appropriate container and transferred to 99 ml sodium citrate solution. Further dilutions were made with 9 ml dilution blanks upto eighth dilution. Decimal dilutions were prepared in duplicate.

#### 3.17.1 Yeast and Mould counts

#### Preparation of potato dextrose agar media

39 g of potato dextrose agar was suspended in 1000 ml distilled water and boiled to dissolve the medium completely. It was then filled in flasks and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min. The pH of the medium was adjusted to 3.5 at the time of plating by using sterile 10% tartaric acid.

#### Procedure

Potato detxrose agar was used as medium to determine the yeast and mold count in yoghurt. In laminar air chamber, under aseptic conditions 1 ml of $10^{-1}$ and $10^{-2}$ dilution were poured in the petriplate followed by potato dextrose agar medium so as to fill 1/3 rd of the volume of the plate with media. The plates were incubated in inverted position at 25°C for 3 to 5 days.

### 3.17.2 Coliform counts

**Preparation of Violet Red Bile Agar (VRBA)**

41.5 g of VRBA agar was suspended in 1000 ml of distilled water and boiled to dissolve completely. The medium was boiled instead of autoclaving as suggested by manufacturers.

**Procedure**

VRBA was used for the enumeration of coliform in yoghurt. In laminar air chamber, under aseptic conditions 1 ml of $10^{-1}$ and $10^{-2}$ dilution were poured in the petriplate followed by agar medium. Agar medium was poured in two layers (to provide anaerobic conditions). Plates were then incubated in inverted position at 37°C for 24 hrs.

### 3.17.3 Enumeration of *Streptopcoccus thermophilus* and *Lactobacillus bulgaricus* (IDF 1993)

**Reagents and culture media**

1. M17 for enumeration of *Streptococcus thermophilus*
2. Acidified MRS for enumeration of *Lactobacillus delbrueckii ssp. bulgaricus.*
3. Glacial acetic acid for addition to MRS agar (to reduce pH to 5.4±1) after sterilisation.
4. 10% lactose solution for addition to molten M17 (add 5 ml of a sterile 10% lactose solution to 95 ml of M17).

**Preparation of acidified MRS Agar**

61.15 g of MRS agar was suspended in 1000 ml distilled water containing 1 ml of tween 80 and boiled to dissolve the medium completely. It was then filled in flasks and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min. The pH of the medium was adjusted to 5.4 at the time of plating by using sterile glacial acetic acid.

**Preparation of M17 Agar**

33.25 g of M17 agar and 19 g of β-Glycerophosphate were suspended in 1000 ml distilled water and boiled to dissolve the medium completely. It was then filled in flasks and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 min. 10% lactose solution was added to molten M17 agar (@ 5 ml of a sterile 10% lactose solution to 95 ml of M17) at the time of plating.

**Procedure**

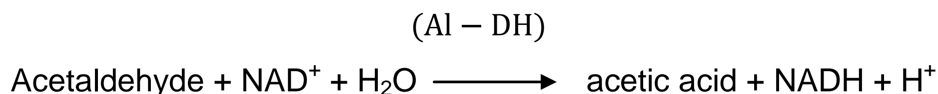
In laminar air chamber, under aseptic conditions 1 ml of $10^{-4}$ to $10^{-8}$ dilution were poured in the petriplate followed by appropriate agar medium (acidified MRS for *L. delbrueckii*, M17 with 10% lactose for *S. thermophilus*) so as to fill 1/3 rd of the volume of the plate with media. Acidified MRS was incubated anaerobically in inverted position at 37ºC for 72 hrs. M17 was incubated inverted at 37ºC for 48 hrs. After the appropriate incubation time, colonies on the plates (containing between 30 and 300 colonies) were counted.

**3.18 Acetaldehyde content in yoghurt**

Acetaldehyde is the principal flavour component in yoghurt produced by the microbial action on milk components. Megazyme assay kit was used for estimation of acetaldehyde in yoghurt samples.

**Principle**

Acetaldehyde is quantitatively oxidised to acetic acid in the presence of aldehyde dehydrogenase (Al-DH) and nicotinamide-adenine dinucleotide ($NAD^+$).



$$\text{Acetaldehyde} + NAD^+ + H_2O \xrightarrow{(\mathrm{Al-DH})} \text{acetic acid} + NADH + H^+$$

The amount of NADH formed in this reaction is stoichiometric with the amount of acetaldehyde. It is the NADH that is measured by the increase in optical density at 340 nm.

**Preparation of reagent solutions**

Solution 1: Buffer (pH 9.0) plus sodium azide (0.02% w/v) as a preservative.

Solution 2: $NAD^+$ (freeze dried powder): the contents were dissolved in 11.0 ml of distilled water and stored at -20ºC in polypropylene tubes after use.

Solution 3: Aldehyde dehydrogenase solution

Solution 4: Acetaldehyde control powder (acetaldehyde ammonia trimer powder): 80 mg of this powder (~50 mg of acetaldehyde) was dissolved in 1 L distilled water and stored in Duran bottle. This solution was prepared to assay where there was some doubt about the accuracy of the spectrophotometer being used or where it was suspected that inhibition was being caused by substances in the sample.

**Sample clarification**

**Reagents**

1. **Carrez I solution:** Dissolve 3.60 g of potassium hexacyanoferrate (II) $\{K_4[Fe(CN)_6].3H_20\}$ in 100 ml of distilled water. Store at room temperature.
2. **Carrez II solution:** Dissolve 7.20 g of zinc sulphate (ZnSO4.7H2O) in 100ml of distilled water. Store at room temperature.
3. **Sodium hydroxide (NaOH, 100 mM):** Dissolve 4 g of NaOH in 1 L of distilled water. Store at room temperature.

**Sample preparation**

60 g yoghurt was weighed accurately into a beaker. Add 5 ml of carrez I solution, 5 ml of carrez II solution and 10 ml of 100 mM NaOH solution. Mix after each addition. The contents were stirred slightly and transferred to a 100 ml volumetric flask with water and filled up to the mark with water.

**Table 3.1: Procedure for acetaldehyde estimation**

| Pipette into curettes | Blank | Sample |
|---|---|---|
| Distilled water<br>Sample solution<br>Buffer 1 solution<br>Solution 2 ($NAD^+$) | 2.10 ml<br>-<br>0.20 ml<br>0.20 ml | 2.00 ml<br>0.10 ml<br>0.20 ml<br>0.20 ml |
| Contents were mixed by gentle inversion, the optical density of the solutions ($A_1$) was read after approximately 2 min and the reactions were started by addition of: | | |
| Solution 3 (Al-DH) | 0.05 ml | 0.05 ml |
| Contents were mixed by gentle inversion, the optical density of the solutions ($A_2$) were read at the end of the reaction (approx. 3-4 min). If the reaction had not stopped after 3-4 min, optical densities were continuously read at 2 min intervals until the optical density increased constantly over 2 min. | | |

The contents were shaken and mixed well and filtered through Whatman No. 1 filter paper. The clear solution was used for the assay. The main steps involved in acetaldehyde estimation are listed in table 3.1.

**Calculation**

The optical density difference ($A_2$-$A_1$) was determined for both blank and sample. The optical density difference of the blank was subtracted from the optical density difference of the sample, thereby obtaining $\Delta A_{acetaldehyde}$.

The concentration of acetaldehyde can be calculated as follows:

$$C_{acetaldehyde} = \frac{V * MW}{\varepsilon * d * v} * \Delta A_{acetaldehyde} \quad (g/L)$$

where, V = final volume (ml)

MW = molecular weight of acetaldehyde (g/mol)

ε = extinction coefficient of NADH at 340 nm= 6300 ($l \times mol^{-1} \times cm^{-1}$)

d = light path (cm)

v = sample volume (ml)

It follows for acetaldehyde:

$$C_{acetaldehyde} = \frac{2.55 * 44.05}{6300 * 1 * 0.10} * \Delta A_{acetaldehyde} \quad (g/L)$$

$$= 0.1783 * \Delta A_{acetaldehyde} \; (g/L)$$

$$\text{Content of acetaldehyde} = \frac{C_{acetaldehyde}\,(g/L \text{ sample solution})}{Weight_{sample}\,(g/L \text{ sample solution})} * 100\,(g/100g)$$

**3.19 Statistical analysis**

The results were expressed as means±standard error of mean (SEM). Significance was tested by employing single way analysis of variance (ANOVA) and two way analysis of variance (ANOVA) with replication and comparison between means was made by critical difference (CD) value as described by Snedecor and Cochran (1994). For computation of data, software application programme Microsoft Office Excel 2007 was used.

# CHAPTER –4

# Results and Discussion

# RESULTS AND DISCUSSION

This chapter deals with the various results obtained during the present study. The first phase dealt with the preparation of iron microencapsules with different coating materials but with common core material i.e. ferrous sulphate heptahydrate. In the second phase, method was standardized for estimation of iron content in microencapsules. In the third phase, yoghurt was prepared with different iron microencapsules and finally, the best iron microencapsule for yoghurt fortification was selected on the basis of sensory analysis. In the fourth phase, method was standardized for estimation of iron content in the yoghurt. Yoghurt was also evaluated for the impact of iron fortification through addition of iron salt and iron microencapsules on the physico-chemical, sensory, rheology and microbial attributes compared to control (unfortified) yoghurt. Results of different experiments from the present investigation are listed and discussed under the following heads:

**4.1 Preparation and evaluation of the different iron microencapsules**

Different iron microencapsules were prepared with the different coating materials i.e. phosphatidylcholine, polyglycerol monostearate (PGMS), sodium alginate (SA), blend of SA and pectin, blend of SA and modified starch (HiCap 100) (MS) and blend of gum arabic (GA), maltodextrin (MD) and MS. Highly bioavailable iron salt i.e. ferrous sulphate heptahydrate was used as a core material. Ascorbic acid was also added with the iron salt during microencapsules preparation to prevent the oxidation of iron from ferrous to ferric form. Xia and Xu (2005) also reported that the ascorbic acid act as an antioxidant during liposome preparation.

**4.1.1 Microencapsulation of iron by formation of liposomes**

Iron microencapsules were prepared by the reverse phase evaporation method. These were in the form of liposomes and prepared by using both egg phosphatidylcholine and soy phosphatidylcholine. Encapsulation efficiency (EE) of these microencapsules was estimated by method 1 which has been described earlier in the section 3.6.

During preparation of liposomes, filterate/supernatant was not separated from the microencapsules, therefore EE were not estimated by method 2 and 3. EE data was

analysed by single way ANOVA applying a subsequent least significant difference (LSD) test for multiple sample comparison to test for any significant differences ($p<0.05$) in the mean values of all the samples.

**Table 4.1: EE of microencapsules prepared using phosphatidylcholine**

| Coating material | Encapsulation efficiency (%) |
|---|---|
| Egg phosphatidylcholine | $64.047 \pm 0.4106^{a}$ |
| Soy phosphatidylcholine | $38.370 \pm 0.4325^{b}$ |

Data are presented as means±SEM (n=3)

$^{a\text{-}b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

EE of iron microencapsules prepared using egg phosphatidylcholine was significantly different ($p<0.05$) from soy phosphatidylcholine (Table 4.1 and Figure 4.1). Soy phosphatidylcholine showed lower EE as compared to egg phosphatidylcholine. Our results were in agreement with Xia and Xu (2005) who reported that EE of egg phosphatidylcholine as 67%. Abbasi and Azari (2011) did not specify the type of phosphatidylcholine but reported the EE of liposomes to be 85.5% which was higher than the above obtained results.

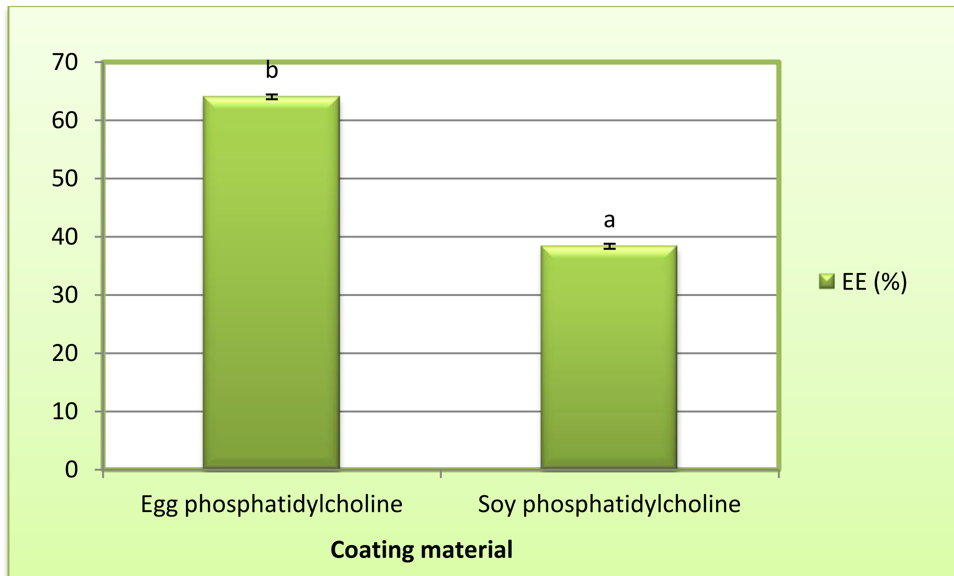



**Figure 4.1: EE of microencapsules prepared using phosphatidylcholine**

$^{a\text{-}b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Among these two microencapsules, microencapsules made using egg phosphatidylcholine were selected for further studies due to higher EE.

### 4.1.2 Preparation of iron microcapsules by spray cooling method using PGMS as coating material

Iron microencapsules were prepared by spray cooling method using PGMS as coating material. Two different PGMS samples were used for the preparation of iron microencapsules and each of them were evaluated for EE. EE of these microencapsules was estimated by both method 1 and 2 which have been described earlier in the section 3.6.

**Table 4.2: EE of microencapsules prepared using PGMS by method 1**

| Coating material | Encapsulation efficiency (%) |
|---|---|
| PGMS Zhengzhou | 19.793 ± 0.4960$^{a}$ |
| PGMS Oleon | 34.790 ± 0.5795$^{b}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

According to method 1, EE of iron microencapsules prepared using PGMS Zhengzhou was significantly different ($p<0.05$) from PGMS Oleon (Table 4.2 and Figure 4.2). PGMS Oleon showed higher EE as compared to PGMS Zhengzhou. Results

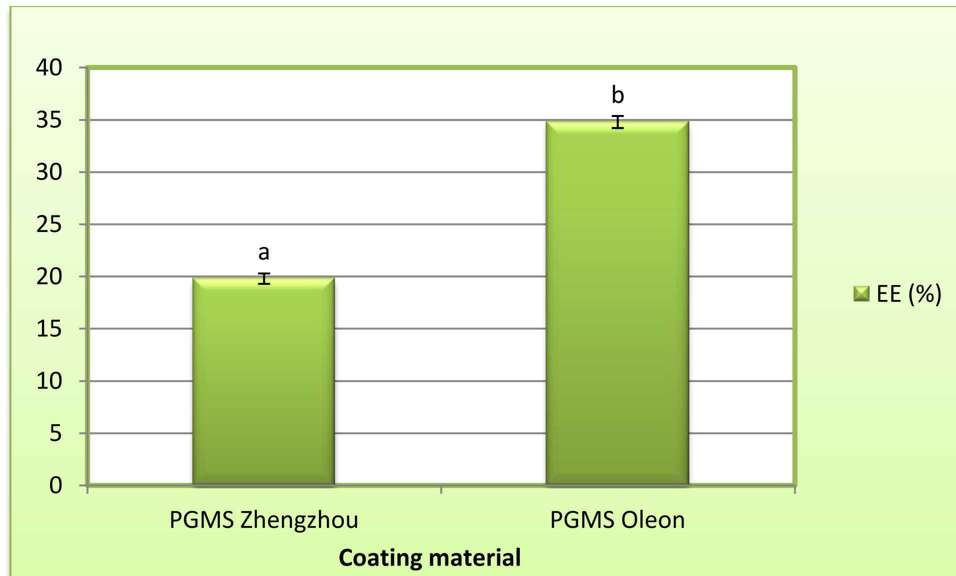



**Figure 4.2: EE of microencapsules prepared using PGMS by method 1**

$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

revealed that the rate of release of iron from the microencapsules prepared using PGMS Oleon was slower than PGMS Zhengzhou, which might be due to inherent properties of

the PGMS supplied by the respective firms. Abbasi and Azari (2011) have also used this method for the liposomes prepared using phosphatidylcholine. Iron microencapsules manufactured using PGMS showed very low EE.

**Table 4.3: EE of microencapsules prepared using PGMS by method 2**

| S.No. | Coating material | Encapsulation efficiency (%) |
|---|---|---|
| 1 | PGMS Zhengzhou | 26.413 ± 0.6004[a] |
| 2 | PGMS Oleon | 27.313 ± 0.4239[a] |

Data are presented as means±SEM (n=3)
[a-b]Means within columns with different superscript are significantly different ($p<0.05$) from each other.

According to method 2, EE of iron microencapsules prepared using PGMS Zhengzhou was not significantly different ($p>0.05$) from PGMS Oleon (Table 4.3 and Figure 4.3). Abbasi and Azari (2011) also evaluated the PGMS iron microencapsules for EE using the same method but found highest EE as 81.8%. Kim *et al.* (2003) also reported higher values for EE of PGMS iron microencapsules as 73%.

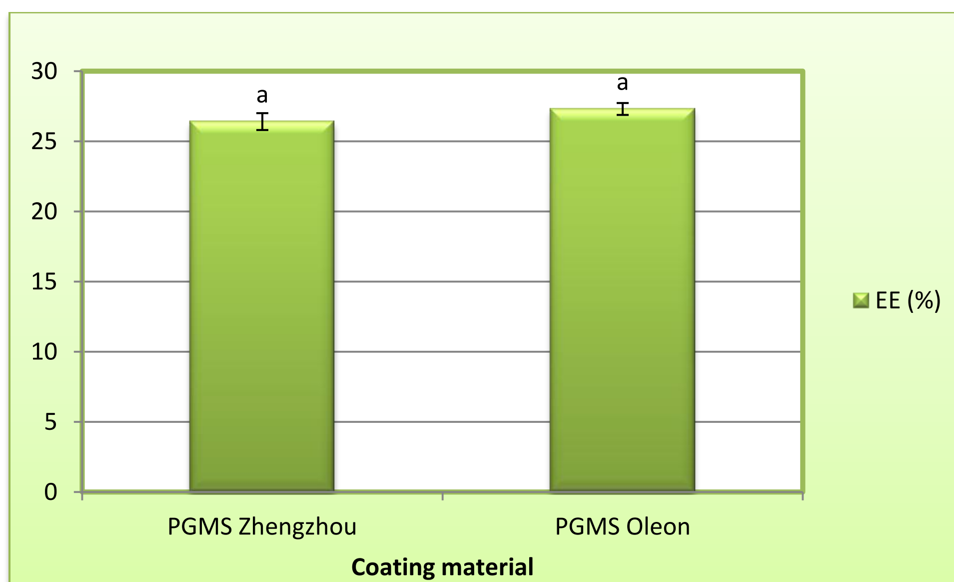



**Figure 4.3: EE of microencapsules prepared using PGMS by method 2**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Both the microencapsules showed low EE, therefore rejected for further studies. The low EE observed for method 2 could be because of less entrapment of iron inside the microencapsules during centrifugation. Both the microencapsules showed low EE, therefore rejected for further studies.

### 4.1.3 Preparation of iron microcapsules using sodium alginate (SA), blend of SA and pectin and blend of SA and MS as coating material

Iron microencapsules were formulated by spray cooling and deemulsification method using SA, blend of SA and pectin and blend of SA and MS powder as coating material.

#### 4.1.3.1 Preparation of iron microcapsules by spray cooling method using SA, blend of SA and pectin, blend of SA and MS as coating material

Three coating material compositions were used for iron microencapsulation.

##### 4.1.3.1.1 Preparation of iron microcapsules by spray cooling method using blend of SA and pectin as coating material

Initially, iron microencapsules were prepared by using a blend of SA and pectin as coating material. 1 g SA and 1 g pectin were dissolved in 100 ml deionised water. 200 mg iron salt and 20.54 mg ascorbic acid were also dissolved in it. EE of these microencapsules were estimated by both the method 1 and 2 which have been described earlier in section 3.6. Different concentrations of $CaCl_2$ (0.1 M, 0.5 M and 1 M) were used to standardise the process parameters.

**Table 4.4: EE of microencapsules prepared using SA + pectin by method 1**

| Coating material with different concentration of $CaCl_2$ solution | Encapsulation Efficiency (%) |
|---|---|
| SA + pectin (0.1 M $CaCl_2$) | $30.673 \pm 0.5617^{b}$ |
| SA + pectin (0.5 M $CaCl_2$) | $15.533 \pm 0.4514^{a}$ |

Data are presented as means±SEM (n=3)
$^{a\text{-}b}$Means within columns with different superscript are significantly different (p<0.05) from each other.

According to method 1, EE of iron microencapsules prepared using SA + pectin (0.1 M $CaCl_2$) was significantly different ($p<0.05$) than SA + pectin (0.5 M $CaCl_2$) (Table 4.4 and Figure 4.4). SA + pectin (0.1 M $CaCl_2$) showed higher EE as compared to SA + pectin (0.5 M $CaCl_2$). It was difficult to dry the microencapsules comprised of SA + pectin 1 M which results in lower EE. $CaCl_2$ is hygroscopic at higher concentration and it also interfered with the drying of the microencapsules as it formed a sticky mass on

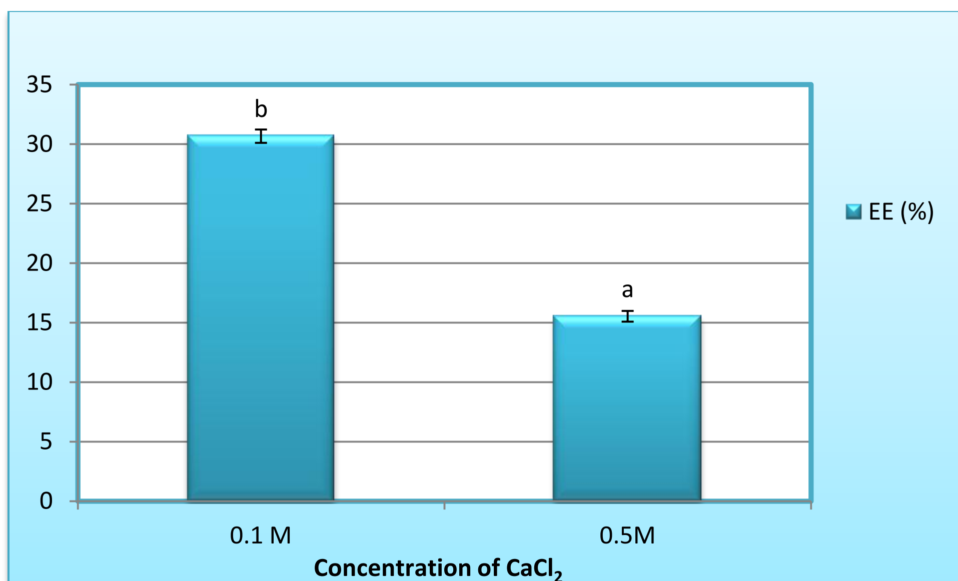



**Figure 4.4: EE of microencapsules prepared using SA + pectin by method 1**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

drying. It was evident from the above results that EE of microencapsules decreased with an increase in the concentration of $CaCl_2$.

**Table 4.5: EE of microencapsules prepared using SA + pectin by method 2**

| Coating material with different concentration of $CaCl_2$ solution | Encapsulation Efficiency (%) |
|---|---|
| SA + pectin (0.1 M $CaCl_2$) | $57.158 \pm 0.3777^c$ |
| SA + pectin (0.5 M $CaCl_2$) | $45.798 \pm 0.3455^b$ |
| SA + pectin (1 M $CaCl_2$) | $33.248 \pm 0.3376^a$ |

Data are presented as means±SEM (n=3)
[a-b]Means within columns with different superscript are significantly different ($p<0.05$) from each other.

According to method 2, EE of all the iron microencapsules were significantly different ($p<0.05$) from each other (Table 4.5 and Figure 4.5). SA + pectin 0.1 M showed higher EE than SA + pectin 0.5 M and SA + pectin 1 M. It was evident from the above results that EE of microencapsules decreased with increase in the concentration of $CaCl_2$.

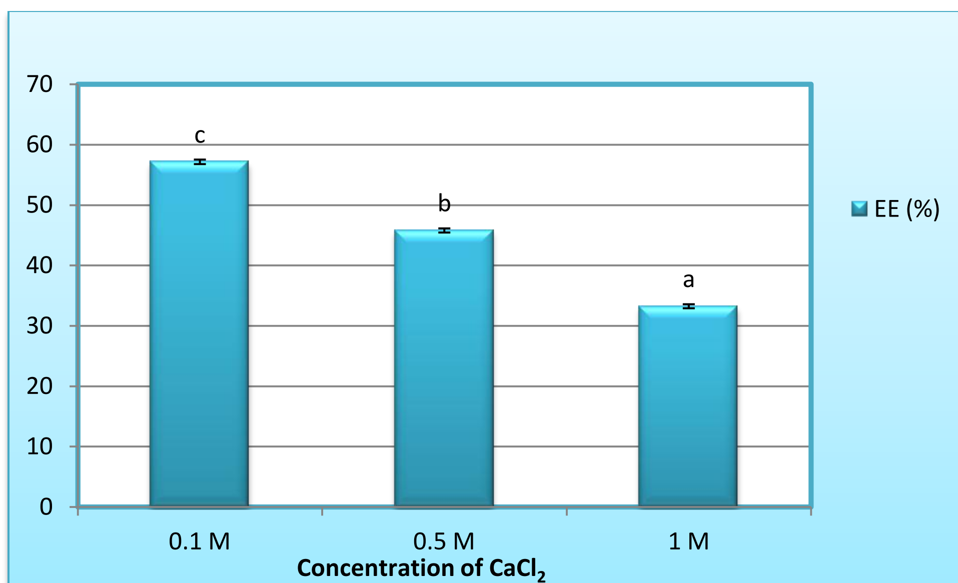



**Figure 4.5: EE of microencapsules prepared using SA + pectin by method 2**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

It was concluded from the above two methods used for calculating the EE that 0.1 M $CaCl_2$ was most suitable cooling and hardening media for microencapsulation of iron. Our results were in agreement with Chandramouli *et al.* (2004) who evaluated the effect of different concentration of $CaCl_2$ solution (0.1 M, 0.2 M and 1 M) on viability of microencapsulated *Lactobacillus spp.* and found that increase in $CaCl_2$ concentration beyond 0.1 M during encapsulation did not improve the capsules efficacy in protecting the viable cells.

These two methods (1 & 2) have been reported in the literature for calculation of the EE. Abbasi and Azari (2011) used method 1 for estimating the EE of liposomes and method 2 for iron microencapsules prepared using PGMS. Estimation of EE by method 2 was fast as compared to method 1 as no dialysis was required for the separation of the free iron, which took around 24 hrs. Similar trend was observed by both the methods for EE of all the iron microencapsules formulated. However, higher value of EE was estimated by method 2 as compared to method 1. Method 2 estimated the iron content in the filterate whereas method 1 in the dialysed water. Higher EE of microencapsules by method 2 revealed higher entrapment of iron inside the microencapsules during preparation and lower EE of microencapsules by method 1 showed higher release of iron in the aqueous system.

#### 4.1.3.1.2 Preparation of iron microcapsules by spray cooling method using SA and blend of SA and MS as coating material

Two samples were prepared using SA and blend of SA and MS as coating material. 2 g SA (I) and 1.8 g SA and 1 g MS (II) were dissolved in 100 ml deionised water. 200 mg iron salt and 20.54 mg ascorbic acid were also dissolved in it. After sample preparation sprayed it in 0.1 M $CaCl_2$ solution. EE of these microencapsules was estimated by the two methods which have been described earlier in section 3.6.

**Table 4.6: EE of microencapsules prepared using SA and SA + MS by method 1**

| Coating material | Encapsulation Efficiency (%) |
|---|---|
| SA (0.1 M $CaCl_2$) | $22.389 \pm 0.2838^a$ |
| SA + MS (0.1 M $CaCl_2$) | $23.214 \pm 0.1534^b$ |

Data are presented as means±SEM (n=3)
$^{a\text{-}b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

According to method 1, EE of iron microencapsules prepared using SA + MS (0.1 M $CaCl_2$) was significantly different ($p<0.05$) from SA (0.1 M $CaCl_2$) (Table 4.6 and Figure 4.6). SA + MS (0.1 M $CaCl_2$) showed slightly higher EE as compared to SA (0.1 M $CaCl_2$).

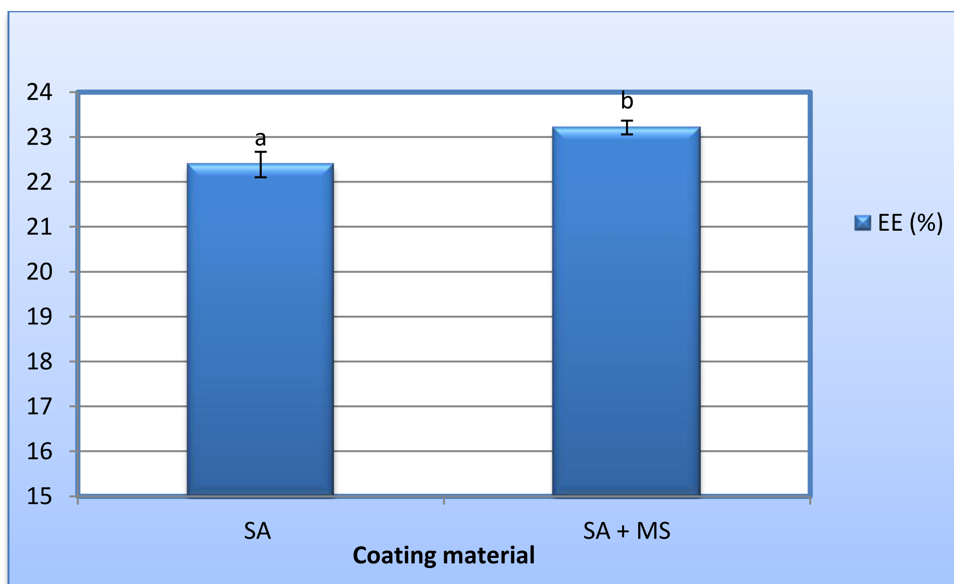



**Figure 4.6: EE of microencapsules prepared using SA and SA + MS by method 1**

$^{a\text{-}b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

It was evident from the above results that MS enhances the EE of iron microencapsules.

**Table 4.7: EE of microencapsules prepared using SA and SA + MS by method 2**

| Coating material | Encapsulation Efficiency (%) |
|---|---|
| SA 0.1 M | 60.398 ± 0.3327[a] |
| SA + MS 0.1 M | 62.972 ± 0.2285[b] |

Data are presented as means±SEM (n=3)
[a-b]Means within columns with different superscript are significantly different ($p<0.05$) from each other.

According to method 2, EE of iron microencapsules prepared using SA + MS (0.1 M $CaCl_2$) was significantly different ($p<0.05$) from SA (0.1 M $CaCl_2$) (Table 4.7 and Figure 4.7). SA + MS (0.1 M $CaCl_2$) showed higher EE as compared to SA (0.1 M $CaCl_2$). It was evident from the above results that MS enhances the EE of iron microencapsules.

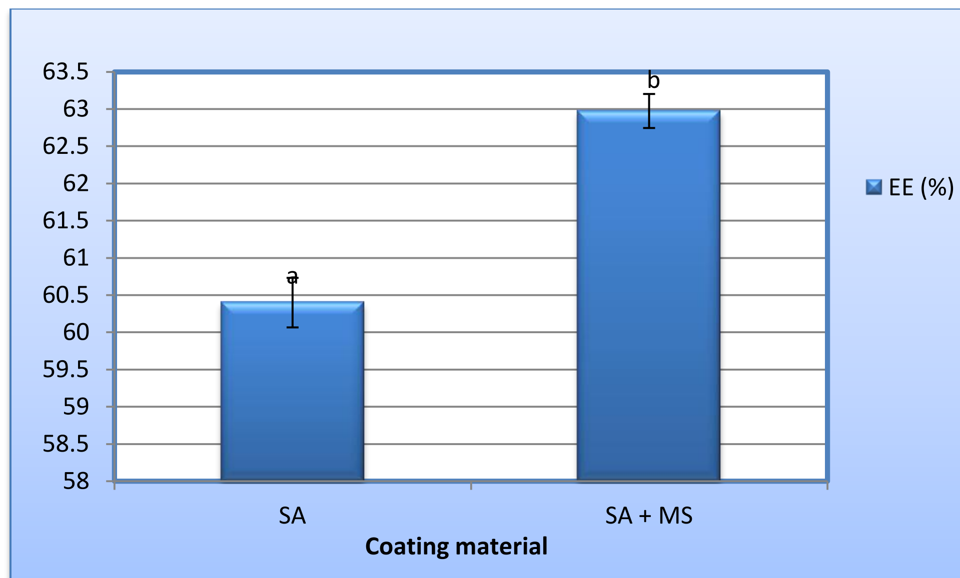



**Figure 4.7: EE of microencapsules prepared using SA and SA + MS by method 2**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

The results obtained were in agreement with the observations of Sultana *et al.* (2000) who reported that incorporation of Hi-Maize starch (a prebiotic) with SA improved encapsulation of viable bacteria.

Tan *et al.* (2009) microencapsulated fish oil by spray drying using a blend of SA and starch and observed that the addition of SA to the wall component resulted in

rounder microspheres with higher oil encapsulation efficiencies. Microencapsulated oil was found to be more stable to degradation than unencapsulated oil.

**4.1.3.2 Deemulsification method**

In this method the emulsion of oil and water phase was disrupted by chilled 0.1 M $CaCl_2$ solution. 0.1 M $CaCl_2$ solution was added both slowly and rapidly for disruption of emulsion and the effect of addition process on EE was elucidated. EE of these microencapsules was estimated by method 1 which has been described earlier in section 3.6.

**Table 4.8: EE of microencapsules prepared using SA by method 1**

| Rate of addition of $CaCl_2$ | Encapsulation Efficiency (%) |
|---|---|
| Slow addition | $69.187 \pm 0.3835^a$ |
| Fast addition | $74.850 \pm 0.3799^b$ |

Data are presented as means±SEM (n=3)
$^{a\text{-}b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

EE of iron microencapsules prepared by slow addition of $CaCl_2$ was significantly different ($p<0.05$) from fast addition of $CaCl_2$ (Table 4.8 and Figure 4.8). Slow addition of $CaCl_2$ resulted in lower EE as compared to fast addition of $CaCl_2$. It was evident from the above results that deemulsification should be fast for higher EE.

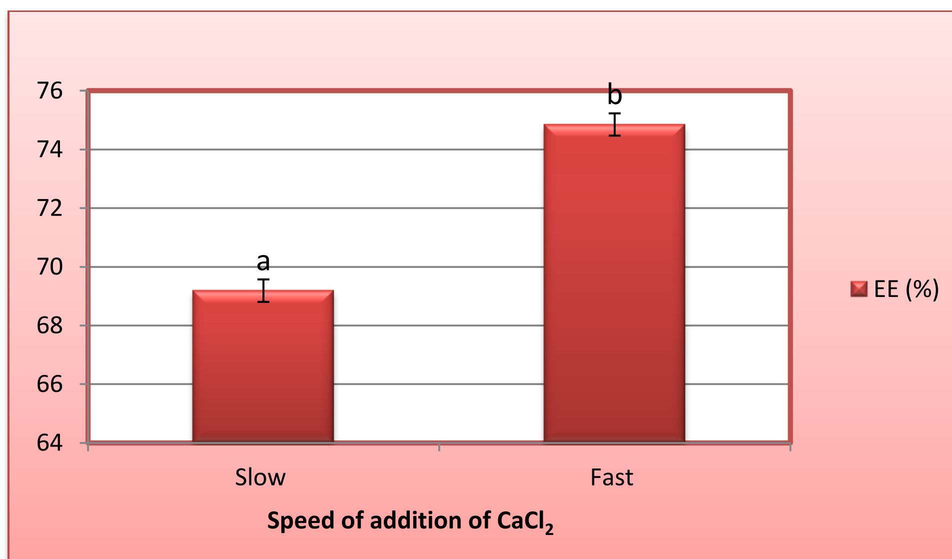



**Figure 4.8: EE of microencapsules prepared using SA by method 1**

$^{a\text{-}b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Mandal *et al.* (2006) encapsulated Lactobacillus casei NCDC-298 using sodium alginate and evaluated the effect of different alginate concentrations (2%, 3% or 4%) on survivability of bacteria under gastrointestinal conditions and reported that survivability increased proportionately with increasing alginate concentrations without affecting the release of entrapped cells in solution of colonic pH. In this study, fast addition of $CaCl_2$ was preferred. Khosroyar *et al.* (2012) encapsulated ferric saccharate with fast addition of calcium alginate coating by emulsification and observed a decrease in organoleptic and appearance problem and enhanced bioavailability.

Fast addition of $CaCl_2$ solution resulted in immediate disruption of the emulsion and provides very less time for coalescence, therefore small size microencapsules were formed with higher EE. Slow addition of $CaCl_2$ solution provides sufficient time for coalescence, therefore bigger size microencapsules were formed with lower EE.

Among all the microencapsules formulated, SA+MS 0.1 M and SA with fast addition of $CaCl_2$ were selected for further studies due to higher EE.

### 4.1.4 Preparation of iron microcapsules by using blend of GA, MD and MS

In previous studies, blend of GA, MD and MS was mainly used for encapsulation of the oils, oleoresins, aromatic esters, ascorbic acid and α tocopherol (Zilberboim *et al.* 1986a, 1986b; Trindade and Grosso 2000; Krishnan *et al.* 2005; Vaidya *et al.* 2006; Shaikh *et al.* 2006; Kanakdande *et al.* 2007; Laine *et al.* 2008; Yu *et al.* 2012). In the present study, it was tried for the microencapsulation of the iron.

Iron microencapsules were prepared by two methods using a blend of GA, MD and MS. In the first method, tween (0.05%) was used as a cooling media and in the second, alcohol was used as a dehydrating media.

#### 4.1.4.1 Preparation of iron microcapsules by spray cooling method using the blend of GA, MD and MS

Standardisation of the process was done by varying the different parameters required for maximum EE of iron. The ratio of GA, MD, MS was 4:1:1, respectively in 20 ml deionised water and the amount of iron salt, ascorbic acid was 200 mg, 13.7 mg, respectively in 10 ml phosphate citrate buffer (PCB) was kept constant for standardisation of process parameters. EE of these microencapsules was estimated only by method 1 which has been described earlier in section 3.6. During preparation of

iron microencapsules using PGMS, filterate/supernatant was not separated from the microencapsules, therefore EE were not estimated by method 2 and 3. In this standardisation process major coating material used in the blend was gum arabic and quantity of iron salt used was 200 mg, therefore, samples were represented as GA 200.

Optimisation conditions were as follows:

1) Addition of tween and suitability of different tweens for microencapsulation of iron
2) Effect of addition of PCB
3) Composition of GA, MD and MS
4) Amount of iron salt

**1) Addition of tween and suitability of different tweens for microencapsulation of iron**

For standardisation, different samples were prepared. Notations were as follows:

A) Without tween (WT) addition (no tween prior to sonication and no tween was used during spraying)

B) Addition of tween @ 0.1% of mixture (30 ml) prior to sonication and spraying directly (SD) into petridish

C) Addition of tween 40 @ 0.1% of mixture (30 ml) prior to sonication and spraying into 0.05% tween 40 solution (ST40)

**Table 4.9: EE of microencapsules as affected by tween**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation Efficiency (%) |
|---|---|
| GA 200 WT | $54.113 \pm 0.3788^{b}$ |
| GA 200 T40 SD | $51.370 \pm 0.7506^{a}$ |
| GA 200 T60 SD | $57.683 \pm 0.4205^{c}$ |
| GA 200 T80 SD | $53.500 \pm 0.7286^{b}$ |
| GA 200 T40 ST40 | $59.313 \pm 0.2791^{d}$ |
| GA 200 T60 ST60 | $67.497 \pm 0.3892^{f}$ |
| GA 200 T80 ST80 | $62.187 \pm 0.2671^{e}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

D) Addition of tween 60 @ 0.1% of mixture (30 ml) prior to sonication and spraying into 0.05% tween 60 solution (ST60)

E) Addition of tween 80 @ 0.1% of mixture (30 ml) prior to sonication and spraying into 0.05% tween 80 solution (ST80)

Different tweens i.e. tween 40 (T40), tween 60 (T60) and tween 80 (T80) were used for the microencapsulation of iron.

EE of GA 200 WT and GA 200 T80 SD were non significantly different ($p>0.05$) from each other, whereas, GA 200 T60 SD, GA 200 T40 ST40, GA 200 T60 ST60 and GA 200 T80 ST 80 were significantly higher ($p<0.05$) than GA 200 T40 SD, GA 200 T80 SD and GA 200 WT (Table 4.9 and Figure 4.9). GA 200 T60 ST60 showed highest EE as compared to other microencapsules. It was evident from the results obtained that iron microencapsules prepared by addition of tween 60 @ 0.1% prior to sonication and spraying into 0.05% tween 60 solution resulted in highest EE. Tween had positive effect on EE of iron microencapsules and Tween 60 was the most suitable stabilizer for iron microencapsulation.

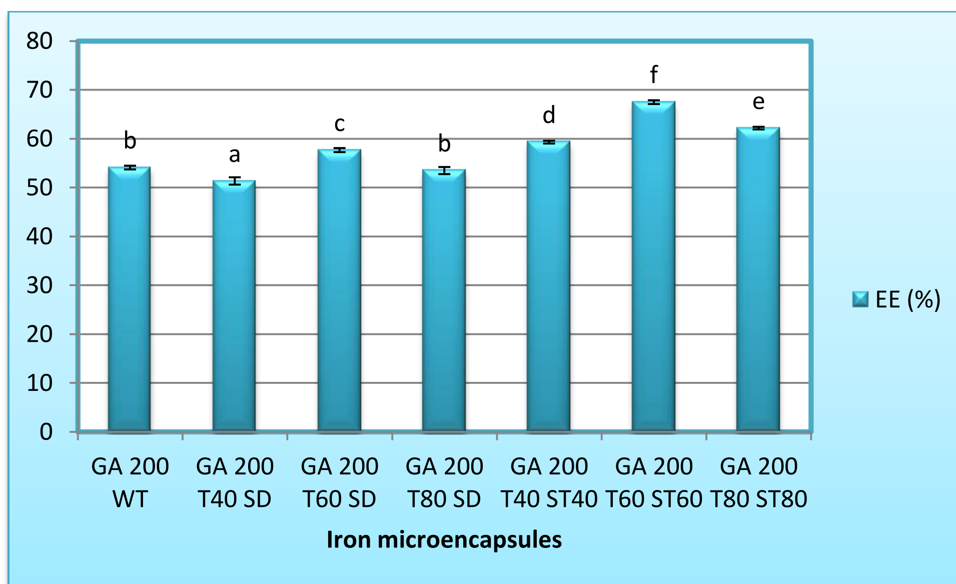



**Figure 4.9: EE of microencapsules as affected by tween**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Tween was used for stabilization of the microencapsules. Our results were in agreement with the Kwak *et al.* (2003a) who microencapsulated iron using PGMS as wall material and 0.05% tween 60 as cooling and stabilizing media for the

microencapsulation. Most effective stabilizing agent for the iron microencapsules using blend of GA, MD and MS was tween 60.

**2) Effect of addition of PCB**

For evaluating the effect of addition of PCB, four samples were prepared. All the samples were prepared by the process standardized in the first step.

A) Rehydration (RH) of blend was done in deionised water and iron salt was also dissolved in deionised water (without PCB)

B) Rehydration of blend was done in deionised water and iron salt was dissolved in PCB (with PCB)

C) Rehydration of blend was done in PCB and iron salt was dissolved in deionised water (without PCB)

D) Rehydration of blend was done in PCB and iron salt was also dissolved in PCB (with PCB)

**Table 4.10: EE of microencapsules as affected by PCB**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation Efficiency (%) |
|---|---|
| GA 200 RH in water (without PCB) **(A1)** | $68.147 \pm 0.3170^{c}$ |
| GA 200 RH in water (with PCB) **(A2)** | $67.803 \pm 0.2463^{c}$ |
| GA 200 RH in PCB (without PCB) **(A3)** | $65.453 \pm 0.4563^{b}$ |
| GA 200 RH in PCB (with PCB) **(A4)** | $63.743 \pm 0.4203^{a}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

EE of GA 200 RH in water (without PCB) and GA 200 RH in water (with PCB) were non significantly different ($p>0.05$) from each other, whereas, GA 200 RH in PCB (without PCB) and GA 200 RH in PCB (with PCB) were significantly lower ($p<0.05$) than GA 200 RH in water (without PCB) and GA 200 RH in water (with PCB) (Table 4.10 and Figure 4.10). It was evident from the results obtained that PCB had slight detrimental effect on the iron microencapsules. Therefore, the process without PCB was selected for the microencapsulation of iron. GA is a complex and variable mixture of arabinogalactan, oligosaccharides, polysaccharides and glycoproteins. It also contain significant amount of 4-O-methyl-D-glucuronic acid due to which it has negative charge on dissolution in water. Iron salt (ferrous sulphate heptahydrate) has positive charge;

therefore, they might interact and form strong bonds. PCB act as a chelating agent and it chelates ferrous ion and thus interferes with the interaction of iron salt with gum arabic, hence reduces the EE of iron microencapsules made using PCB (Williams and Phillips 2000).

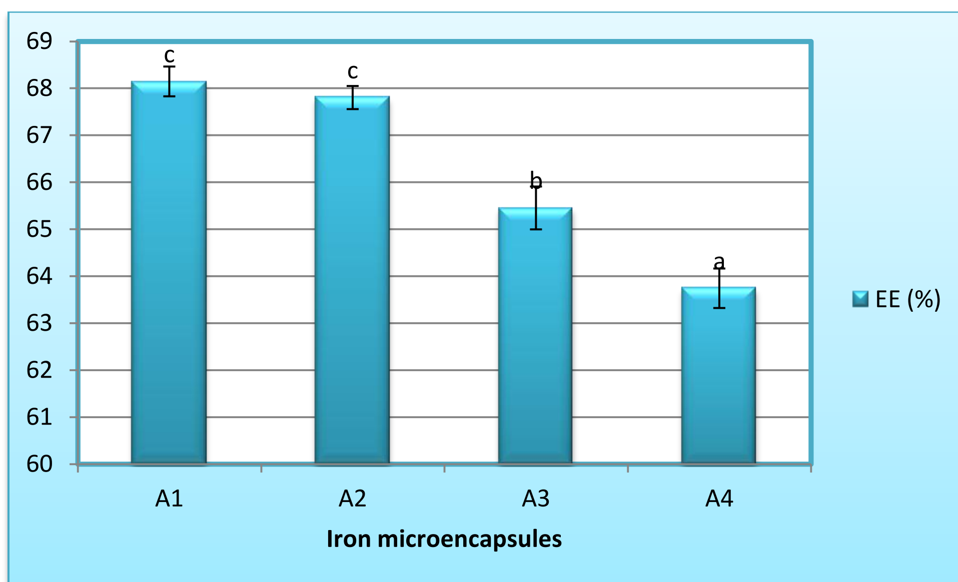



**Figure 4.10: EE of microencapsules as affected by PCB**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 3) Composition of GA, MD and MS

Ratio of GA, MD, and MS was varied for this purpose. Process standardised in the above two steps was followed for the microencapsules preparation. Ratio of GA, MD, and MS was varied as follows:

A) 4:1:1

B) 1:4:1

C) 1:1:4

D) 2:2:2

E) 3:2:1

F) 3:1:2

G) 6:0:0

H) 0:6:0

I) 0:0:6

**Table 4.11: EE of microencapsules as affected by composition of GA, MD and MS**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation Efficiency (%) |
|---|---|
| GA 200 4:1:1 **(A)** | 68.167 ± 0.3755[i] |
| GA 200 1:4:1 **(B)** | 37.850 ± 0.3493[d] |
| GA 200 1:1:4 **(C)** | 33.613 ± 0.1934[b] |
| GA 200 2:2:2 **(D)** | 44.713 ± 0.5983[e] |
| GA 200 3:2:1 **(E)** | 49.437 ± 0.6942[g] |
| GA 200 3:1:2 **(F)** | 46.087 ± 0.2115[f] |
| GA 200 6:0:0 **(G)** | 60.403 ± 0.4380[h] |
| GA 200 0:6:0 **(H)** | 35.657 ± 0.3460[c] |
| GA 200 0:0:6 **(I)** | 32.170 ± 0.3460[a] |

Data are presented as means±SEM (n=3)
[a-b]Means within columns with different superscript are significantly different ($p<0.05$) from each other.

EE of all the microencapsules were significantly different ($p<0.05$) from each other. GA 200 4:1:1 showed highest EE as compared to other microencapsules (Table 4.11 and Figure 4.11). It was evident from the results that 4:1:1 ratio of GA, MD and MS was the most suitable composition for microencapsulation of iron as it resulted in higher EE.

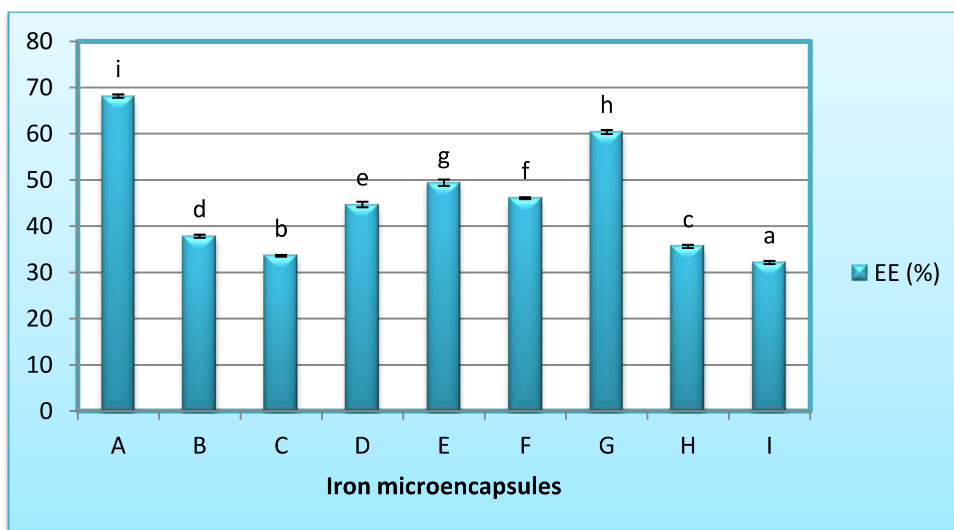



**Figure 4.11: EE of microencapsules as affected by composition of GA, MD and MS**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Our results were in agreement with Krishnan *et al.* (2005); Kanakdande *et al.* (2007) who observed that the blend of GA, MD and MS in the ratio of 4:1:1 gave better results as compared to 100% GA. They also observed the microencapsules by SEM and found GA, MD and MS in the ratio of 4:1:1 gave spherical microencapsules with smooth surface. Microencapsules from gum arabic alone were found to be nearly spherical but had many dents on the surface, whereas the microencapsules obtained from MD and MS were partially disrupted.

4) **Amount of iron salt**

Iron salt (ferrous sulphate heptahydrate) was used as a core material. Optimisation of concentration of iron salt was performed with the addition of different amount of iron salt to the blend of GA, MD and MS in the ratio 4:1:1. Amount of iron salt added were:

A) 200 mg
B) 300 mg
C) 400 mg
D) 600 mg
E) 800 mg
F) 1000 mg

**Table 4.12: EE of microencapsules as affected by iron concentration**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation Efficiency (%) |
|---|---|
| GA 200 T60 ST60 **(A)** | $67.447 \pm 0.5171^{e}$ |
| GA 300 T60 ST60 **(B)** | $67.463 \pm 0.3379^{e}$ |
| GA 400 T60 ST60 **(C)** | $59.510 \pm 0.4821^{d}$ |
| GA 600 T60 ST60 **(D)** | $53.670 \pm 0.3236^{c}$ |
| GA 800 T60 ST60 **(E)** | $45.777 \pm 0.2916^{b}$ |
| GA 1000 T60 ST60 **(F)** | $31.300 \pm 0.4937^{a}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

EE of GA 200 T60 ST 60 and GA 300 T60 ST 60 were non significantly different ($P>0.05$) from each other, whereas, EE of GA 400 T60 ST60, GA 600 T60 ST60, GA 800 T60 ST60, GA 1000 T60 ST60 were significantly lower ($p<0.05$) than GA 200 T60 ST60 and GA 300 T60 ST60 (Table 4.12 and Figure 4.12). It was evident from the

above results that 300 mg of iron salt could be microencapsulated with the blend of GA, MD and MS in the ratio of 4:1:1 and this process resulted in a maximum of EE 67.48%.

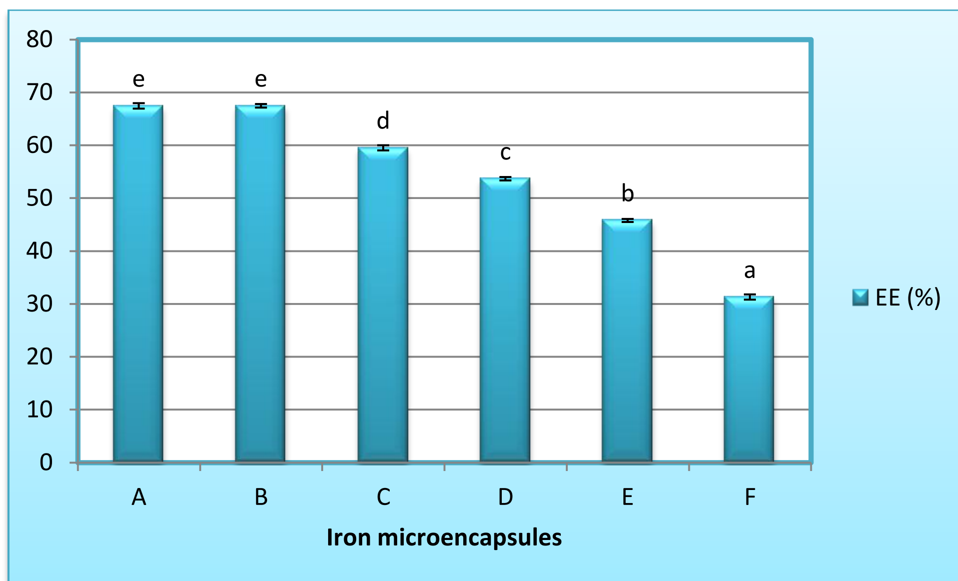



**Figure 4.12: EE of microencapsules as affected by iron concentration**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other. Error bars show the variations of three determinations in terms of standard error of mean.

According to the above results, the process for preparation of iron microcapsules by spray cooling method using a blend of GA, MD and MS was standardized and is discussed below:

Microencapsules were prepared by dissolving GA, MD and MS in ratio of 4:1:1 in deionised water at 60ºC and kept it for rehydration under refrigerated condition (4-7ºC) for 12 hrs. Iron salt (300 mg) and ascorbic acid (20.54 mg) were dissolved in deionised water containing tween 60 @ 0.1% of mixture. Solution of core and coating material were mixed and kept in the ice bath. This mixture was sonicated using a probe sonicator at 5ºC and 5.0 sec pulse rate for 15 min. This resulting emulsion was then sprayed in the solution of 0.05% tween 60 using an airless paint sprayer at 1.5 kg/cm$^2$. It was then frozen at -20ºC and freeze dried using lyophiliser to obtain a free flowing powder. Iron microencapsules prepared by the above method was subjected for further analysis.

**4.1.4.2 Preparation of iron microcapsules using alcohol as dehydrating media with the blend of GA, MD and MS**

Standardisation of the process was carried out by varying the different parameters for maximum EE of iron. The ratio of GA, MD, MS as 4:1:1 in 10 ml deionised water and amount of iron salt, ascorbic acid as 300 mg, 20.54 mg, respectively in 10 ml deionised water was kept constant for standardisation of the process parameters. Absolute alcohol was used as a dehydrating media in the ratio of mixture to alcohol 1:10. EE of these microencapsules was estimated by method 3 which has been described earlier in section 3.6. In this process major coating material in the blend was gum arabic and iron salt was 300 mg, therefore, samples were represented as GA 300. Optimisation conditions were as follows:

1) Different concentration of alcohol
2) Different ratio of mixture and alcohol
3) Composition of GA, MD and MS
4) Amount of iron salt

**1) Different concentration of alcohol**

80%, 90% and absolute alcohol were tried as dehydrating media for preparation of microencapsules with maximum EE.

**Table 4.13: EE of microencapsules as affected by different concentration of alcohol estimated by method 3**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation efficiency (%) |
|---|---|
| GA 300 80% alcohol | $77.564 \pm 0.2233^{a}$ |
| GA 300 90% alcohol | $89.066 \pm 0.1787^{b}$ |
| GA 300 absolute alcohol | $91.579 \pm 0.1201^{c}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

EE of all the microencapsules were significantly different ($p<0.05$) from each other. GA 300 absolute alcohol showed highest EE as compared to GA 300 80% alcohol and GA 300 90% alcohol (Table 4.13 and Figure 4.13). It was evident from the results obtained that absolute alcohol was the most suitable dehydrating media required for dehydration of iron microencapsules.

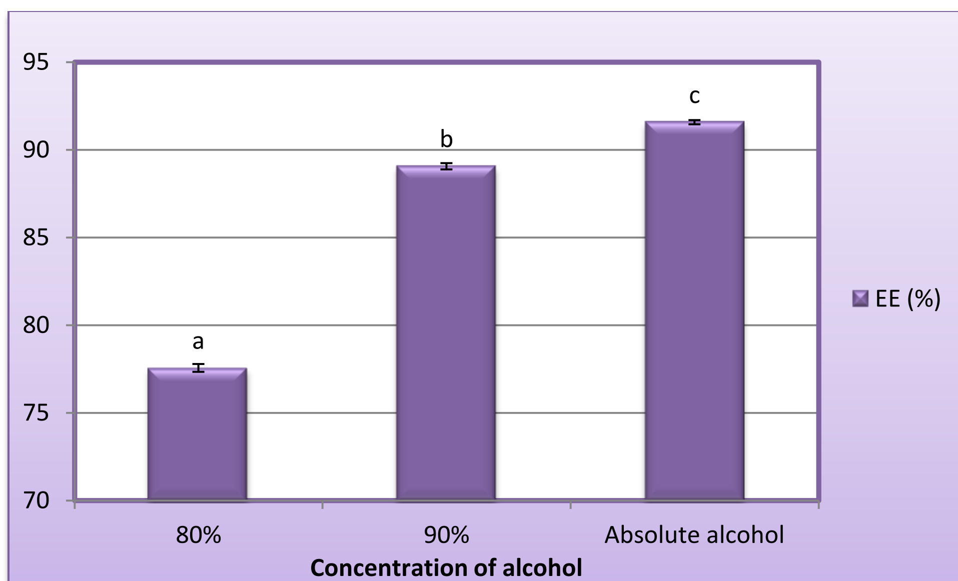



**Figure 4.13: EE of microencapsules as affected by different concentration of alcohol estimated by method 3**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Ethanol was used as a dehydrating media for various microencapsules preparations. It was used for various vitamin, paprika oleoresin and ethyl caprylate encapsulations by Richmond and Moss (1983), Zilberboim *et al.* (1986a) and Zilberboim *et al.* (1986b).

Our results were in agreement with Zilberboim *et al.* (1986a) who observed that the reduction in alcohol concentration reduced the retention capacity of the encapsules as lower concentration resulted in slow release of water from the encapsules and longer dehydration time. Lower concentration of alcohol resulted in particle agglomeration into sticky mass which was difficult to filter. They used vacuum drier for the drying of the microencapsules at 50$^{o}$C.

**2) Different ratio of mixture to alcohol**

According to the above results, absolute alcohol was the most suitable dehydrating media for dehydration of iron microencapsules, therefore the ratio of mixture to absolute alcohol was varied as 1:5, 1:7.5 and 1:10 for the preparation of microencapsules with maximum EE.

**Table 4.14: EE of microencapsules as affected by different ratio of mixture to alcohol ratio estimated by method 3**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation efficiency (%) |
|---|---|
| GA 300 1:5 | 68.703 ± 0.2685[a] |
| GA 300 1:7.5 | 80.223 ± 0.1889[b] |
| GA 300 1:10 | 91.579 ± 0.1201[c] |

Data are presented as means±SEM (n=3)
[a-b]Means within columns with different superscript are significantly different (p<0.05) from each other.

EE of all the microencapsules were significantly different (p<0.05) from each other. GA 300 1:10 resulted in highest EE as compared to GA 300 1:5 and GA 300 1:7.5 (Table 4.14 and Figure 4.14). It was evident from the results that the most suitable, mixture to alcohol ratio was 1:10 for microencapsulation of iron.

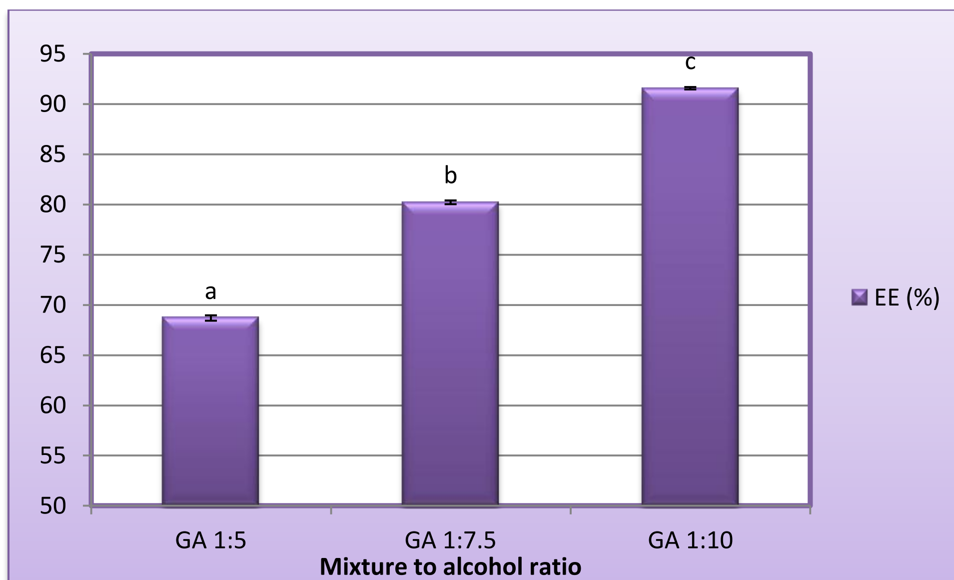



**Figure 4.14: EE of microencapsules as affected by different ratio of mixture to alcohol ratio estimated by method 3**

[a-b]Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Our results are in agreement with Zilberboim *et al.* (1986a) who reported that alcohol to emulsion ratio below 10:1 reduced the retention efficiency of the microencapsules but at higher concentration reduced the water content without affecting the retention efficiency as higher ratio of alcohol to emulsion resulted in rapid drying of encapsules and formed a protective crust that prevented the extensive leaching of core material.

Cho and Park (2003) evaluated the process parameters for O/W/O multiple emulsion method for flavour encapsulation and reported that increase in the gum arabic content resulted in more stable emulsion and highest flavour retention (71%) was observed with ethanol to mixture ratio of 9:1 as a dehydrating agent.

**3) Composition of GA, MD and MS**

According to the above two parameters ratio of mixture to alcohol, 1:10 and use of absolute alcohol as dehydrating media were most suitable for microencapsulation of iron. Ratio of GA, MD and MS was now varied by keeping these two parameters constant. Ratio of GA, MD and MS was varied as:

A) 4:1:1
B) 1:4:1
C) 1:1:4
D) 2:2:2
E) 3:2:1
F) 3:1:2
G) 6:0:0

**Table 4.15: EE of microencapsules as affected by composition of GA, MD and MS estimated by method 3**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation Efficiency (%) |
|---|---|
| GA 300 4:1:1 **(A)** | $91.579 \pm 0.1201^{g}$ |
| GA 300 1:4:1 **(B)** | $65.699 \pm 0.4015^{a}$ |
| GA 300 1:1:4 **(C)** | $69.432 \pm 0.3458^{b}$ |
| GA 300 2:2:2 **(D)** | $82.854 \pm 0.1812^{d}$ |
| GA 300 3:2:1 **(E)** | $79.628 \pm 0.1687^{c}$ |
| GA 300 3:1:2 **(F)** | $83.548 \pm 0.3566^{e}$ |
| GA 300 6:0:0 **(G)** | $88.796 \pm 0.2614^{f}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

EE of all the microencapsules were significantly different ($p<0.05$) from each other. GA 300 4:1:1 showed highest EE as compared to all other microencapsules (Table 4.15 and Figure 4.15). GA 300 6:0:0 showed significantly lower ($p<0.05$) EE as

compared to GA 300 4:1:1. It was evident from the results that 4:1:1 ratio of GA, MD, and MS was the most suitable ratio for microencapsulation of iron.

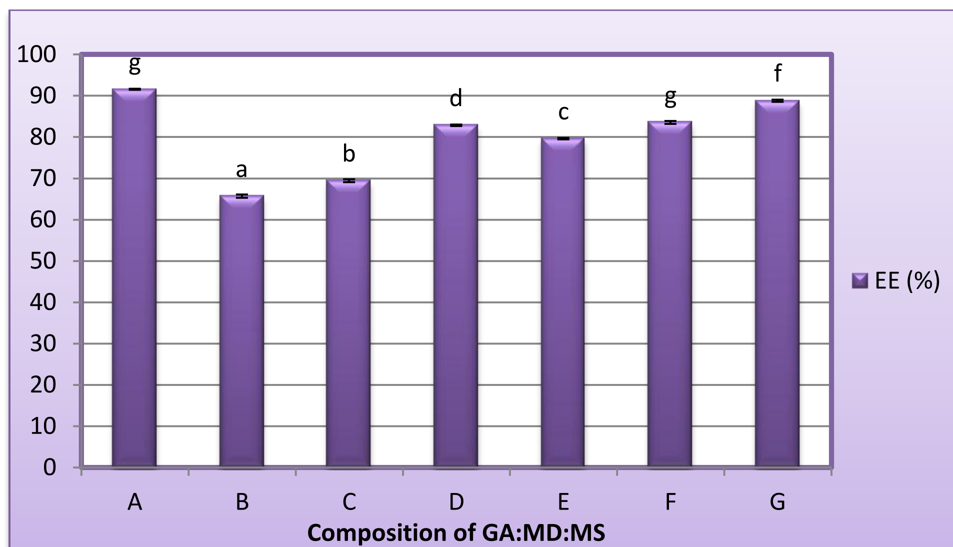



**Figure 4.15: EE of microencapsules as affected by composition of GA, MD and MS estimated by method 3**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other. Error bars show the variations of three determinations in terms of standard error of mean.

The observed results were in agreement with Krishnan *et al.* (2005); Kanakdande *et al.* (2007) observed that the blend of GA, MD and MS in the ratio of 4:1:1 gave better results as compared to 100% GA. They also observed microencapsules by SEM and found GA, MD and MS in the ratio of 4:1:1 gave spherical microencapsules with smooth surface. Microencapsules from gum arabic alone were found to be nearly spherical but had many dents on the surface, whereas the microencapsules obtained from MD and MS were partially disrupted.

Microencapsules prepared with GA, MD, MS with a ratio of 1:4:1 and 1:1:4 were difficult to prepare as they blocked the filter paper and it was very difficult to filter using Whatman No 1 filter paper. EE of these microencapsules were also less as compared to other microencapsules, therefore GA 300 0:6:0 and GA 300 0:0:6 were not tried for microencapsulation of iron. It was also evident from the above results that MS enhanced the EE more as compared to the MD.

**4) Amount of iron salt**

Most suitable microencapsulation process was finally selected on the basis of EE estimated by method 3 which has been described earlier in section 3.6. Amount of iron

salt was standardized by estimating the EE by method 1 and 3 both of which have also been described earlier in section 3.6. All the samples were prepared using the process standardised in the above three steps.

Iron salt (ferrous sulphate heptahydrate) was used as a core material. Optimisation of the concentration of iron salt was done with the addition of different amount of iron salt to the blend of GA, MD and MS. Amount of iron salt added was as follows

A) 300 mg
B) 500 mg
C) 800 mg
D) 1000 mg

**Table 4.16: EE of microencapsules as affected by iron concentration estimated by method 3**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation Efficiency (%) |
|---|---|
| GA 300 **(A)** | 91.579 ± 0.1201[d] |
| GA 500 **(B)** | 89.857 ± 0.1628[c] |
| GA 800 **(C)** | 87.641 ± 0.2298[b] |
| GA 1000 **(D)** | 85.574 ± 0.1661[a] |

Data are presented as means±SEM (n=3)
[a-b]Means within columns with different superscript are significantly different (p<0.05) from each other.

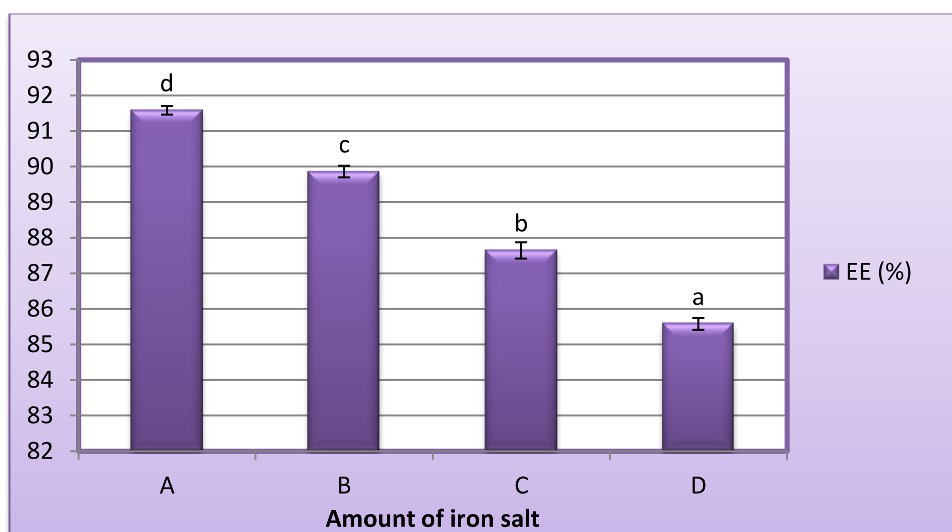



**Figure 4.16: EE of microencapsules as affected by iron concentration estimated by method 3**

[a-b]Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

EE of all the microencapsules was significantly different ($p<0.05$) from each other. It was evident from the results that GA 300 showed highest EE as compared to GA 500, GA 800 and GA 1000 (Table 4.16 and Figure 4.16).

**Table 4.17: EE of microencapsules as affected by iron concentration estimated by method 1**

| Iron microencapsules using GA, MD and MS as coating material | Encapsulation Efficiency (%) |
|---|---|
| GA 300 **(A)** | $75.101 \pm 0.1366^{d}$ |
| GA 500 **(B)** | $71.858 \pm 0.2663^{c}$ |
| GA 800 **(C)** | $63.312 \pm 0.3245^{b}$ |
| GA 1000 **(D)** | $60.019 \pm 0.1939^{a}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

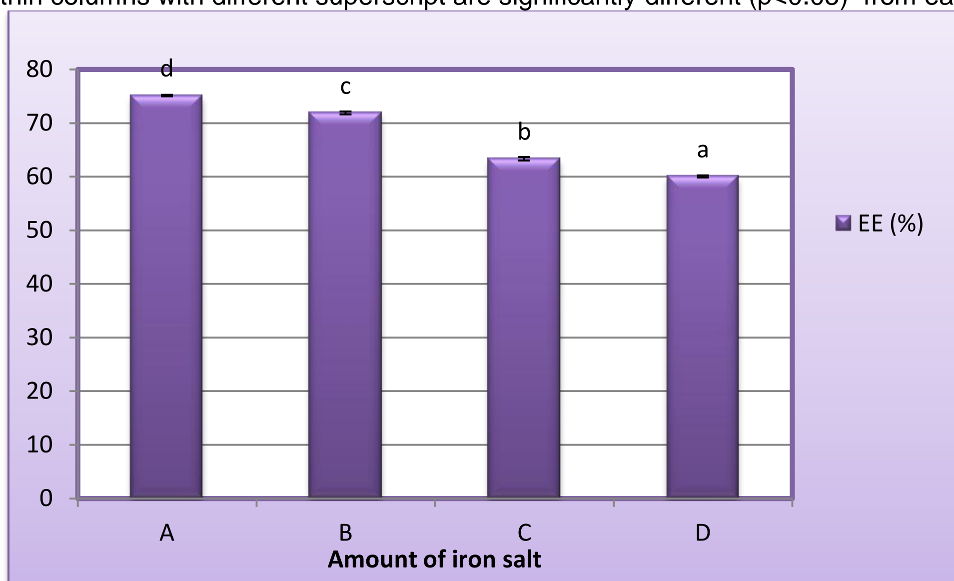



**Figure 4.17: EE of microencapsules as affected by iron concentration estimated by method 1**

$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

EE of all the microencapsules were significantly different ($p<0.05$) from each other. It was evident from the results that GA 300 showed highest EE as compared to GA 500, GA 800 and GA 1000 (Table 4.17 and Figure 4.17).

Higher EE of microencapsules by method 3 revealed higher entrapment of iron inside the microencapsules during preparation and lower EE of microencapsules by method 1 showed higher release of iron in the aqueous system.

Trindade and Grosso (2000) microencapsulated ascorbic acid by spray drying method using gum arabic and rice starch as coating material. He reported that microencapsules coated with gum arabic were more stable than rice starch coated. Laine *et al.* (2008) microencapsulated α-tocopherol with gum arabic and spruce galactomannan and reported that highest encapsulation yield was obtained with gum arabic (59%).

GA 300 and GA 500 were selected from the above microencapsules for further analysis. At a higher iron concentration, amount of coating material reduced. However, at a higher iron concentration the release of iron from microencapsules was also fast.

According to the above results, standardised process for preparation of iron microcapsules by spray cooling method using the blend of GA, MD and MS was as follows:

Microencapsules were prepared by dissolving GA, MD and MS in ratio of 4:1:1 in deionised water at 60ºC and kept it for rehydration under refrigeration (4-7ºC) for 12 hrs. Iron salt (300 or 500 mg) and ascorbic acid (20.54 or 34.25 mg) were dissolved in deionised water. Solution of core and coating material were mixed and kept in the ice bath. This mixture was then sonicated using a probe sonicator at 5ºC with 5.0 sec pulse rate for 15 min. This mixture was then sprayed in absolute alcohol with the ratio of mixture to absolute alcohol 1:10. Alcohol solution was kept on magnetic stirrer and stirred at 500 rpm. Airless paint sprayer was used for spraying of the mixture in chilled alcohol which was operated at 1.5 $kg/cm^2$. After spraying, mixture was left undisturbed for 5 min. Finally, filtration was carried out under vacuum by Whatman No. 1 filter paper using vacuum filtration assembly. The retentate was then spread in petridish and stored at 4-7ºC/12-14 hrs for complete removal of alcohol.

**4.2 Standardisation of AAS method for estimation of iron content in iron microencapsules**

Different process parameters were utilized for standardisation of AAS method for estimation of iron content in microencapsules. These parameters were as follows:

1) Weight of the sample and dilution factor
2) Period of ashing
3) Different method of digestion

**1) Weight of the sample and dilution factor**

Weigh 100, 200, 300, 400 and 500 mg iron microencapsules in different crucibles. Charred it on hot plate and kept it in the muffle furnace for 16 hrs for proper ashing of the microencapsules. Ash was dissolved in 10 ml $HNO_3$ and then transferred to the 100 ml volumetric flask and made up the volume with deionised water. Dissolved ash was filtered with Whatman No. 42 filter paper. During estimation of iron content with $100^{th}$ dilution, absorbance was higher than the absorbance of standard curve. Samples were then diluted 1000 times and iron content was estimated in these samples. Iron content in the 100 mg sample was in the range of the standard curve. Therefore, weight of the sample was selected as 100 mg with a dilution factor 1000.

**2) Period of ashing**

The parameter standardized in the above step was kept constant for further standardisation. Ashing time was estimated by keeping the same sample (100 mg) in different crucibles. Crucibles were taken out from the muffle at different time intervals (4 hrs, 6 hrs, 8 hrs, 10 hrs, 12 hrs, 14 hrs and 16 hrs). Samples were prepared by dry ashing method. Iron content in these samples was estimated by AAS.

**Table 4.18: Optimisation of ashing time for microencapsules**

| Sample | Iron content (ppm) |
|---|---|
| GA 200 T80 SD (4 hrs) | $4942.541 \pm 57.7294^{a}$ |
| GA 200 T80 SD (6 hrs) | $5437.143 \pm 31.2652^{b}$ |
| GA 200 T80 SD (8 hrs) | $5896.083 \pm 41.1936^{c}$ |
| GA 200 T80 SD (10 hrs) | $5903.333 \pm 20.5778^{c}$ |
| GA 200 T80 SD (12 hrs) | $5894.691 \pm 19.7710^{c}$ |
| GA 200 T80 SD (14 hrs) | $5901.691 \pm 15.1547^{c}$ |
| GA 200 T80 SD (16 hrs) | $5941.264 \pm 4.4189^{c}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

Iron content of the microencapsules after 8 hrs i.e.10 hrs, 12 hrs, 14 hrs and 16 hrs were not significantly different ($p>0.05$) from each other, whereas, iron content of

the microencapsules after 4 hrs and 6 hrs were significantly lower ($p<0.05$) than 8 hrs (Table 4.18 and Figure 4.18). According to the above results ashing time for the microencapsules was selected as 8 hrs.

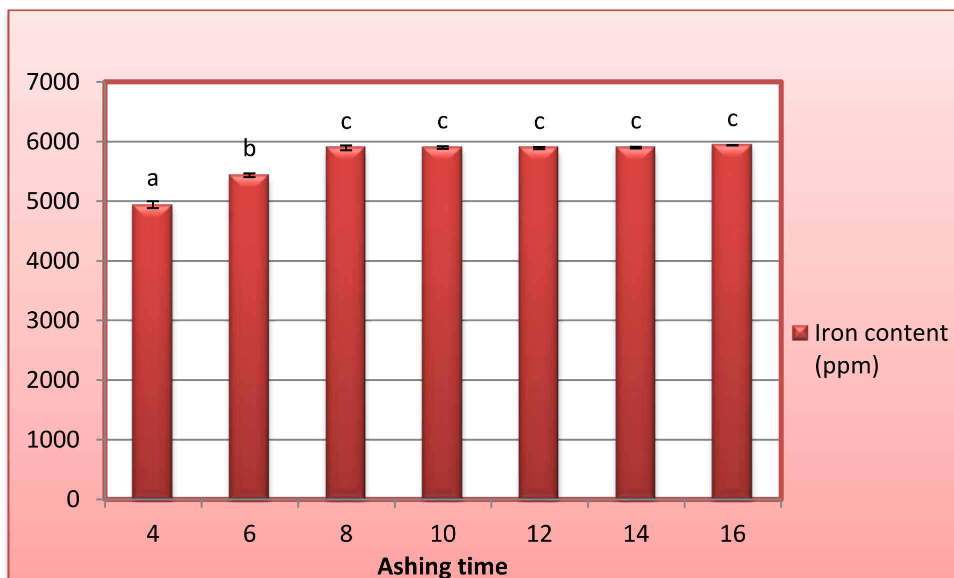



**Table 4.18: Optimisation of ashing time for microencapsules**

$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 3) Different methods of digestion

Three different methods of digestion were followed for standardisation of process for estimation of iron content in iron microencapsules. These methods were as follows:

a) Dry ashing
b) Wet digestion
c) Combination of dry and wet digestion

**Table 4.19: Optimisation of digestion method for microencapsules**

| Sample | Iron content (ppm) |
|---|---|
| GA 200 T80 SD (dry ashing) | 5896.083 ± 41.1936$^{b}$ |
| GA 200 T80 SD (wet digestion) | 5072.677 ± 82.7647$^{a}$ |
| GA 200 T80 SD (combination of dry and wet digestion) | 6231.590 ± 45.4118$^{c}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

Iron content of all the microencapsules were significantly different ($p<0.05$) from each other (Table 4.19 and Figure 4.19). Lowest iron content was estimated by wet digestion followed by dry ashing and combination of dry and wet digestion.

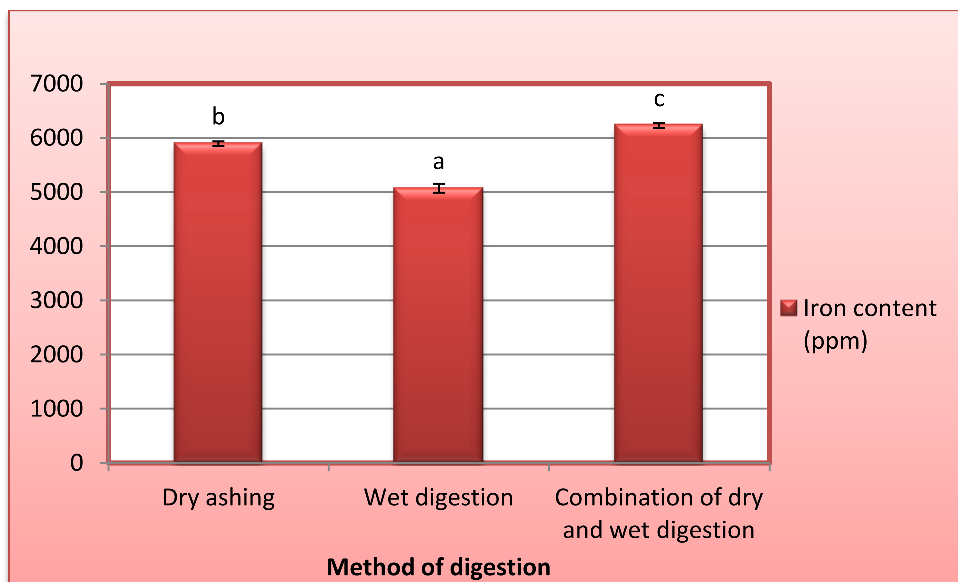



**Figure 4.19: Optimisation of digestion method for microencapsules**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

It was observed that during dry ashing, ash particles did not dissolve properly in acid and hence some were lost during filtration. Therefore, lower iron content was estimated by the dry ashing method as compared to the combination of dry and wet digestion method in which all the suspended particles properly dissolved. Therefore combination of dry and wet digestion was selected as most suitable method for estimation of iron content in iron microencapsules.

Wet digestion is carried out at 250-300ºC, whereas dry ashing at 550ºC. Wet digestion leads to complete solubilization of the material but incomplete decomposition resulting in erroneous results regarding iron content. Complete decomposition is required for achieving reproducible and accurate elemental analysis (*www.pg.gda.pl/chem/CEEAM/Dokumenty/CEEAM/chapter13.pdf*, 2013)

### 4.3 Selection of most suitable microencapsules

Microencapsules were prepared using phosphatidylcholine, PGMS, SA, blend of SA and pectin, blend of SA and MS and blend of GA, MD and MS. Microencapsules

were selected for sensory evaluation on the basis of their high EE. These microencapsules are as follows:

1) Liposome using egg phosphatidylcholine as coating material
2) Microencapsules using blend of SA and MS as coating material solidified in 0.1 M $CaCl_2$ (SA 0.1 M $CaCl_2$)
3) Microencapsules using SA as coating material by deemulsification method. (SA fast addition of $CaCl_2$)
4) Microencapsules using blend of GA, MD and MS as coating material using spray cooling method (GA 300 T60 ST60)
5) Microencapsules using blend of GA, MD and MS as coating material and absolute alcohol as dehydrating media (GA 300 1:10 & GA 500 1:10)

#### 4.3.1 Sensory analysis of yoghurt

The selected iron microencapsules were fortified in yoghurt and the product was evaluated by selected panelists from the institute, on the basis of BIS composite sensory score card for yoghurt (Annexure I). Sensory evaluation was based on following important characteristics i.e. flavour, body and texture, colour and appearance, acidity, container and closure and over all acceptability. Control (unfortified), iron salt fortified and iron microencapsules fortified yoghurt samples were prepared for the sensory evaluation. Iron salt and iron microencapsules were added @ 25 ppm iron.

##### 4.3.1.1 Sensory scores of iron salt fortified and liposomes fortified yoghurt

Total sensory scores of control (unfortified), iron salt and liposome fortified yoghurt were significantly different ($p<0.05$) from each other (Table 4.20). Lowest sensory scores were observed for liposome fortified yoghurt. Flavour scores of control (unfortified), iron salt and liposome fortified yoghurt were significantly different ($p<0.05$) from each other. Lowest flavour scores were observed for liposome fortified yoghurt. Body and texture scores of control and fortified yoghurt samples did not differ significantly ($p>0.05$) from each other. Acidity and colour and appearance scores of liposome fortified yoghurt were significantly lower ($p<0.05$) than control (unfortified) and iron salt fortified yoghurt.

Liposomes were hard to dissolve, and it took around 45 min for their complete dissolution in milk. Yoghurt fortified with liposomes had an oily and unacceptable

flavour. Lower flavour scores of this yoghurt was not due to the oxidized flavour but was due to the inherent flavour of phosphatidylcholine, which is an integral component of liposomes. It also imparted slight yellow-brown colour to yoghurt.

**Table 4.20: Effect of addition of iron salt (ferrous sulphate) and liposomes on sensory scores of yoghurt**

| Characteristics | Maximum scores | Control (Unfortified) | Iron salt fortified | Liposomes fortified |
|---|---|---|---|---|
| Flavour | 45 | $41.90 \pm 0.34^{C}$ | $36.55 \pm 0.27^{B}$ | $31.80 \pm 0.49^{A}$ |
| Body and texture | 30 | $27.20 \pm 0.21^{A}$ | $27.10 \pm 0.27^{A}$ | $26.50 \pm 0.37^{A}$ |
| Acidity | 10 | $8.95 \pm 0.17^{B}$ | $8.90 \pm 0.18^{B}$ | $7.80 \pm 0.20^{A}$ |
| Colour and appearance | 10 | $8.95 \pm 0.17^{B}$ | $8.90 \pm 0.18^{B}$ | $7.80 \pm 0.20^{A}$ |
| Container and closure | 5 | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ |
| Total scores | 100 | $91.90 \pm 0.44^{C}$ | $86.50 \pm 0.29^{B}$ | $82.05 \pm 0.60^{A}$ |

Data are presented as means±SEM (n=10).
$^{A-B}$Means within rows with different superscript are significantly different ($p<0.05$) from each other.

Our results were in agreement with Abbasi and Azari (2011) who fortified milk with liposomes and reported a significant difference between treated and control samples regarding smell and metallic taste, whereas non significant differences were observed in terms of astringency, bitterness and colour scores.

**4.3.1.2 Sensory scores of iron salt fortified and SA + MS (0.1 M $CaCl_2$) microencapsules fortified yoghurt**

Total sensory scores of control (unfortified) and SA + MS (0.1 M $CaCl_2$) microencapsules fortified yoghurt were significantly higher ($p<0.05$) than iron salt fortified yoghurt (Table 4.21). Lowest total sensory scores were observed for iron salt fortified yoghurt. Flavour scores of control (unfortified) and SA + MS (0.1 M $CaCl_2$) microencapsules fortified yoghurt were significantly higher ($p<0.05$) from iron salt fortified yoghurt. Body and texture, acidity, colour and appearance and container and closure scores of control and fortified yoghurt samples did not differ significantly ($p>0.05$) from each other.

**Table 4.21: Effect of addition of iron salt and SA + MS (0.1 M $CaCl_2$) microencapsules on sensory scores of yoghurt**

| Characteristics | Maximum scores | Control (Unfortified) | Iron salt fortified | SA + MS (0.1 M $CaCl_2$) fortified |
|---|---|---|---|---|
| Flavour | 45 | $42.10 \pm 0.27^{B}$ | $36.65 \pm 0.31^{A}$ | $41.40 \pm 0.28^{B}$ |
| Body and texture | 30 | $27.10 \pm 0.19^{A}$ | $27.00 \pm 0.29^{A}$ | $26.55 \pm 0.19^{A}$ |
| Acidity | 10 | $8.75 \pm 0.17^{A}$ | $8.8 \pm 0.19^{A}$ | $8.85 \pm 0.15^{A}$ |
| Colour and appearance | 10 | $9.00 \pm 0.15^{A}$ | $8.95 \pm 0.16^{A}$ | $8.90 \pm 0.18^{A}$ |
| Container and closure | 5 | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ |
| Total scores | 100 | $91.95 \pm 0.36^{B}$ | $86.45 \pm 0.30^{A}$ | $90.80 \pm 0.40^{B}$ |

Data are presented as means±SEM (n=10).
$^{A-B}$Means within rows with different superscript are significantly different ($p<0.05$) from each other.

These microcapsules produced a fibrous network upon freeze drying. There was problem regarding sedimentation of capsules at the bottom when these microcapsules were added to milk for yoghurt preparation due to the difference in density of milk and microencapsules and some microcapsules tended to stick to the walls of container as well, thereby decreasing the amount of iron that would be finally made available to the consumer.

#### 4.3.1.3 Sensory scores of iron salt fortified and SA (Fast addition of $CaCl_2$) fortified yoghurt

**Table 4.22: Effect of addition of iron salt and SA (Fast addition of $CaCl_2$) microencapsules on sensory scores of yoghurt**

| Characteristics | Maximum scores | Control (Unfortified) | Iron salt fortified | SA (Fast addition of $CaCl_2$) fortified |
|---|---|---|---|---|
| Flavour | 45 | $42.20 \pm 0.21^{C}$ | $36.75 \pm 0.32^{B}$ | $34.20 \pm 0.42^{A}$ |
| Body and texture | 30 | $27.00 \pm 0.25^{B}$ | $26.90 \pm 0.34^{B}$ | $25.60 \pm 0.27^{A}$ |
| Acidity | 10 | $8.90 \pm 0.16^{B}$ | $8.90 \pm 0.16^{B}$ | $8.35 \pm 0.08^{A}$ |
| Colour and appearance | 10 | $9.00 \pm 0.00^{B}$ | $9.00 \pm 0.00^{B}$ | $8.50 \pm 0.15^{A}$ |
| Container and closure | 5 | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ |
| Total scores | 100 | $91.90 \pm 0.30^{C}$ | $86.4 \pm 0.33^{B}$ | $80.5 \pm 0.57^{A}$ |

Data are presented as means±SEM (n=10).
$^{A-B}$Means within rows with different superscript are significantly different ($p<0.05$) from each other.

Total sensory scores of control (unfortified), iron salt fortified and SA (Fast addition of $CaCl_2$) microencapsules fortified yoghurt were significantly different ($p<0.05$) from each other (Table 4.22).

Lowest total sensory scores were observed for SA (Fast addition of $CaCl_2$) microencapsules fortified yoghurt. Flavour scores of control (unfortified), iron salt fortified and SA (Fast addition of $CaCl_2$) microencapsules fortified yoghurt were significantly different ($p<0.05$) from each other. Lowest flavour scores were observed for SA (Fast addition of $CaCl_2$) microencapsules. Body and texture, acidity and colour and appearance scores of SA (Fast addition of $CaCl_2$) microencapsules were also significantly lower ($p<0.05$) than control (unfortified) and iron salt fortified yoghurt.

SA (Fast addition of $CaCl_2$) microencapsules were prepared using sunflower oil and after deemulsification, some amount of oil adhered to the microencapsules. During preparation of the yoghurt these oil droplets settled on the surface of the yoghurt and adversely affected the flavour and colour and appearance of yoghurt.

#### 4.3.1.4 Sensory scores of iron salt fortified and GA 300 T60 ST60 fortified yoghurt

Total sensory scores of control (unfortified) and GA 300 T60 ST60 microencapsules fortified yoghurt were significantly higher ($p<0.05$) than iron salt fortified yoghurt (Table 4.23). Lowest total sensory scores were observed for iron salt fortified yoghurt.

**Table 4.23: Effect of addition of iron salt and GA 300 T60 ST60 microencapsules on sensory scores of yoghurt**

| Characteristics | Maximum scores | Control (Unfortified) | Iron salt fortified | GA 300 T60 ST60 fortified |
|---|---|---|---|---|
| Flavour | 45 | $42.30 \pm 0.23^B$ | $36.85 \pm 0.37^A$ | $41.20 \pm 0.41^B$ |
| Body and texture | 30 | $26.90 \pm 0.27^A$ | $26.80 \pm 0.38^A$ | $27.05 \pm 0.24^A$ |
| Acidity | 10 | $8.75 \pm 0.15^A$ | $8.80 \pm 0.17^A$ | $8.85 \pm 0.15^A$ |
| Colour and appearance | 10 | $8.85 \pm 0.17^A$ | $8.80 \pm 0.19^A$ | $8.85 \pm 0.20^A$ |
| Container and closure | 5 | $5.00 \pm 0.00^A$ | $5.00 \pm 0.00^A$ | $5.00 \pm 0.00^A$ |
| Total scores | 100 | $91.95 \pm 0.29^B$ | $86.45 \pm 0.37^A$ | $91.10 \pm 0.67^B$ |

Data are presented as means±SEM (n=10).
$^{A-B}$Means within rows with different superscript are significantly different ($p<0.05$) from each other.

Flavour scores of control (unfortified) and GA 300 T60 ST60 microencapsules fortified yoghurt were significantly higher ($p<0.05$) than iron salt fortified yoghurt. Body and texture, acidity, colour and appearance and container and closure scores of the fortified yoghurt samples did not differ significantly ($p>0.05$) from control.

Control (unfortified) and GA 300 T60 ST60 fortified yoghurt were not significantly different ($p>0.05$) from each other in all aspects of sensory analysis.

**4.3.1.5 Sensory scores of iron salt fortified and GA 300 1:10 fortified yoghurt**

Total sensory scores of control (unfortified) and GA 300 1:10 microencapsules fortified yoghurt were significantly higher ($p<0.05$) than iron salt fortified yoghurt (Table 4.24). Lowest total sensory scores were observed for iron salt fortified yoghurt. Flavour scores of control (unfortified) and GA 300 1:10 microencapsules fortified yoghurt were significantly higher ($p<0.05$) than iron salt fortified yoghurt. Body and texture, acidity, colour and appearance and container and closure scores of the fortified yoghurt samples did not differ significantly ($p>0.05$) from control. Control (unfortified) and GA 300 1:10 fortified yoghurt were not significantly different ($p>0.05$) from each other in all aspects of sensory analysis.

**Table 4.24: Effect of addition of iron salt and GA 300 1:10 microencapsules on sensory scores of yoghurt**

| Characteristics | Maximum scores | Control (Unfortified) | Iron salt fortified | GA 300 1:10 fortified |
|---|---|---|---|---|
| Flavour | 45 | $42.30 \pm 0.23^{B}$ | $38.50 \pm 0.27^{A}$ | $41.30 \pm 0.28^{B}$ |
| Body and texture | 30 | $26.90 \pm 0.27^{A}$ | $26.80 \pm 0.38^{A}$ | $27.25 \pm 0.23^{A}$ |
| Acidity | 10 | $8.70 \pm 0.13^{A}$ | $8.75 \pm 0.15^{A}$ | $8.75 \pm 0.13^{A}$ |
| Colour and appearance | 10 | $8.90 \pm 0.18^{A}$ | $8.85 \pm 0.21^{A}$ | $8.90 \pm 0.24^{A}$ |
| Container and closure | 5 | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ |
| Total scores | 100 | $91.95 \pm 0.30^{B}$ | $88.45 \pm 0.38^{A}$ | $91.35 \pm 0.45^{B}$ |

Data are presented as means±SEM (n=10).
$^{A-B}$Means within rows with different superscript are significantly different ($p<0.05$) from each other.

**4.3.1.6 Sensory scores of iron salt fortified and GA 500 1:10 fortified yoghurt**

Total sensory scores of control (unfortified) and GA 500 1:10 microencapsules fortified yoghurt were significantly higher ($p<0.05$) than iron salt fortified yoghurt (Table

4.25). Lowest total sensory scores were observed for iron salt fortified yoghurt. Flavour scores of control (unfortified) and GA 500 1:10 microencapsules fortified yoghurt were significantly higher ($p<0.05$) from iron salt fortified yoghurt. Body and texture, acidity, colour and appearance and container and closure scores of the fortified yoghurt samples did not differ significantly ($p>0.05$) from control. Control (unfortified) and GA 500 1:10 fortified yoghurt were not significantly different ($p>0.05$) from each other in all aspects of sensory analysis.

**Table 4.25: Effect of addition of iron salt and GA 500 1:10 microencapsules on sensory scores of yoghurt**

| Characteristics | Maximum scores | Control (Unfortified) | Iron salt fortified | GA 500 1:10 fortified |
|---|---|---|---|---|
| Flavour | 45 | $42.00 \pm 0.27^B$ | $38.70 \pm 0.24^A$ | $41.10 \pm 0.22^B$ |
| Body and texture | 30 | $26.70 \pm 0.25^A$ | $26.10 \pm 0.40^A$ | $26.15 \pm 0.25^A$ |
| Acidity | 10 | $8.60 \pm 0.10^A$ | $8.20 \pm 0.11^A$ | $8.30 \pm 0.11^A$ |
| Colour and appearance | 10 | $8.85 \pm 0.17^A$ | $8.75 \pm 0.17^A$ | $8.90 \pm 0.18^A$ |
| Container and closure | 5 | $5.00 \pm 0.00^A$ | $5.00 \pm 0.00^A$ | $5.00 \pm 0.00^A$ |
| Total scores | 100 | $91.15 \pm 0.46^B$ | $86.75 \pm 0.44^A$ | $89.55 \pm 0.49^B$ |

Data are presented as means±SEM (n=10).
$^{A-B}$Means within rows with different superscript are significantly different ($p<0.05$) from each other.

GA 300 T60 ST60, GA 300 1:10 and GA 500 1:10 microencapsules gave satisfactory results which were comparable to control and hence, they were chosen for fortification in yoghurt. These selected microencapsules were further analysed by scanning electron microscopy (SEM) for gaining insight about the structure and surface characteristics of the microencapsules. Oxidative stability (TBA value) of the fortified yoghurt as affected by addition of iron microencapsules was also studied.

### 4.3.2 Scanning electron microscopy (SEM) of the selected microencapsules

Above selected microencapsules were analysed by SEM for selection of most suitable microencapsules. Fig 4.20, 4.21, 4.22 showed the images of GA 300 T60 ST60, GA 300 1;10 and GA 500 1:10, respectively.

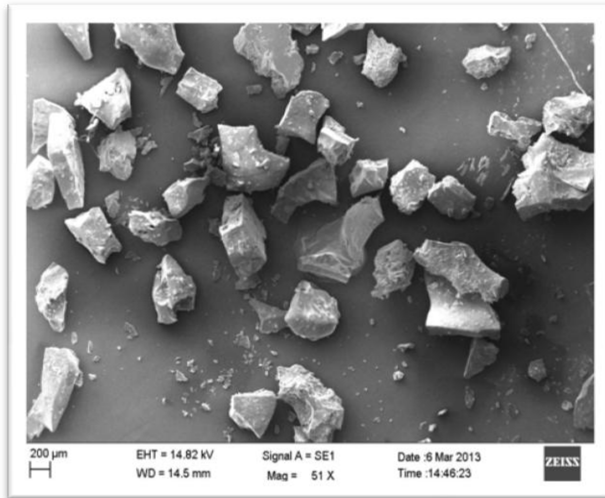



**Figure 4.20: SEM structure of GA 300 T60 ST60**

**Figure 4.21: SEM structure of GA 300 1:10**

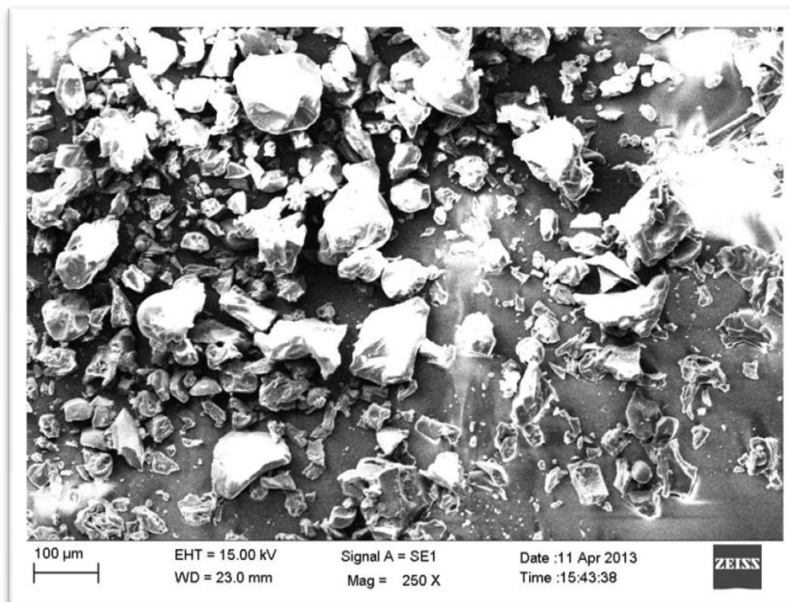



**Figure 4.22: SEM structure of GA 500 1:10**

SEM structure of GA 300 1:10 and GA 500 1:10 were spherical, however GA 300 T60 ST60 was not spherical, therefore GA 300 T60 ST60 was rejected. Spherical structures have maximum stability due to minimum surface area to volume ratio, therefore spherical microencapsules were preferred over non spherical microencapsules.

Krishnan *et al.* (2005); Vaidya *et al.* (2006); Kanakdande *et al.* (2007) reported that blend of GA, MD and MS in the ratio of 4:1:1 produced spherical microencapsules suitable for encapsulation. They also reported that gum arabic alone formed nearly spherical microencapsules but with many dents, whereas maltodextrin and modified starch microencapsules were broken and not completely spherical.

GA 300 1:10 and GA 500 1:10 were further analysed by TBA value.

### 4.3.3 TBA value of fortified yoghurt

TBA value of control (unfortified), GA 300 1:10 and GA 500 1:10 fortified yoghurt was estimated for selection of most suitable microencapsules. TBA value of GA 300 1:10 and GA 500 1:10 fortified yoghurt were significantly different ($p<0.05$) from control (unfortified) yoghurt, whereas no significant difference ($p>0.05$) was observed between GA 300 1:10 and GA 500 1:10 (Table 4.26 and Figure 4.23).

**Table 4.26: TBA value of control (unfortified), GA 300 1:10 and GA 500 1:10 fortified yoghurt**

| Yoghurt samples | TBA value |
|---|---|
| Control (unfortified) | $0.0293 \pm 0.0008^{a}$ |
| GA 300 1:10 | $0.0377 \pm 0.0003^{b}$ |
| GA 500 1:10 | $0.0387 \pm 0.0003^{b}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

According to the above result, GA 500 1:10 was selected as the most suitable microencapsule for yoghurt fortification, since higher concentration of iron could be fortified in the product without causing any adverse oxidative effect in the product.

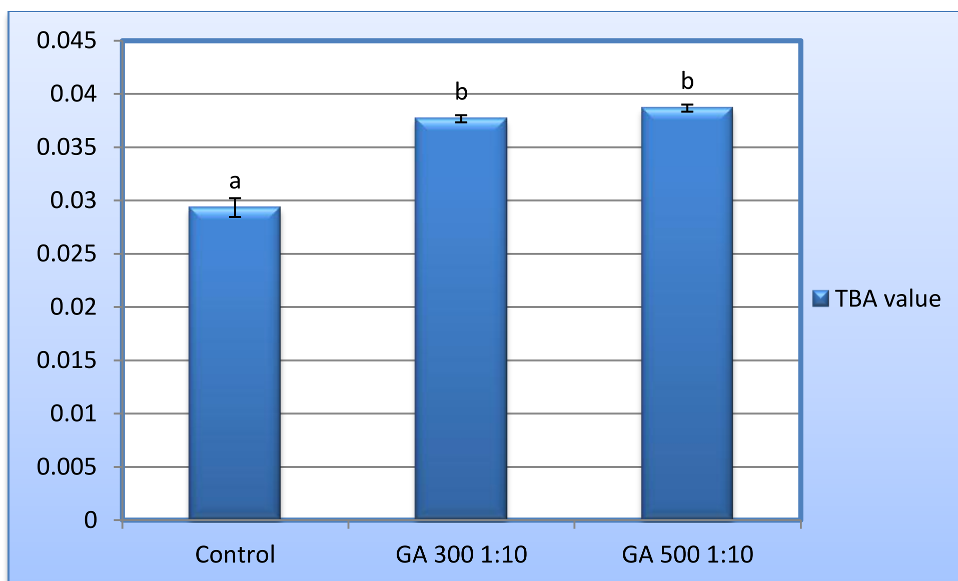



**Figure 4.23: TBA value of control (unfortified), GA 300 1:10 and GA 500 1:10 fortified yoghurt**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.3.4 Particle size analysis of microencapsules

Particle size of selected microencapsules were analysed by observing the microencapsules (magnification 400-fold) under an inverted light microscope and size was measured with the help of inbuilt software with microscope. Fig 4.24 shows the microscopic observation of GA 500 1:10. Average size of microencapsules was observed as 15.54 µm (range 6.84 – 33.42 µm).

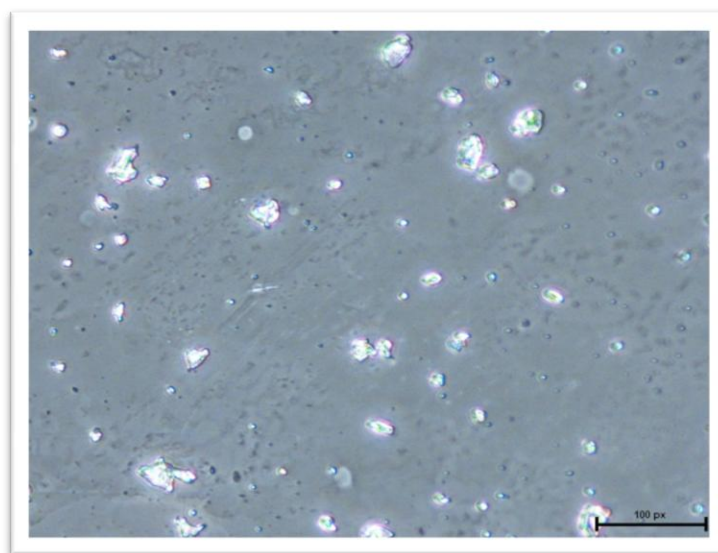



**Fig 4.24: Microscopic observation of GA 500 1:10**

## 4.4 Optimisation of rate of addition of GA 500 1:10 microencapsules in yoghurt

Recommended dietary allowance (RDA) of iron is 20 mg/day (ICMR 2009). The major problem associated with iron is its absorption rather than its consumption. Higher levels of iron were tried for the fortification of yoghurt. Above 35 ppm level of iron addition, there were noticeable changes in flavour and odour of fortified yoghurt samples; therefore, the fortification levels were restricted to 25 ppm and 30 ppm of iron.

The iron fortified yoghurt samples were analysed by the sensory panel from the institute, on the basis of BIS composite sensory score card for yoghurt (Annexure I). The sensory scores of control were similar to that of the fortified yoghurt for all the sensory parameters.

**Table 4.27: Optimization of concentration of iron microencapsules for fortification in yoghurt**

| Characteristics | Maximum scores | Control (Unfortified) | GA 500 1:10 (@25ppm) | GA 500 1:10 (@30ppm) |
|---|---|---|---|---|
| Flavour | 45 | $41.90 \pm 0.34^{A}$ | $41.35 \pm 0.34^{A}$ | $41.20 \pm 0.33^{A}$ |
| Body and texture | 30 | $27.20 \pm 0.21^{A}$ | $27.15 \pm 0.21^{A}$ | $27.05 \pm 0.24^{A}$ |
| Acidity | 10 | $8.80 \pm 0.17^{A}$ | $8.80 \pm 0.15^{A}$ | $8.75 \pm 0.17^{A}$ |
| Colour and appearance | 10 | $8.95 \pm 0.17^{A}$ | $8.85 \pm 0.22^{A}$ | $8.95 \pm 0.19^{A}$ |
| Container and closure | 5 | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ | $5.00 \pm 0.00^{A}$ |
| Total scores | 100 | $91.90 \pm 0.44^{A}$ | $91.35 \pm 0.44^{A}$ | $91.20 \pm 0.35^{A}$ |

Data are presented as means±SEM (n=30).
$^{A\text{-}B}$Means within rows with different superscript are significantly different ($p<0.05$) from each other.

According to the above results, 30 ppm iron concentration was finally selected for fortification of yoghurt with the selected microencapsules. As higher level of fortification did not reduced the acceptability of the yoghurt, therefore higher level of fortification was selected for fortification.

## 4.5 Standardisation of AAS method for estimation of iron content in yoghurt

Different process parameters were utilized for standardization of AAS method for estimation of iron content in yoghurt. These parameters were as follows:

1) Weight of the sample and dilution factor
2) Period of ashing
3) Different method of digestion

1) **Weight of the sample and dilution factor**

1 g, 2 g and 5 g control yoghurt and iron fortified yoghurt samples were weighed in different crucibles. They were charred on the hot plate and kept in a muffle furnace for 16 hrs for proper ashing of the microencapsules. Dissolved the ash in 10 ml $HNO_3$ and different dilutions were prepared ($25^{th}$, $50^{th}$, $100^{th}$, $250^{th}$ and $500^{th}$) by making up the volume upto the mark in volumetric flask. Dissolved ash was filtered with Whatman No. 42 filter paper if suspended particles were present in it. Samples were diluted to a particular dilution, for measurement of iron concentration in the range of the standard curve. For 1 g, 2 g and 5 g sample, suitable dilutions were $25^{th}$, $50^{th}$ and $100^{th}$, respectively.

2) **Period of ashing**

Ashing time was estimated by keeping the same sample in different crucibles. 1g of iron fortified yoghurt sample was weighed in different crucibles. Crucibles were taken out from the muffle at different time intervals (4 hrs, 6 hrs, 8 hrs, 10 hrs, 12 hrs, 14 hrs and 16 hrs). Samples were prepared by dry ashing method and diluted 25 times (Table 4.28 and Figure 4.25). Iron content in these samples was estimated by AAS.

**Table 4.28: Optimisation of ashing time for yoghurt**

| Sample | Iron content (ppm) |
|---|---|
| Iron fortified yoghurt 30ppm (4 hrs) | $24.921 \pm 0.2874^{a}$ |
| Iron fortified yoghurt 30ppm (6 hrs) | $25.885 \pm 0.0975^{b}$ |
| Iron fortified yoghurt 30ppm (8 hrs) | $26.133 \pm 0.0244^{c}$ |
| Iron fortified yoghurt 30ppm (10 hrs) | $26.160 \pm 0.0460^{c}$ |
| Iron fortified yoghurt 30ppm (12 hrs) | $26.178 \pm 0.0832^{c}$ |
| Iron fortified yoghurt 30ppm (14 hrs) | $26.175 \pm 0.0393^{c}$ |
| Iron fortified yoghurt 30ppm (16 hrs) | $26.165 \pm 0.0154^{c}$ |

Data are presented as means±SEM (n=3)
[a-b]Means within columns with different superscript are significantly different ($p<0.05$) from each other.

Iron content in the fortified yoghurt after 8 hrs of ashing time i.e. 10 hrs, 12 hrs, 14 hrs and 16 hrs were not significantly different ($p>0.05$) from each other, whereas, iron content of the microencapsules after 4 hrs and 6 hrs were significantly lower

($p<0.05$) than 8 hrs (Table 4.28 and Figure 4.22). According to the above results ashing time for the yoghurt was selected as 8 hrs.

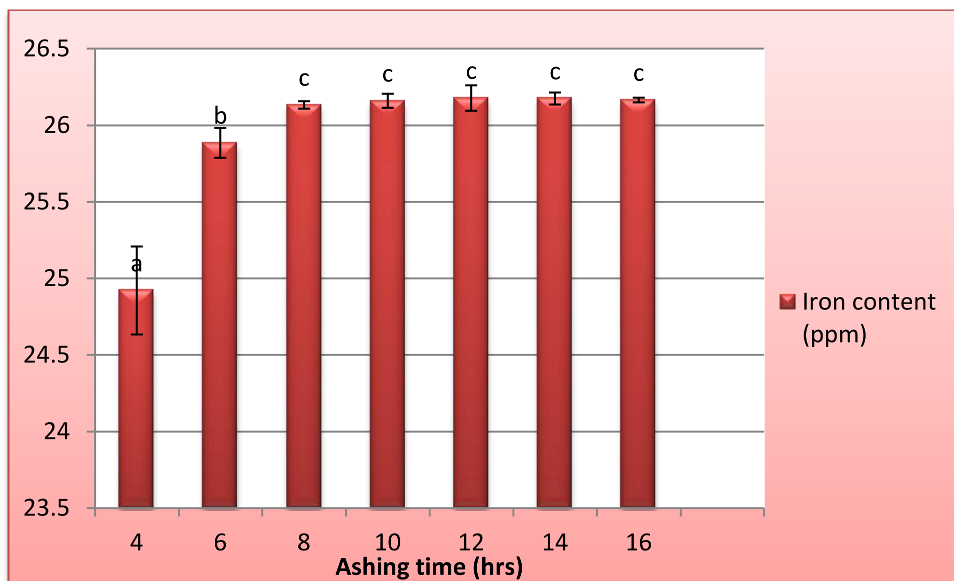



**Figure 4.25: Optimisation of ashing time for yoghurt**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 3) Different methods of digestion

Three different methods of digestion were followed for standardisation of process for estimation of iron content in yoghurt sample (Table 4.29). Both the control and iron fortified yoghurt (30 ppm iron) samples were evaluated for the iron content. Steps standardized in the above two steps were used for the sample preparation. These methods were as follows:

a) Dry ashing
b) Wet digestion
c) Combination of dry and wet digestion

**Table 4.29: Optimization of digestion method for control yoghurt**

| Sample | Iron content (ppm) |
|---|---|
| Control yoghurt (dry ashing) | $0.162 \pm 0.0029^b$ |
| Control yoghurt (wet digestion) | $0.075 \pm 0.0058^a$ |
| Control yoghurt (combination of dry and wet digestion) | $0.264 \pm 0.0049^c$ |

Data are presented as means±SEM (n=3)
[a-b]Means within columns with different superscript are significantly different ($p<0.05$) from each other.

Iron content of all the control yoghurt samples were siginificantly different ($p<0.05$) from each other. Lowest iron content was estimated by wet digestion followed by dry ashing and combination of dry and wet digestion.

**Table 4.30: Optimisation of digestion method for fortified yoghurt**

| Sample | Iron content (ppm) |
|---|---|
| Iron fortified yoghurt (dry ashing) | $26.212 \pm 0.0216^{b}$ |
| Iron fortified yoghurt (wet digestion) | $22.691 \pm 0.1063^{a}$ |
| Iron fortified yoghurt (combination of dry and wet digestion) | $30.057 \pm 0.1230^{c}$ |

Data are presented as means±SEM (n=3)
$^{a\text{-}b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

Iron content of all the control yoghurt samples were significantly different ($p<0.05$) from each other. Lowest iron content was estimated by wet digestion followed by dry ashing and combination of dry and wet digestion.

It was observed that during dry ashing, ash particles did not dissolve properly in the acid and during filteration they were lost, therefore, lower iron content was estimated by the dry ashing.

### 4.5.1 Recovery (%) of iron by different digestion methods

Recovery (%) of all these methods were calculated by excluding the iron content in the control (unfortified) yoghurt. Yoghurt samples were fortified with iron microencapsules @ 30 ppm iron.

**Table 4.31: Recovery (%) of iron by different digestion methods**

| Method of digestion | Fortified iron content (ppm) | Estimated iron content (ppm) | Recovery (%) |
|---|---|---|---|
| Dry ashing | 30 | $26.050 \pm 0.0189^{b}$ | $86.832 \pm 0.0631^{a}$ |
| Wet digestion | 30 | $22.426 \pm 0.1083^{a}$ | $74.755 \pm 0.3608^{b}$ |
| Combination of dry and wet digestion | 30 | $29.724 \pm 0.1203^{c}$ | $99.081 \pm 0.4008^{c}$ |

Data are presented as means±SEM (n=3)
$^{a\text{-}b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

%Recovery of all the methods were significantly different ($p<0.05$) from each other. Lowest recovery (%) was estimated by wet digestion followed by dry ashing and

combination of dry and wet digestion, therefore combination of dry and wet digestion was selected as the most suitable method for estimation of iron content in yoghurt (Table 4.31 and Figure 4.26).

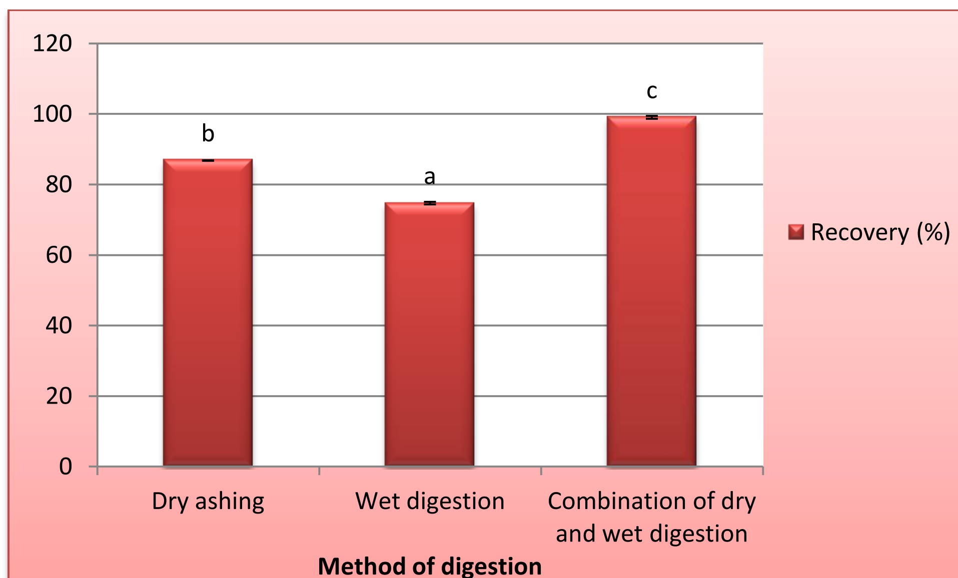



**Figure 4.26: % Recovery of iron by different digestion methods**

[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

## 4.6 Effect of fortification on sensory, physicochemical, rheological and microbiological properties of yoghurt during storage

### 4.6.1 Sensory evaluation of iron salt fortified and iron microencapsules fortified yoghurt

Yoghurt samples were fortified with iron salt and iron microencapsules and were served to a panel of 10 judges for sensory evaluation.

#### 4.6.1.1 Flavour scores

Flavour scores of iron salt fortified yoghurt were significantly lower ($p<0.05$) than control (unfortified) and iron microencapsules fortified yoghurt for the initial period of three days (Table 4.32 and Figure 4.27). After three days of storage, flavour scores of control (unfortified) yoghurt were significantly higher ($p<0.05$) than iron salt and iron microencapsules fortified yoghurt. After 7 days of storage, flavour scores of iron microencapsules fortified yoghurt were higher than iron salt fortified yoghurt. However, this difference was not statistically significantly ($p>0.05$). As storage time increased, flavour scores of all yoghurt samples decreased significantly ($p<0.05$).

**Table 4.32: Flavour scores of fortified yoghurt during storage at 4-7$^{o}$C**

| Sample → <br> Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $40.80 \pm 0.490^{Bc}$ | $34.60 \pm 2.039^{Ac}$ | $39.40 \pm 0.927^{Bd}$ |
| 3rd day | $40.40 \pm 0.748^{Bc}$ | $35.00 \pm 2.738^{Ac}$ | $39.60 \pm 1.208^{Bd}$ |
| 7th day | $37.20 \pm 0.970^{Bb}$ | $34.40 \pm 1.166^{Ac}$ | $35.40 \pm 1.535^{Ac}$ |
| 10th day | $37.00 \pm 0.707^{Bb}$ | $31.80 \pm 0.916^{Ab}$ | $32.20 \pm 0.663^{Ab}$ |
| 15th day | $35.80 \pm 0.583^{Ba}$ | $28.00 \pm 1.224^{Aa}$ | $29.00 \pm 1.183^{Aa}$ |

Data are presented as means±SEM (n=10)
$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

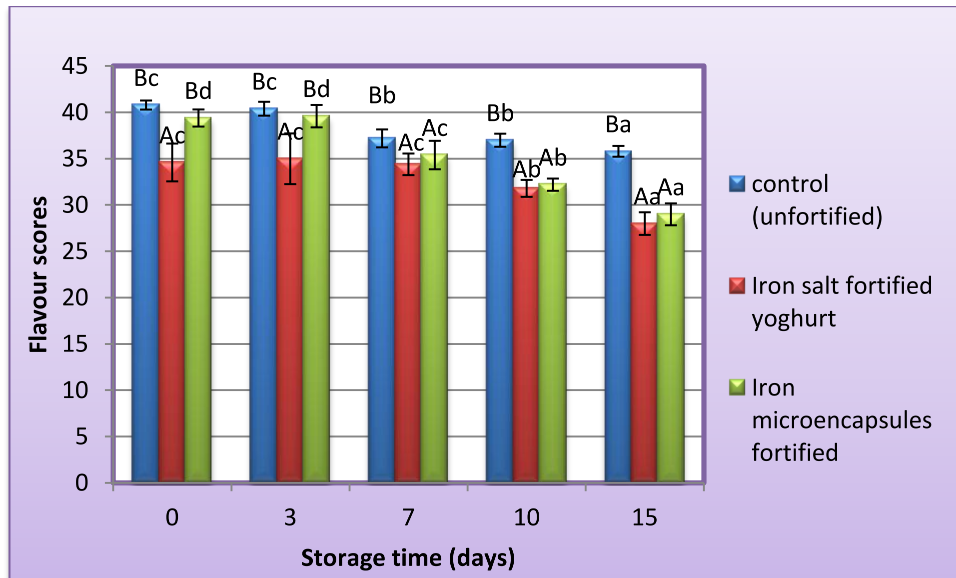



**Figure 4.27: Flavour scores of fortified yoghurt during storage at 4-7$^{o}$C**

$^{A-B}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.1.2 Body and texture scores

The scores observed for iron salt fortified yoghurt were significantly different ($p<0.05$) from control (unfortified) and iron microencapsules fortified yoghurt during storage. As storage time proceeded, body and texture scores of all yoghurt samples did not differ significantly ($p>0.05$) from each other (Table 4.33 and Figure 4.28).

**Table 4.33: Body and texture scores of fortified yoghurt during storage at 4-7ºC**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $28.00 \pm 0.316^{Ba}$ | $27.40 \pm 0.400^{Aa}$ | $27.60 \pm 0.678^{Ba}$ |
| 3rd day | $27.40 \pm 0.600^{Ba}$ | $26.00 \pm 0.316^{Aa}$ | $27.80 \pm 0.200^{Ba}$ |
| 7th day | $28.20 \pm 0.200^{Ba}$ | $26.00 \pm 1.549^{Aa}$ | $27.60 \pm 0.510^{Ba}$ |
| 10th day | $27.70 \pm 0.200^{Ba}$ | $26.40 \pm 0.510^{Aa}$ | $27.20 \pm 0.200^{ABa}$ |
| 15th day | $27.60 \pm 0.245^{Aa}$ | $27.40 \pm 0.400^{Aa}$ | $27.40 \pm 0.245^{Aa}$ |

Data are presented as means±SEM (n=10)
$^{A\text{-}B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.
$^{a\text{-}b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

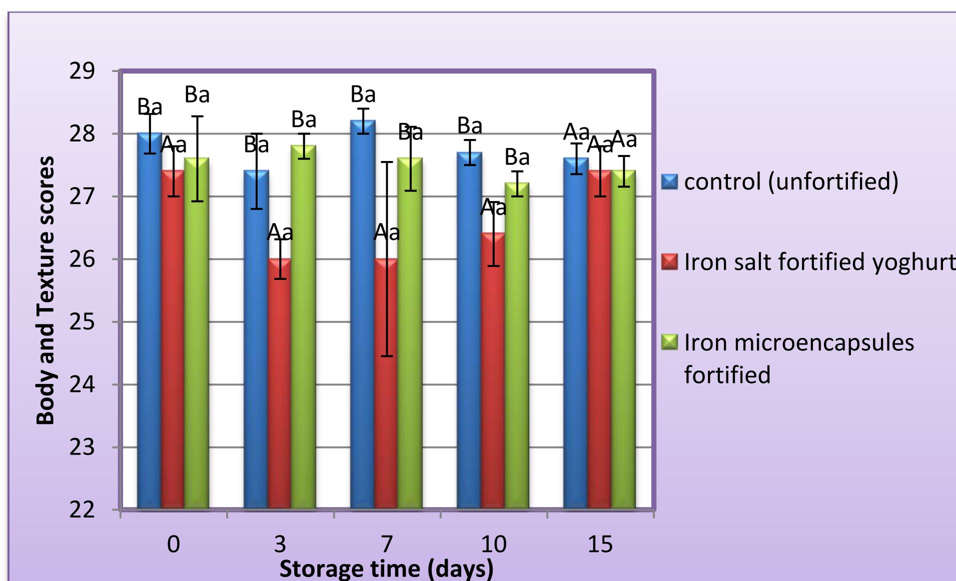



**Figure 4.28: Body and texture scores of fortified yoghurt during storage at 4-7ºC**

$^{A\text{-}B}$Samples represented with different letters are significantly different (p<0.05) from each other.
$^{a\text{-}b}$Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.1.3 Acidity scores

Acidity scores of iron salt and iron microencapsules fortified yoghurt did not differ significantly (p>0.05) from each other during storage. Control (unfortified) yoghurt showed higher (p<0.05) acidity scores from iron salt and iron microencapsules fortified yoghurt on $0^{th}$, $7^{th}$ and $15^{th}$ day. As storage time proceeded, acidity scores of all yoghurt samples significantly (p>0.05) decreased (Table 4.34 and Figure 4.29).

**Table 4.34: Acidity scores of fortified yoghurt during storage at 4-7$^{o}$C**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $9.00 \pm 0.316^{Bd}$ | $8.20 \pm 0.374^{Ad}$ | $8.40 \pm 0.510^{Ad}$ |
| 3rd day | $8.40 \pm 0.245^{Ac}$ | $8.20 \pm 0.374^{Ad}$ | $8.40 \pm 0.245^{Ad}$ |
| 7th day | $8.20 \pm 0.374^{Bc}$ | $7.40 \pm 0.400^{Ac}$ | $7.60 \pm 0.245^{Ac}$ |
| 10th day | $7.40 \pm 0.245^{Ab}$ | $7.00 \pm 0.316^{Ab}$ | $7.20 \pm 0.200^{Ab}$ |
| 15th day | $7.10 \pm 0.100^{Ba}$ | $6.50 \pm 0.447^{Aa}$ | $6.70 \pm 0.300^{Aa}$ |

Data are presented as means±SEM (n=10)
$^{A-B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

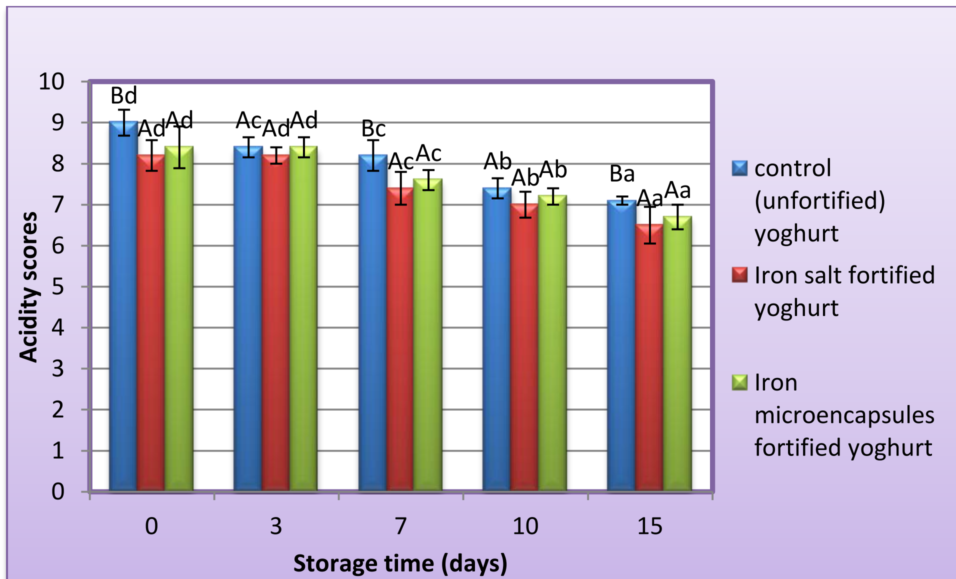



**Figure 4.29: Acidity scores of fortified yoghurt during storage at 4-7$^{o}$C**

$^{A-B}$Samples represented with different letters are significantly different (p<0.05) from each other.
$^{a-b}$Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.1.4 Colour and appearance scores

Colour and appearance scores of all the yoghurt samples were non significantly (p>0.05) affected by both fortification and storage time (Table 4.35 and Figure 4.30).

**Table 4.35: Colour and appearance scores of fortified yoghurt during storage at 4-7ºC**

| Sample → <br> Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules Fortified |
|---|---|---|---|
| 0th day | $8.40 \pm 0.400^{Aa}$ | $8.00 \pm 0.316^{Aa}$ | $8.10 \pm 0.332^{Aa}$ |
| 3rd day | $8.20 \pm 0.200^{Aa}$ | $8.00 \pm 0.316^{Aa}$ | $8.00 \pm 0.367^{Aa}$ |
| 7th day | $8.00 \pm 0.316^{Aa}$ | $7.60 \pm 0.510^{Aa}$ | $7.80 \pm 0.374^{Aa}$ |
| 10th day | $8.40 \pm 0.245^{Aa}$ | $7.60 \pm 0.678^{Aa}$ | $8.00 \pm 0.447^{Aa}$ |
| 15th day | $8.40 \pm 0.245^{Aa}$ | $8.20 \pm 0.374^{Aa}$ | $8.00 \pm 0.547^{Aa}$ |

Data are presented as means±SEM (n=10)
$^{A-B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

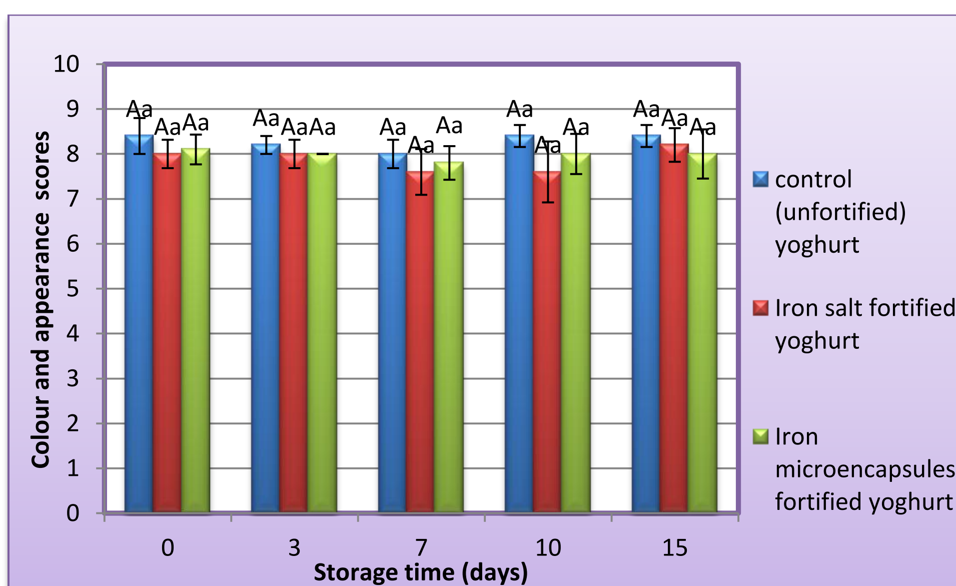



**Figure 4.30: Colour and appearance scores of fortified yoghurt during storage at 4-7ºC**

$^{A-B}$Samples represented with different letters are significantly different (p<0.05) from each other.
$^{a-b}$Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.1.5 Closure and container scores

Closure and container scores of all the yoghurt samples were non significantly (p>0.05) affected by both fortification and storage time (Table 4.36 and Figure 4.31).

**Table 4.36: Closure and container scores of fortified yoghurt during storage at 4-7ºC**

| Sample → <br> Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | 4.40 ± 0.400$^{Aa}$ | 4.40 ± 0.400$^{Aa}$ | 4.40 ± 0.400$^{Aa}$ |
| 3rd day | 4.40 ± 0.400$^{Aa}$ | 4.40 ± 0.400$^{Aa}$ | 4.40 ± 0.400$^{Aa}$ |
| 7th day | 4.60 ± 0.245$^{Aa}$ | 4.60 ± 0.246$^{Aa}$ | 4.60 ± 0.247$^{Aa}$ |
| 10th day | 4.40 ± 0.400$^{Aa}$ | 4.40 ± 0.400$^{Aa}$ | 4.40 ± 0.403$^{Aa}$ |
| 15th day | 4.40 ± 0.400$^{Aa}$ | 4.40 ± 0.400$^{Aa}$ | 4.40 ± 0.404$^{Aa}$ |

Data are presented as means±SEM (n=10)

$^{A-B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.

$^{a-b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

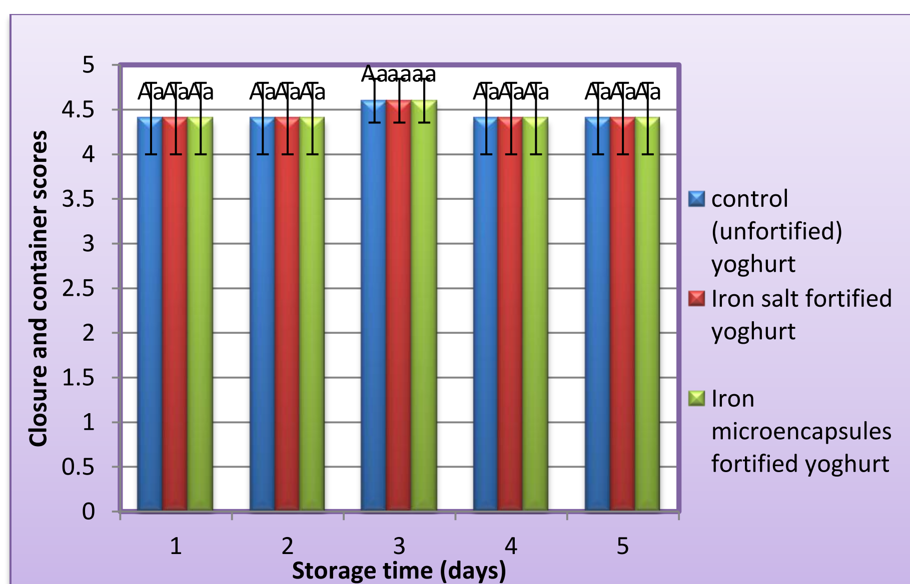



**Figure 4.31: Closure and container scores of fortified yoghurt during storage at 4-7ºC**

$^{A-B}$Samples represented with different letters are significantly different (p<0.05) from each other.

$^{a-b}$Samples represented with different letters are significantly different (p<0.05) from each other.

Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.1.6 Total scores

Total scores of iron salt fortified yoghurt were significantly lower (p<0.05) than control (unfortified) and iron microencapsules fortified yoghurt on 0$^{th}$ and 3$^{rd}$ day of storage (Table 4.37 and Figure 4.32). On 7$^{th}$ day, all samples showed significant

difference ($p<0.05$) from each other. Iron salt fortified yoghurt showed lowest total scores followed by iron microencapsules and control (unfortified) sample. On 10$^{th}$ and 15$^{th}$ day, total scores of iron salt fortified and iron microencapsules fortified yoghurt did not differ significantly ($p>0.05$) from each other, whereas significant difference was observed from control (unfortified) yoghurt.

**Table 4.37: Total scores of fortified yoghurt during storage at 4-7$^{o}$C**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | 90.00 ± 0.707$^{Be}$ | 82.60 ± 2.501$^{Ad}$ | 88.10 ± 1.030$^{Bd}$ |
| 3rd day | 88.80 ± 1.114$^{Bd}$ | 81.60 ± 2.657$^{Ad}$ | 88.20 ± 0.970$^{Bd}$ |
| 7th day | 86.20 ± 1.319$^{Cc}$ | 80.00 ± 2.738$^{Ac}$ | 83.00 ± 2.098$^{Bc}$ |
| 10th day | 84.90 ± 0.510$^{Bb}$ | 77.20 ± 1.934$^{Ab}$ | 79.00 ± 1.265$^{Ab}$ |
| 15th day | 83.30 ± 0.663$^{Ba}$ | 74.50 ± 1.688$^{Aa}$ | 75.50 ± 1.517$^{Aa}$ |

Data are presented as means±SEM (n=10)
$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

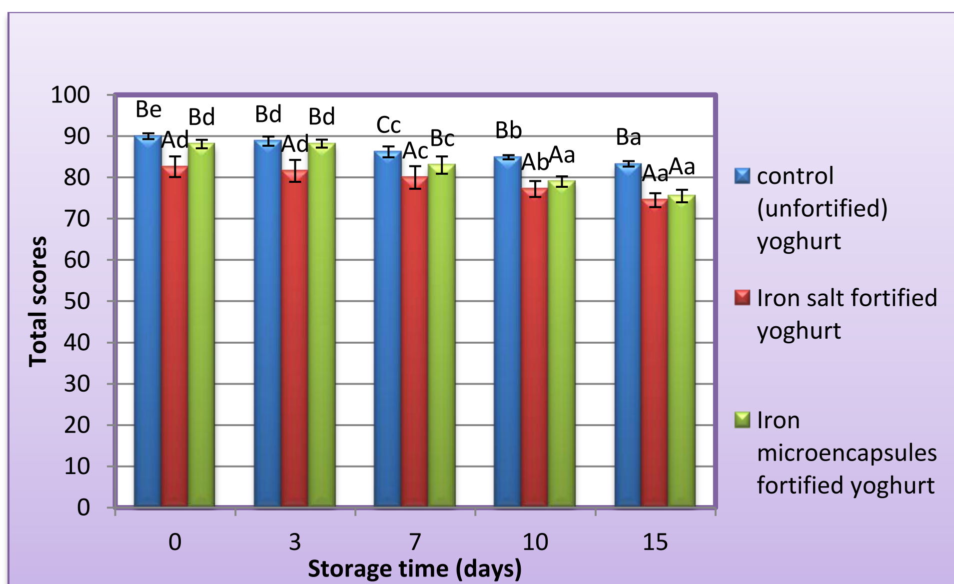



**Figure 4.32: Total scores of fortified yoghurt during storage at 4-7$^{o}$C**

$^{A-B}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

El-Behairy and Mohamed (2010) reported that yoghurt samples had acceptable flavour upto 20 and 40 mg/kg ferric chloride and 20 mg/kg ferrous sulphate. Simova *et al.* (2008) also reported that iron-fortified yoghurts (ferrous lactate) did not differ significantly in their characteristics from unfortified yoghurt. Hekmat and McMahon (1997) reported that yoghurts (non fat and low fat) made from iron fortified milk (casein chelated iron and whey protein-chelated iron @ 20 ppm) had slightly higher oxidised flavour scores than control yoghurt as detected by trained panelists. There was no increase in metallic, bitter and other off-flavours. The consumer panel did not detect any significant differences in the appearance, mouthfeel, flavour or overall quality between fortified and unfortified flavoured yoghurts. All yoghurt samples were liked by the consumer panelists. They suggested that yoghurt was a suitable vehicle for iron fortification.

Umbelino *et al.* (2001) also reported that the addition of iron (microencapsulated ferrous sulphate) does not interfere with the sensory properties of soy yoghurt. Achanta *et al.* (2007) observed that non significant ($p>0.05$) differences was observed for flavour and appearance scores of the yoghurts fortified with the iron (ferric orthophosphate) compared to the control. Umbelino and Rossi (2009) reported that the addition of iron (microencapsulated ferrous sulphate @ 12 ppm iron) did not significantly affect the sensory scores for all evaluated attributes. Azzam (2009) reported that the source of iron used for fortification did not have a significant effect on the smoothness of yoghurt samples when fresh and also during storage.

It is well known that off-flavours may be associated with fortified dairy milk products i.e. oxidised flavour resulting from catalysis of lipid oxidation by iron and metallic flavour contributed by iron salts (Jackson and Lee 1992). No oxidative rancidity was detected in fresh yoghurt and during storage (Abd-Rabou *et al.* 1999; Osman and Ismail 2004) while, Mehanna *et al.* (2000) concluded that fresh yoghurt fortified with iron from different sources seem to be slightly affected by iron concentration.

El-Kholy *et al.* (2011) reported that the sensory qualities of iron-fortified dairy foods are affected by the type of iron salt used. They observed highest flavour score for control and yoghurt fortified with iron-whey protein chelate (Fe-WP) when fresh and during storage. The lowest score was observed for yoghurt fortified with ammonium

ferrous sulphate which showed a slight metallic flavour. Also, the iron treatments did not affect significantly the body and texture of yoghurt. The colour and appearance of yoghurt fortified with iron was not affected by the sources of iron used (ammonium ferric sulphate, ammonium ferrous sulphate, iron-casein complex and iron-whey protein complex). The total score of yoghurt fortified with iron from different sources revealed that the addition of Fe-WP resulted in yoghurt quite similar to that of control yoghurt.

It was evident from the above results that iron microencapsules fortified yoghurt was significantly different ($p<0.05$) from iron salt fortified yoghurt upto $7^{th}$ day and also resembled control (unfortified) yoghurt. However, after $7^{th}$ day of storage its sensory scores were similar ($p>0.05$) to yoghurt containing iron salts.

### 4.6.2 Effect of fortification on physicochemical properties of yoghurt

#### 4.6.2.1 Setting time

Setting time of the yoghurt is the time needed for complete setting of the milk in the form of firm coagulum. It is a time interval between the time of addition of cultures and the time at which the pH of the yoghurt had fallen to 4.6 (Puvanenthiran *et al.* 2002). Setting time of all the samples was non significantly different ($p>0.05$) from each other.

**Table 4.38: Effect of fortification on setting time (hrs) of yoghurt during storage at 4-7ºC**

| Sample | Setting time (hrs) |
|---|---|
| Control (Unfortified) | $5.85 \pm 0.0763^{a}$ |
| Iron salt fortified | $5.88 \pm 0.0726^{a}$ |
| Iron microencapsules fortified | $5.80 \pm 0.0577^{a}$ |

Data are presented as means±SEM (n=3)
$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

#### 4.6.2.2 pH

Appropriate acid concentration is one of the important factors ensuring the quality of fermented products. pH of all the yoghurt samples were different from each other (Table 4.39 and Figure 4.33). However, this difference was non significant ($p>0.05$). Iron microencapsules fortified yoghurt showed a minimum pH followed by iron salt and control (unfortified) yoghurt. pH of all the yoghurt samples decreased during storage.

**Table 4.39: Effect of fortification on pH of yoghurt during storage at 4-7ºC**

| Sample → / Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | 4.493 ± 0.0233$^{Ae}$ | 4.483 ± 0.0384$^{Ae}$ | 4.470 ± 0.0265$^{Ae}$ |
| 3rd day | 4.370 ± 0.0529$^{Ad}$ | 4.350 ± 0.0346$^{Ad}$ | 4.343 ± 0.0233$^{Ad}$ |
| 7th day | 4.194 ± 0.0260$^{Ac}$ | 4.187 ± 0.0202$^{Ac}$ | 4.163 ± 0.0405$^{Ac}$ |
| 10th day | 4.013 ± 0.0417$^{Ab}$ | 4.003 ± 0.0533$^{Ab}$ | 3.990 ± 0.0361$^{Ab}$ |
| 15th day | 3.873 ± 0.0617$^{Aa}$ | 3.860 ± 0.0404$^{Aa}$ | 3.847 ± 0.0593$^{Aa}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

Lower pH of yoghurt manufactured using iron microencapsules containing gum arabic might be because of the low pH of gum arabic solution i.e. 5.20 (Yaseen *et al.* 2005).

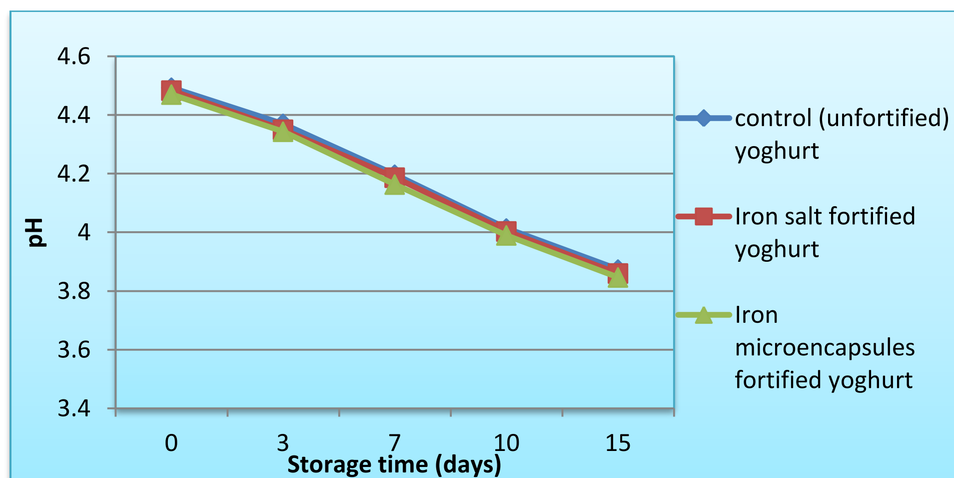



**Figure 4.33: Effect of fortification on pH of yoghurt during storage at 4-7ºC**

Our results were in accordance with El-Behairy and Mohamed (2010) who reported a decrease in pH and increase in acidity of yoghurt samples prepared from iron fortified milk compared to those prepared from control milk. El-Kholy *et al.* (2011) reported that the changes in the pH values for all yoghurt treatments with iron (Fe-WP complex) were not significantly (p>0.05) different.

However, Umbelino and Rossi (2009) reported that the initial titratable acidity of control soy yoghurt (0.87±0.01% lactic acid) was higher than iron fortified product

(0.85±0.01% lactic acid). They also observed that control (unfortified) and iron fortified soy yoghurt required 4.5 and 5.15 h to reduce pH to the 4.4-4.5 range, respectively, indicating that the starter culture was capable to produce enough acids in a suitable time period and that the fortification does not make the fermentation process unviable.

#### 4.6.2.3 Acidity

Acidity of all the yoghurt samples were not significantly different ($p>0.05$) from each other (Table 4.40 and Figure 4.34). Iron microencapsules fortified yoghurt showed highest acidity followed by iron salt and control (unfortified) yoghurt. Acidity of all the yoghurt samples increased during storage.

**Table 4.40: Effect of fortification on acidity (%LA) of yoghurt during storage at 4-7⁰C**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $0.872 \pm 0.0011^{Aa}$ | $0.873 \pm 0.0044^{Aa}$ | $0.875 \pm 0.0002^{Aa}$ |
| 3rd day | $0.898 \pm 0.0011^{Ab}$ | $0.896 \pm 0.0019^{Ab}$ | $0.899 \pm 0.0006^{Ab}$ |
| 7th day | $0.935 \pm 0.0002^{Ac}$ | $0.933 \pm 0.0011^{Ac}$ | $0.935 \pm 0.0004^{Ac}$ |
| 10th day | $0.953 \pm 0.0002^{Ad}$ | $0.954 \pm 0.0004^{Ad}$ | $0.953 \pm 0.0017^{Ad}$ |
| 15th day | $0.969 \pm 0.0006^{Ae}$ | $0.972 \pm 0.0010^{Ae}$ | $0.976 \pm 0.0020^{Ae}$ |

Data are presented as means±SEM (n=3)

$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.

$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

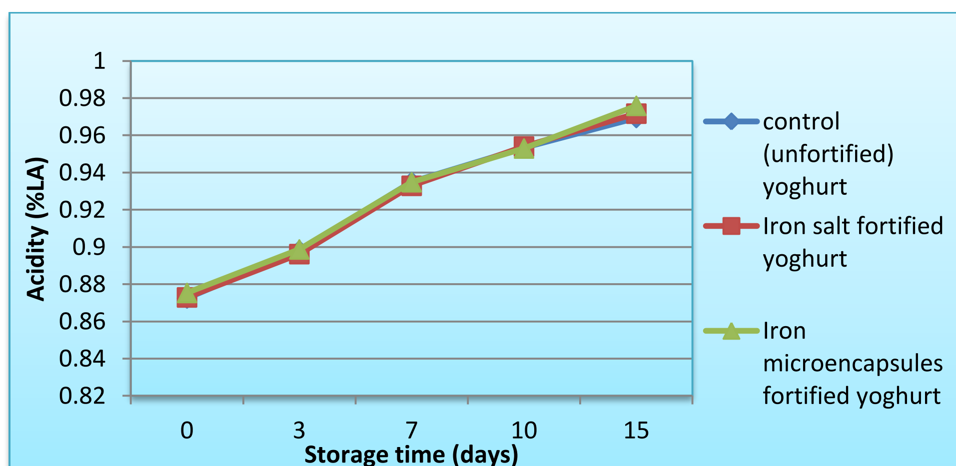



**Figure 4.34: Effect of fortification on acidity (%LA) of yoghurt during storage at 4-7⁰C**

#### 4.6.2.4 Water holding capacity

Water holding capacity of iron salt and iron microencapsules fortified yoghurt did not differ significantly (p>0.05) from control (unfortified) yoghurt upto the $7^{th}$ day of storage (Table 4.41 and Figure 4.35). Control (unfortified) yoghurt showed lowest water holding capacity followed by iron salt and iron microencapsules fortified yoghurt. Increased water holding capacity of iron microencapsules fortified yoghurt might be due to the high water binding property of gum arabic (Bhattacharya *et al.* 2003). After 7 days, control (unfortified) and iron salt fortified yoghurt did not differ significantly (p>0.05) in water holding capacity, whereas, water holding capacity of iron microencapsules fortified yoghurt was significantly higher (p<0.05) than control (unfortified) and iron salt fortified yoghurt. As storage proceeded water holding capacity of all yoghurt samples increased.

**Table 4.41: Effect of fortification on water holding capacity (%) of yoghurt during storage at 4-7ºC**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $81.669 \pm 0.1260^{Aa}$ | $82.582 \pm 0.3375^{Ba}$ | $82.623 \pm 0.0522^{Ba}$ |
| 3rd day | $81.674 \pm 0.1560^{Aa}$ | $82.749 \pm 0.5697^{Bab}$ | $82.754 \pm 0.4405^{Ba}$ |
| 7th day | $81.815 \pm 0.3324^{Aa}$ | $82.905 \pm 0.1859^{Bb}$ | $83.199 \pm 0.1163^{Bb}$ |
| 10th day | $82.550 \pm 0.0903^{Ab}$ | $82.895 \pm 0.1409^{Ab}$ | $83.833 \pm 0.2513^{Bc}$ |
| 15th day | $82.777 \pm 0.5818^{Ab}$ | $82.911 \pm 0.3921^{Ab}$ | $83.644 \pm 0.0415^{Bc}$ |

Data are presented as means±SEM (n=3)

$^{A-B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.

$^{a-b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

Our findings were in accordance with Achanta *et al.* (2007) who studied the rheological properties of yoghurts fortified with various minerals and reported that the mean syneresis values of yoghurts fortified with iron (ferric orthophosphate) were significantly (p<0.05) lower than control yoghurt. They reported that yoghurt fortified with iron had better water-holding capacities compared to control.

Gaucheron *et al.* (1997) reported that there may be a change in casein structure when iron binds to micellar casein. This probable change in the structure of casein may explain better water holding capacity or lower released whey in iron fortified yoghurts.

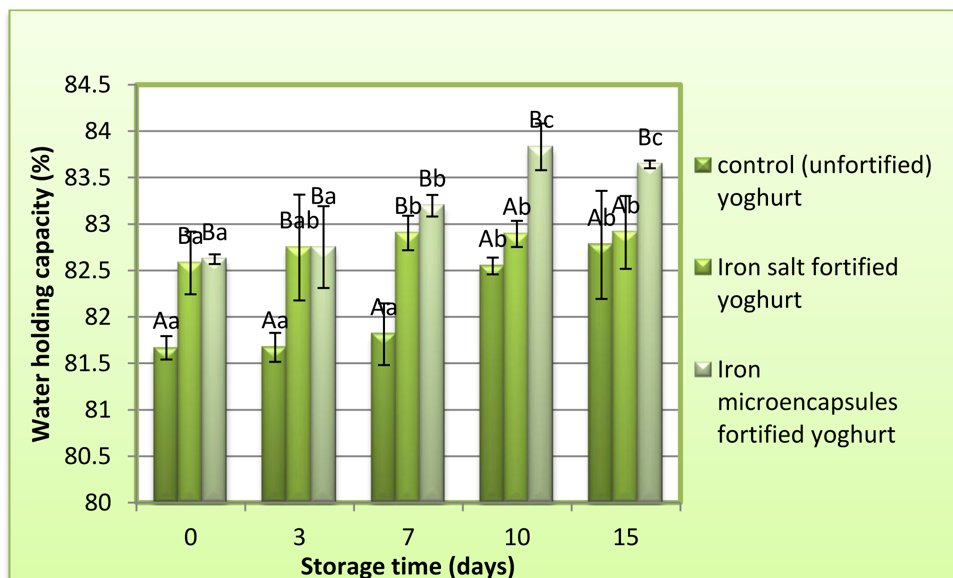



**Figure 4.35: Effect of fortification on water holding capacity (%) of yoghurt during storage at 4-7ºC**

[A-B]Samples represented with different letters are significantly different ($p<0.05$) from each other.
[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

El-Kholy *et al.* (2011) observed non significant difference in syneresis among most of the yoghurts fortified with different iron salts viz. ammonium ferric sulphate, ammonium ferrous sulphate, iron-casein complex and iron-whey protein complex.

#### 4.6.2.5 Spontaneous syneresis

Control (unfortified), iron salt and iron microencapsules fortified yoghurt showed no syneresis during storage. Smooth body and texture, without any syneresis was observed for all the samples during storage.

#### 4.6.2.6 Colour estimation

Colour is an important criterion while fortifying light coloured foods with iron fortificants. Colour of control and fortified milks was determined by Hunter lab coordinates. The colour measured with colourflex was described in terms of L* value (lightness), a* value (red-green) and b* value (yellow-blue).

#### 4.6.2.6.1 L value

L value of control (unfortified) and iron microencapsules fortified yoghurt was significantly different ($p<0.05$) from iron salt fortified yoghurt on 0 day (Table 4.42 and Figure 4.36). After the 3$^{rd}$ day of storage, L value of all the samples were significantly different ($p<0.05$) from each other. Iron salt fortified yoghurt showed the lowest value followed by iron microencapsules and control (unfortified) yoghurt. On 15$^{th}$ day, L value of control (unfortified) yoghurt was significantly different ($p<0.05$) from iron salt and iron microencapsules fortified yoghurt.

**Table 4.42: Effect of fortification on L value of yoghurt during storage at 4-7ºC**

| Sample → / Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | 89.72 ± 0.0433$^{Ba}$ | 88.52 ± 0.1162$^{Aa}$ | 88.78 ± 0.0371$^{Ba}$ |
| 3rd day | 89.67 ± 0.0797$^{Ca}$ | 88.51 ± 0.1438$^{Aa}$ | 88.85 ± 0.0252$^{Ba}$ |
| 7th day | 89.64 ± 0.0546$^{Ca}$ | 88.60 ± 0.0821$^{Aa}$ | 89.01 ± 0.0470$^{Ba}$ |
| 10th day | 89.62 ± 0.0115$^{Ca}$ | 88.56 ± 0.1004$^{Aa}$ | 88.99 ± 0.0484$^{Ba}$ |
| 15th day | 89.36 ± 0.3477$^{Ba}$ | 88.90 ± 0.0033$^{Aa}$ | 88.99 ± 0.0219$^{Aa}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

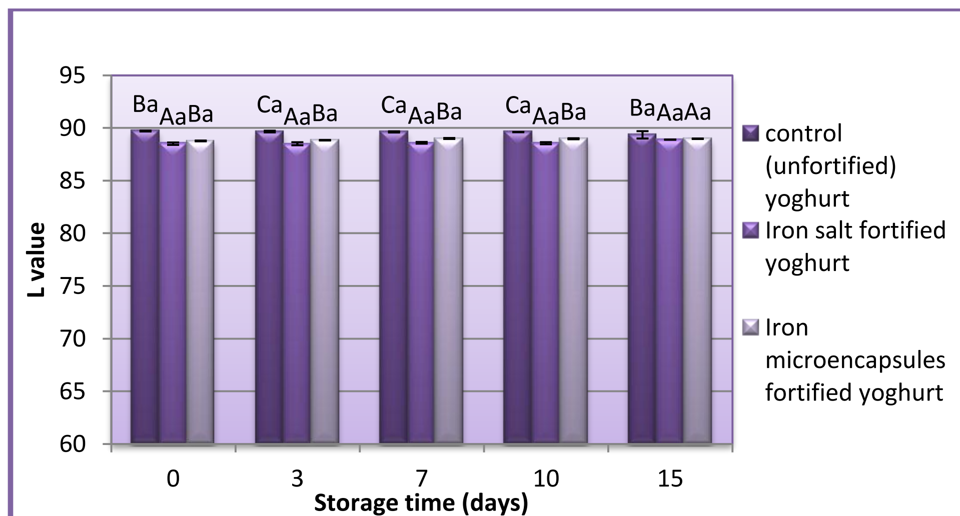



**Figure 4.36: Effect of fortification on L value of yoghurt during storage at 4-7ºC**

$^{A-B}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

#### 4.6.2.6.2 a value

a value of all the samples were significantly different ($p<0.05$) from each other (Table 4.43 and Figure 4.37). Iron salt fortified yoghurt showed the lowest value followed by control (unfortified) and iron microencapsules fortified yoghurt.

**Table 4.43: Effect of fortification on a value of yoghurt during storage at 4-7ºC**

| Sample → <br> Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $-0.787 \pm 0.0185^{Bc}$ | $-0.610 \pm 0.0153^{Ac}$ | $-0.813 \pm 0.0186^{Cbc}$ |
| 3rd day | $-0.763 \pm 0.0296^{Bbc}$ | $-0.600 \pm 0.0252^{Ac}$ | $-0.837 \pm 0.0219^{Cc}$ |
| 7th day | $-0.917 \pm 0.0219^{Bd}$ | $-0.820 \pm 0.0058^{Ad}$ | $-1.010 \pm 0.0252^{Cd}$ |
| 10th day | $-0.723 \pm 0.0067^{Ba}$ | $-0.527 \pm 0.0348^{Aa}$ | $-0.753 \pm 0.0233^{Ca}$ |
| 15th day | $-0.743 \pm 0.0219^{Bab}$ | $-0.573 \pm 0.0088^{Ab}$ | $-0.803 \pm 0.0088^{Cb}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

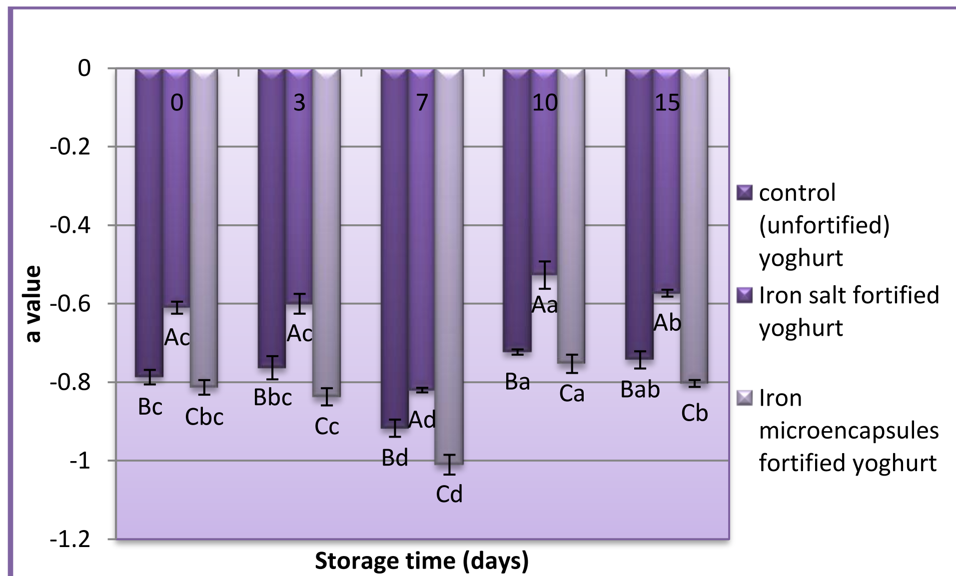



**Figure 4.37: Effect of fortification on a value of yoghurt during storage at 4-7ºC**

$^{A-B}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.2.6.3 b value

b value of all the yoghurt samples were significantly different ($p<0.05$) from each other on 0$^{th}$ and 3$^{rd}$ day of storage (Table 4.44 and Figure 4.38). Iron salt fortified yoghurt showed the lowest value followed by iron microencapsules fortified and control (unfortified) yoghurt. On 7$^{th}$ day, b value of control (unfortified) yoghurt was significantly different ($p<0.05$) from iron salt fortified and iron microencapsules fortified yoghurt.

**Table 4.44: Effect of fortification on b value of yoghurt during storage at 4-7$^{o}$C**

| Sample → / Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $9.050 \pm 0.0100^{Ca}$ | $8.790 \pm 0.0416^{Aa}$ | $8.973 \pm 0.0233^{Bb}$ |
| 3rd day | $9.167 \pm 0.0736^{Cb}$ | $8.813 \pm 0.2117^{Aa}$ | $9.093 \pm 0.0684^{Bc}$ |
| 7th day | $9.020 \pm 0.0666^{Ba}$ | $8.847 \pm 0.0667^{Aa}$ | $8.863 \pm 0.0384^{Aa}$ |
| 10th day | $9.203 \pm 0.0296^{Cb}$ | $9.003 \pm 0.0033^{Ab}$ | $9.097 \pm 0.0491^{Bc}$ |
| 15th day | $9.233 \pm 0.0285^{Bb}$ | $9.163 \pm 0.0533^{Ab}$ | $9.187 \pm 0.0433^{ABd}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

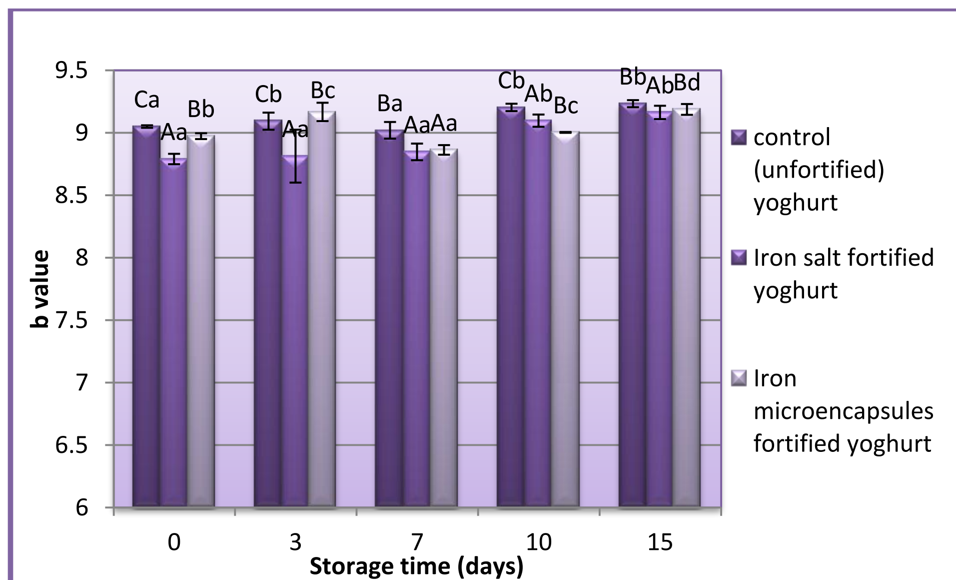



**Figure 4.38: Effect of fortification on b value of yoghurt during storage at 4-7$^{o}$C**

$^{A-B}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Douglas *et al*. (1981) reported darkening of chocolate milk fortified with FeAC and FPP at 10 ppm level and suggested that the difference observed could be due to the difference in original colour of milk and chocolate milk.

#### 4.6.2.7 Oxidative properties

##### 4.6.2.7.1 TBA value

TBA value of all the yoghurt samples were significantly different ($p<0.05$) from each other upto 5$^{th}$ day (Table 4.45 and Figure 4.39). Control (unfortified) yoghurt showed lowest TBA value followed by iron microencapsules and iron salt fortified yoghurt. After 7$^{th}$ day of storage, TBA value of iron salt and iron microencapsules fortified yoghurt did not differ significantly ($p>0.05$) from each other, whereas they differed significantly from control (unfortified) yoghurt. TBA value of all the samples increased during storage.

**Table 4.45: Effect of fortification on TBA value of yoghurt during storage at 4-7ºC**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0$^{th}$ day | $0.042 \pm 0.0006^{Aa}$ | $0.083 \pm 0.0006^{Ca}$ | $0.053 \pm 0.0009^{Ba}$ |
| 3$^{rd}$ day | $0.047 \pm 0.0009^{Ab}$ | $0.086 \pm 0.0006^{Cb}$ | $0.063 \pm 0.0007^{Bb}$ |
| 5$^{th}$ day | $0.051 \pm 0.0009^{Ac}$ | $0.089 \pm 0.0006^{Cc}$ | $0.072 \pm 0.0009^{Bc}$ |
| 7$^{th}$ day | $0.053 \pm 0.0003^{Ad}$ | $0.089 \pm 0.0012^{Bd}$ | $0.090 \pm 0.0003^{Bd}$ |
| 10$^{th}$ day | $0.053 \pm 0.0003^{Ae}$ | $0.094 \pm 0.0003^{Be}$ | $0.093 \pm 0.0003^{Be}$ |
| 15$^{th}$ day | $0.055 \pm 0.0006^{Af}$ | $0.100 \pm 0.0009^{Bf}$ | $0.099 \pm 0.0010^{Bf}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

It was evident from the results obtained that iron was released from the microencapsules during storage of the yoghurt. TBA value increased due to the solubilisation of microencapsules in yoghurt system. After the 7$^{th}$ day of storage, the oxidative effect due to iron became more prominent with the solubilisation of microencapsule material.

Rao and Ramamurthy (1987) reported that the presence of metal catalysts not only accelerates the rate of breakdown of peroxides but also affects the course of reaction during the breakdown (Lea and Swaboda 1962; Downey 1969). The breakdown of peroxides leads to the accumulation of monocarbonyls directly responsible for the off-flavours in autooxidised milk fat. The breakdown of peroxides can undergo different reactions to give rise to various secondary oxidation products which include different classes of monocarbonyls (Lea and Swaboda 1962).

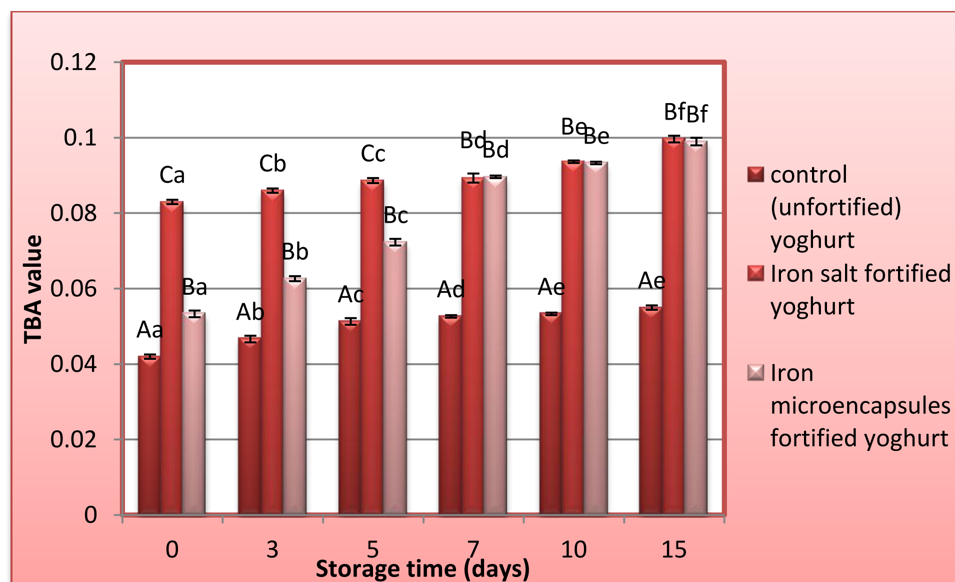



**Figure 4.39: Effect of fortification on TBA value of yoghurt during storage at 4-7ºC**

[A-B]Samples represented with different letters are significantly different ($p<0.05$) from each other.
[a-b]Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

Downey (1969) reported that metal catalyst accelerates the ratio of breakdown of peroxides and also affects the course of reaction during the break down. Our results were in accordance with Wang and King (1973) who reported four times higher TBA values in ferrous sulphate fortified cow milk kept for 48 hrs in a cold storage as compared to the ferric ammonium citrate (chelated salt) fortified counterpart. Edmonson *et al*. (1971) reported that ferrous sulphate addition at 10 ppm resulted in oxidised flavour, whereas ferric ammonium citrate containing milks developed rancid flavours, but not oxidised.

However, Saini *et al*. (1987) observed that there were negligible differences in the TBA values of 48 and 96 hr stored pasteurised milks fortified with 10 ppm of iron as

ferrous and ferric iron with or without ascorbic acid. Heat processing seemed to stabilise the milk against oxidative deterioration, possibly due to SH groups affecting the oxidation-reduction potential of the system. Under certain circumstances, particularly in products with a low protein but high milk fat content, the presence of iron causes oxidative deterioration (Kehagias and Radma 1973). Edmonson *et al*. (1971) have also shown that the enrichment of whole milk with ferric iron results in lipolytic rancidity when milk was pasteurised (below 79ºC). Guzun-Cojocaru *et al*. (2009) cited that iron incorporated into food systems induces oxidation and precipitation.

#### 4.6.2.7.1 Induction time

Induction time of all the yoghurt samples were significantly different ($p<0.05$) from each other upto the 5$^{th}$ day of storage (Table 4.46 and Figure 4.40). Control (unfortified) yoghurt showed maximum induction time followed by iron microencapsules and iron salt fortified yoghurt. After the 7$^{th}$ day of storage, induction time of iron salt and iron microencapsules fortified yoghurt did not differ significantly ($p>0.05$) from each other, whereas they differed significantly from control (unfortified) yoghurt. Induction time of all samples decreased during storage.

**Table 4.46: Effect of iron fortification on induction time (hrs) of yoghurt during storage at 4-7ºC**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $37.670 \pm 0.11^{Ce}$ | $23.945 \pm 0.09^{Ae}$ | $26.960 \pm 0.07^{Be}$ |
| 3rd day | $35.740 \pm 0.12^{Cd}$ | $21.705 \pm 0.06^{Ad}$ | $23.465 \pm 0.10^{Bd}$ |
| 7th day | $32.730 \pm 0.09^{Bc}$ | $19.875 \pm 0.14^{Ac}$ | $19.935 \pm 0.15^{Ac}$ |
| 10th day | $30.120 \pm 0.16^{Bb}$ | $19.715 \pm 0.16^{Ab}$ | $19.810 \pm 0.02^{Ab}$ |
| 15th day | $27.540 \pm 0.11^{Ba}$ | $16.705 \pm 0.15^{Aa}$ | $16.635 \pm 0.145^{Aa}$ |

Data are presented as means±SEM (n=2)
$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

It was evident from the above results that iron was released from the microencapsules during storage of the yoghurt, leading to lower induction period of the

fortified product. Induction time decreased due to the solubilisation of microencapsules in yoghurt system.

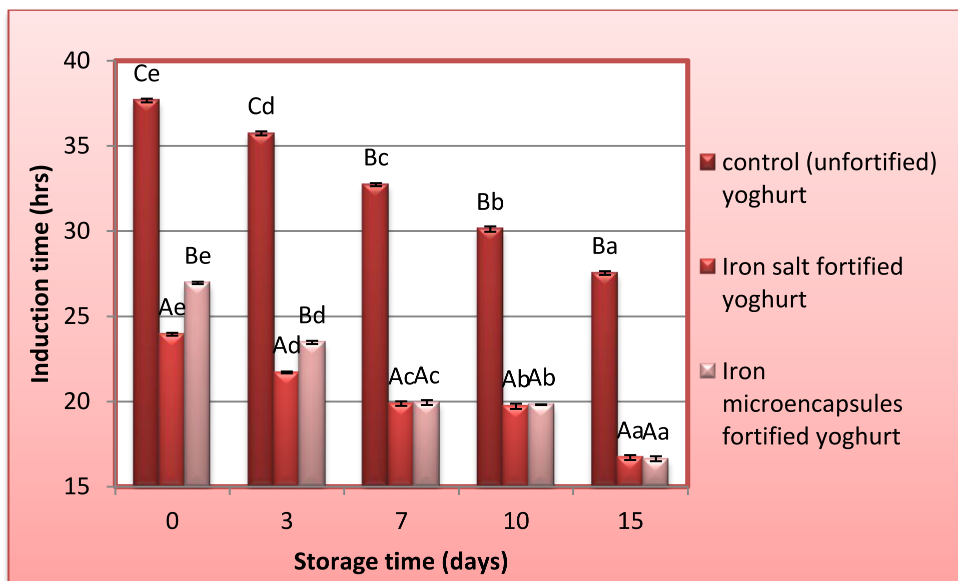



**Figure 4.40: Effect of iron fortification on induction time (hrs) of yoghurt during storage at 4-7ºC**

[A-B]Samples represented with different letters are significantly different (p<0.05) from each other.
[a-b]Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.2.8 Acetaldehyde content

The compounds which impart the distinctive flavour to yoghurt are lactic acid and a variety of volatile organic aroma compounds, produced by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Beshkova *et al.* 1998). Gallardo-Escamilla *et al.* (2005) reported that the volatile compounds such as acetaldehyde and diacetyl are key compounds for typical yoghurt aroma. Acetaldehyde is recognised as a major flavour component in yoghurt (Bottazzi *et al.* 1973; Dumont and Adda 1973; Law 1981). Therefore, acetaldehyde content of yoghurt samples (control and fortified samples) was determined to evaluate the effect of iron fortification on flavour producing activity of yoghurt cultures.

Acetaldehyde content of all the yoghurt samples were significantly different (p<0.05) from each other during storage (Table 4.47 and Figure 4.41). Acetaldehyde content decreased during storage in all the samples, probably due to its conversion into another organic compound which reduced to ethanol or diacetyl (El-Kholy *et al.* 2011).

However, El-Kholy *et al.* (2011) also reported non significant (P>0.05) differences in acetaldehyde content among most iron treatments.

**Table 4.47: Effect of fortification on acetaldehyde content (ppm) of yoghurt during storage at 4-7ºC**

| Sample → <br> Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $3.48 \pm 0.067^{Bc}$ | $3.61 \pm 0.066^{Cc}$ | $3.16 \pm 0.101^{Ac}$ |
| 7th day | $0.52 \pm 0.026^{Bb}$ | $0.65 \pm 0.027^{Cb}$ | $0.44 \pm 0.028^{Ab}$ |
| 15th day | $0.40 \pm 0.022^{Ba}$ | $0.51 \pm 0.016^{Ca}$ | $0.30 \pm 0.018^{Aa}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

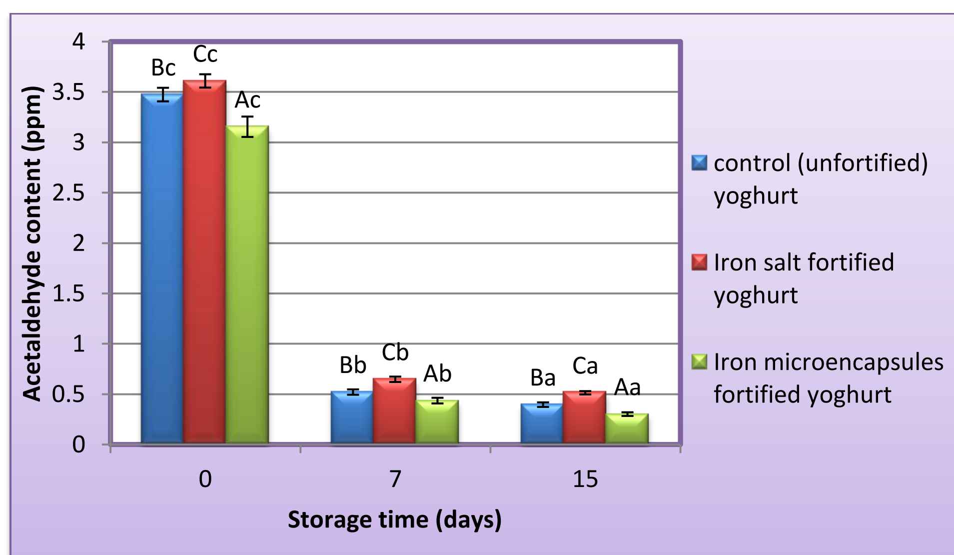



**Figure 4.41: Effect of fortification on acetaldehyde content (ppm) of yoghurt during storage at 4-7ºC**

$^{A-B}$Samples represented with different letters are significantly different (p<0.05) from each other.
$^{a-b}$Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.3 Effect of fortification on rheological properties of yoghurt

#### 4.6.3.1 Texture profile analysis

Texture profile analysis of yoghurt was done by a textural analyser using single bite compression test. Yoghurt was evaluated for the four parameters i.e. firmness, work of adhesion, work of shear and stickiness.

#### 4.6.3.1.1 Firmness

Firmness of all yoghurt samples was significantly different from each other (Table 4.48 and Figure 4.42). Control (unfortified) yoghurt showed lowest value for firmness followed by iron salt and iron microencapsules fortified yoghurt.

**Table 4.48: Effect of iron salt and iron microencapsules fortification on firmness (N)**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | 1.303 ± 0.0231$^{Aa}$ | 1.532 ± 0.0128$^{Ba}$ | 1.695 ± 0.0261$^{Ca}$ |
| 3rd day | 1.376 ± 0.0657$^{Aa}$ | 1.594 ± 0.0274$^{Ba}$ | 1.737 ± 0.0498$^{Ca}$ |
| 7th day | 1.497 ± 0.0500$^{Aa}$ | 1.555 ± 0.0445$^{Ba}$ | 1.517 ± 0.0598$^{Ba}$ |
| 10th day | 1.370 ± 0.0478$^{Aa}$ | 1.592 ± 0.0390$^{Ba}$ | 1.580 ± 0.0258$^{Ba}$ |
| 15th day | 1.316 ± 0.0246$^{Aa}$ | 1.546 ± 0.0377$^{Ba}$ | 1.573 ± 0.0149$^{Ba}$ |

Data are presented as means±SEM (n=3)

$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.

$^{a-b}$Means within columns with different superscript are significantly different ($p<0.05$) from each other.

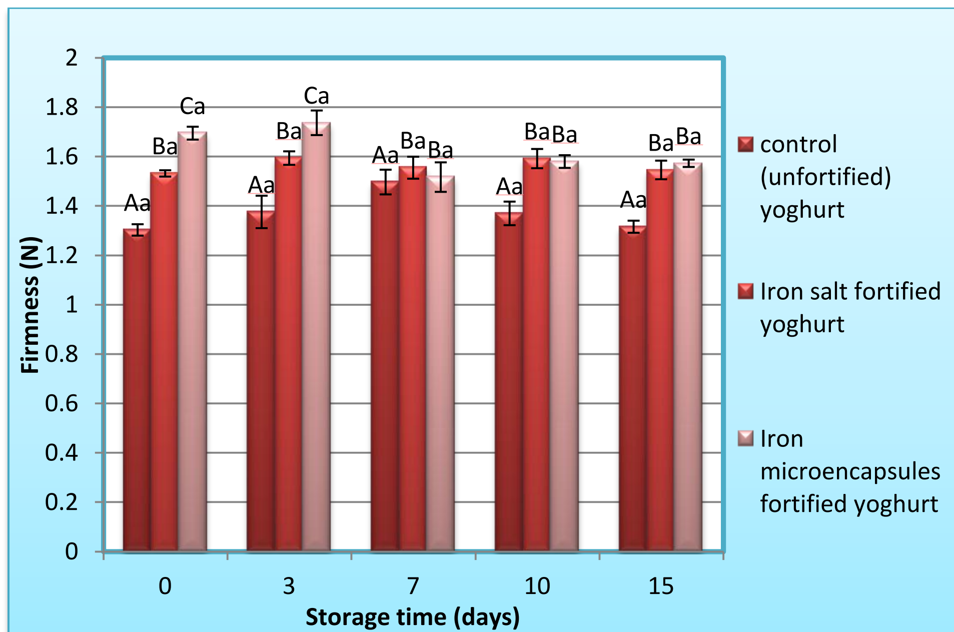



**Figure 4.42: Effect of iron salt and iron microencapsules fortification on firmness (N)**

$^{A-B}$Samples represented with different letters are significantly different ($p<0.05$) from each other.

$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.

Error bars show the variations of three determinations in terms of standard error of mean.

#### 4.6.3.1.2 Work of adhesion

Work of adhesion of all the samples was slightly but significantly different from each other. Control (unfortified) yoghurt showed lowest value for work of adhesion followed by iron salt and iron microencapsules fortified yoghurt (Table 4.49 and Figure 4.43).

**Table 4.49: Effect of iron salt and iron microencapsules fortification on work of adhesion (Ns)**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | -2.045 ± 0.1087$^{Aa}$ | -2.564 ± 0.2065$^{Ba}$ | -2.635 ± 0.0482$^{Ba}$ |
| 3rd day | -2.326 ± 0.1586$^{Ab}$ | -2.606 ± 0.1484$^{Ba}$ | -2.892 ± 0.1528$^{Cbc}$ |
| 7th day | -2.449 ± 0.0690$^{Ac}$ | -2.617 ± 0.0218$^{Bab}$ | -2.896 ± 0.1390$^{Cbc}$ |
| 10th day | -2.101 ± 0.0522$^{Aa}$ | -2.724 ± 0.1720$^{Bb}$ | -2.862 ± 0.0502$^{Bb}$ |
| 15th day | -2.060 ± 0.0645$^{Aa}$ | -2.551 ± 0.0579$^{Ba}$ | -2.971 ± 0.1251$^{Cc}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

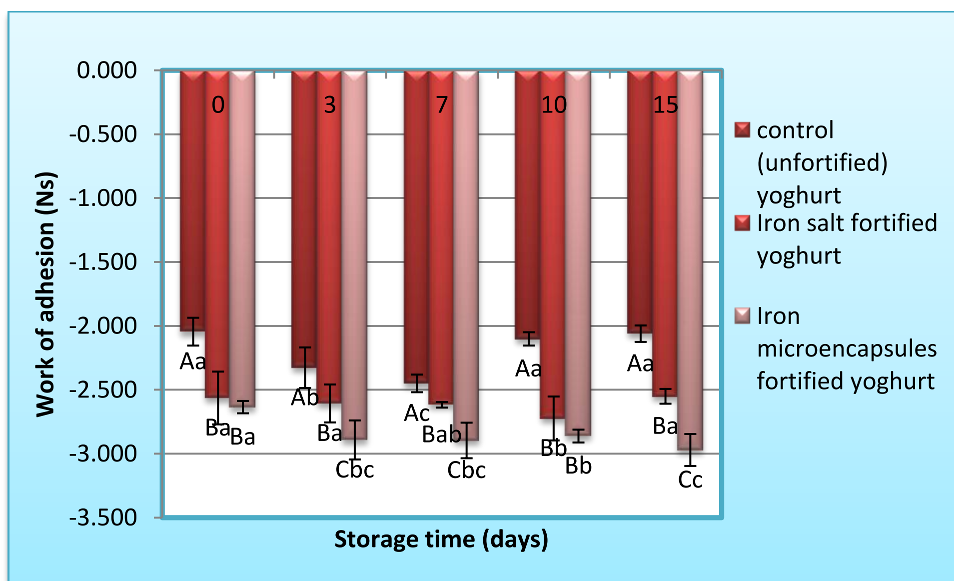



**Figure 4.43: Effect of iron salt and iron microencapsules fortification on work of adhesion (Ns)**

$^{A-B}$Samples represented with different letters are significantly different (p<0.05) from each other.
$^{a-b}$Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.3.1.3 Work of shear

Work of shear of all the samples was significantly different (p<0.05) from each other on $0^{th}$ and $3^{rd}$ day (Table 4.50 and Figure 4.44). Control (unfortified) showed lowest value followed by iron salt and iron microencapsules fortified yoghurt. Control (unfortified) yoghurt was significantly different (p<0.05) from iron salt and iron microencapsules fortified yoghurt on $7^{th}$,$10^{th}$ and $15^{th}$ day.

**Table 4.50: Effect of iron salt and iron microencapsules fortification on work of shear (Ns)**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $25.416 \pm 0.5266^{Aa}$ | $30.519 \pm 0.4621^{Bab}$ | $34.066 \pm 0.0841^{Cd}$ |
| 3rd day | $27.883 \pm 0.6386^{Ab}$ | $32.331 \pm 0.8333^{Bc}$ | $34.932 \pm 1.0425^{Ce}$ |
| 7th day | $30.079 \pm 1.2090^{Ac}$ | $30.271 \pm 0.8123^{Ba}$ | $29.545 \pm 0.5053^{Ba}$ |
| 10th day | $25.828 \pm 0.4453^{Aa}$ | $31.173 \pm 0.4525^{Bb}$ | $31.515 \pm 0.6685^{Bc}$ |
| 15th day | $25.833 \pm 0.4627^{Aa}$ | $30.343 \pm 1.0342^{Ba}$ | $30.504 \pm 1.1324^{Bb}$ |

Data are presented as means±SEM (n=3)
$^{A\text{-}B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.
$^{a\text{-}b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

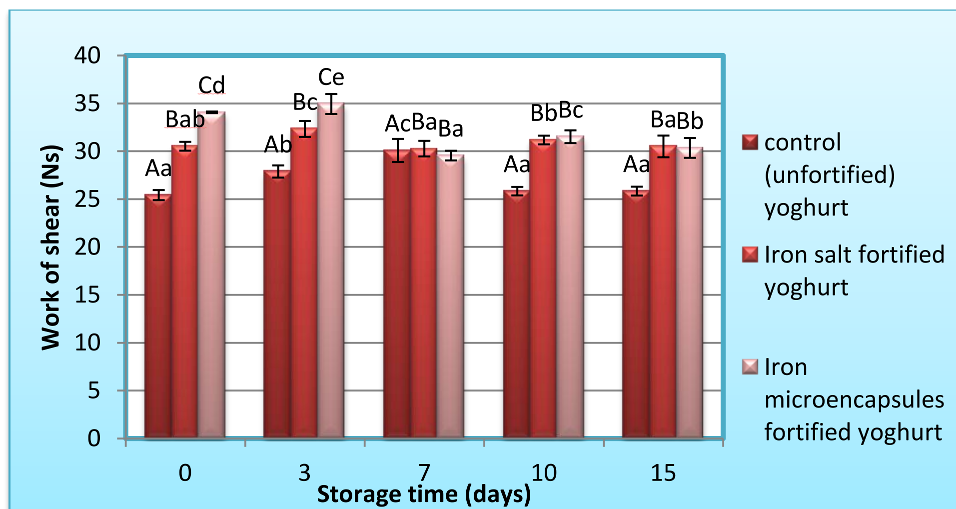



**Figure 4.44: Effect of iron salt and iron microencapsules fortification on work of adhesion (Ns)**

$^{A\text{-}B}$Samples represented with different letters are significantly different (p<0.05) from each other.
$^{a\text{-}b}$Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.3.1.4 Stickiness

Stickiness of all the samples was significantly different ($p<0.05$) from each other on $0^{th}$ day (Table 4.51 and Figure 4.45). After $0^{th}$ day, iron salt and iron microencapsules fortified yoghurt was non significantly different ($p>0.05$) from each other, whereas significantly different form control (unfortified) yoghurt. Stickiness of all the samples was not affected by the storage time.

**Table 4.51: Effect of iron salt and iron microencapsules fortification on stickiness (N)**

| Sample → Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $-0.260 \pm 0.0018^{Aa}$ | $-0.461 \pm 0.1644^{Ca}$ | $-0.362 \pm 0.0075^{Ba}$ |
| 3rd day | $-0.279 \pm 0.0222^{Aa}$ | $-0.323 \pm 0.0184^{Ba}$ | $-0.362 \pm 0.0113^{Ba}$ |
| 7th day | $-0.292 \pm 0.0100^{Aa}$ | $-0.335 \pm 0.0047^{Ba}$ | $-0.363 \pm 0.0136^{Ba}$ |
| 10th day | $-0.277 \pm 0.0101^{Aa}$ | $-0.344 \pm 0.0163^{Ba}$ | $-0.333 \pm 0.0021^{Ba}$ |
| 15th day | $-0.253 \pm 0.0023^{Aa}$ | $-0.317 \pm 0.0052^{Ba}$ | $-0.365 \pm 0.0175^{Ba}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

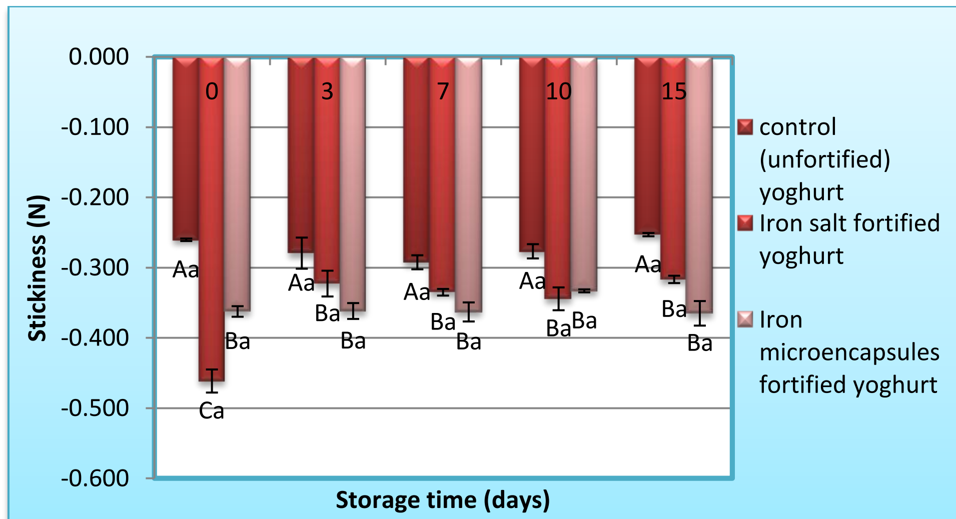



**Figure 4.45: Effect of iron salt and iron microencapsules fortification on stickiness (N)**

$^{A-B}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

During storage no trend was observed for the textural properties of all the samples. Our results were in agreement with Kumar and Mishra (2004) who prepared the mango soy fortified yoghurt and evaluated the effect of stabilizer addition on physicochemical, sensory and textural properties of yoghurt.

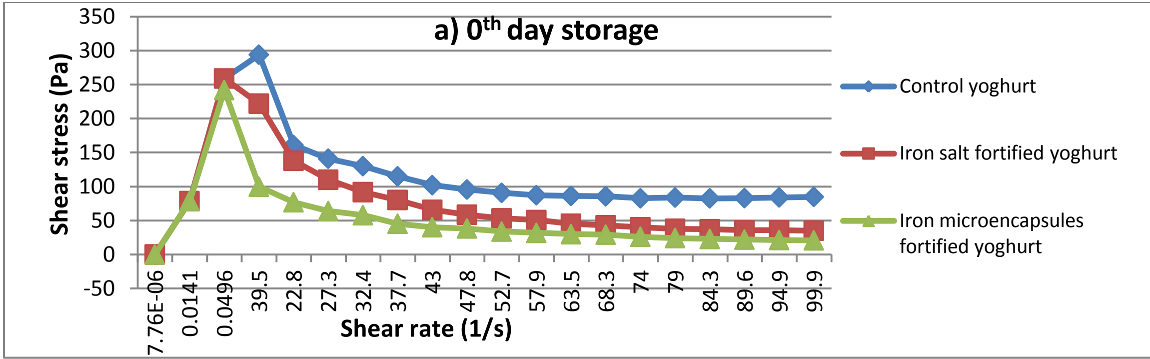
Our results were in consonance with Ocak and Köse (2010) who reported that addition of iron (ferric nitrate @ 0.3 and 0.6 ppm) to milk caused a general increase in firmness and cohesiveness of yoghurt in comparison to the control yoghurt and this was found statistically significant ($P<0.01$). Sandoval-Castilla *et al.* (2004) stated that the protein matrix was responsible for the firmness and springiness of yoghurt.

Unnikrishnan and Rao (1977) reported that around 90% of added iron was associated with skim milk, 71.9% with casein and 19.5% with isoelectric whey. Demott and Park (1973) also reported that 87.28% added iron was associated with skim milk, 67.84% with isoelectric casein and 16.50% with isoelectric whey. Demott and Dincer (1976) reported that iron, when added as $FeCl_2$ to skim milk, 85% is bound to caseins (72, 21 and 4% are bound to $\alpha_{s}$-, $\beta$ and $\kappa$-caseins, respectively). This casein iron network might have resulted in compact network of yoghurt.

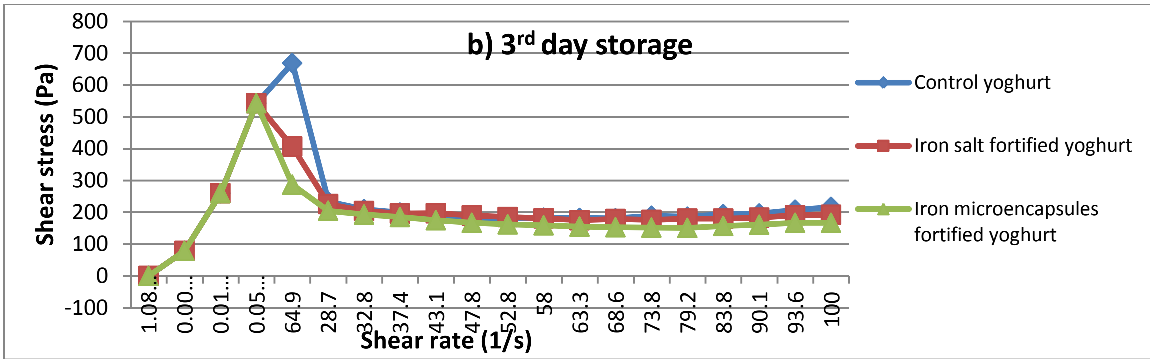
**4.6.3.2 Flow behaviour**

Figure 4.46 depicts flow behaviour curve of control (unfortified), iron salt and iron microencapsules fortified yoghurt during storage a) $0^{th}$ day, b) $3^{rd}$ day, c) $7^{th}$ day, d) $10^{th}$ day and e) $15^{th}$ day. Shear stress of control (unfortified) yoghurt was maximum followed by iron salt and iron microencapsules fortified yoghurt. Maximum shear stress was obtained on $3^{rd}$ day of storage for all the samples.

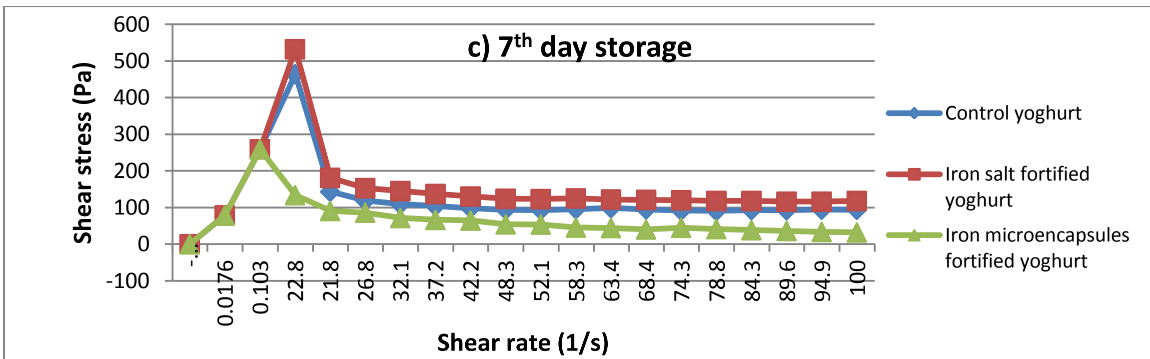
Figure 4.47 depicts viscosity of control (unfortified), iron salt and iron microencapsules fortified yoghurt as affected by different shear stress during storage a) $0^{th}$ day, b) $3^{rd}$ day, c) $7^{th}$ day, d) $10^{th}$ day and e) $15^{th}$ day. Viscosity of control (unfortified) yoghurt was highest followed by iron salt and iron microencapsules fortified yoghurt. Maximum viscosity was obtained on $3^{rd}$ day of storage for all the samples.



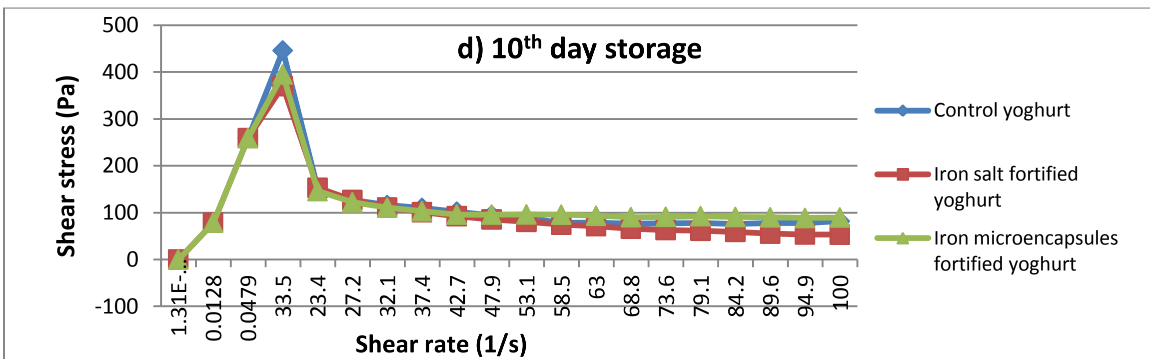
a) 0th day storage
Shear stress (Pa)
Shear rate (1/s)
Control yoghurt
Iron salt fortified yoghurt
Iron microencapsules fortified yoghurt

b) 3rd day storage
Shear stress (Pa)
Shear rate (1/s)
Control yoghurt
Iron salt fortified yoghurt
Iron microencapsules fortified yoghurt

c) 7th day storage
Shear stress (Pa)
Shear rate (1/s)
Control yoghurt
Iron salt fortified yoghurt
Iron microencapsules fortified yoghurt

d) 10th day storage
Shear stress (Pa)
Shear rate (1/s)
Control yoghurt
Iron salt fortified yoghurt
Iron microencapsules fortified yoghurt

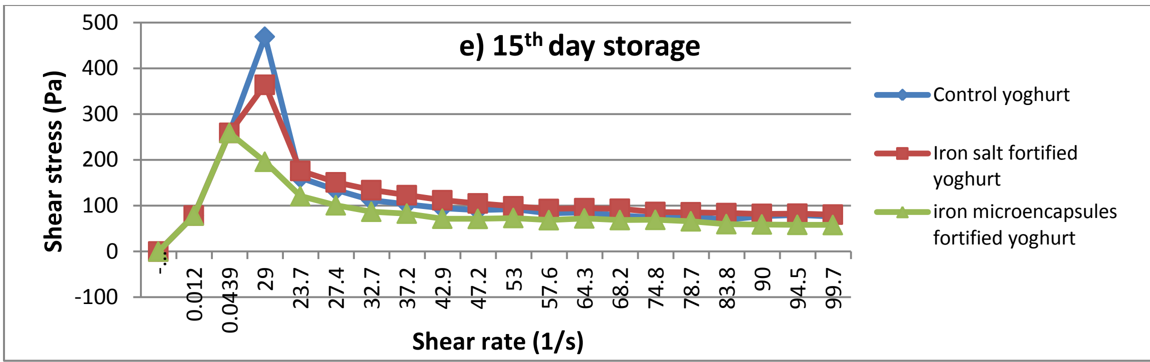
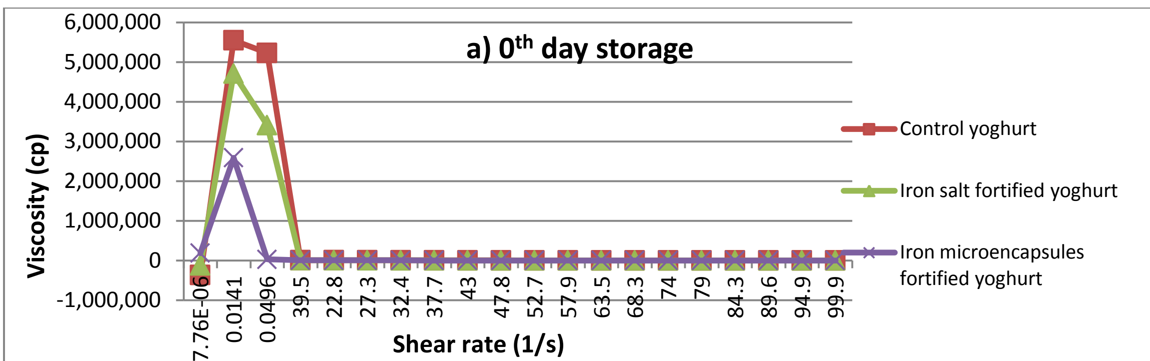
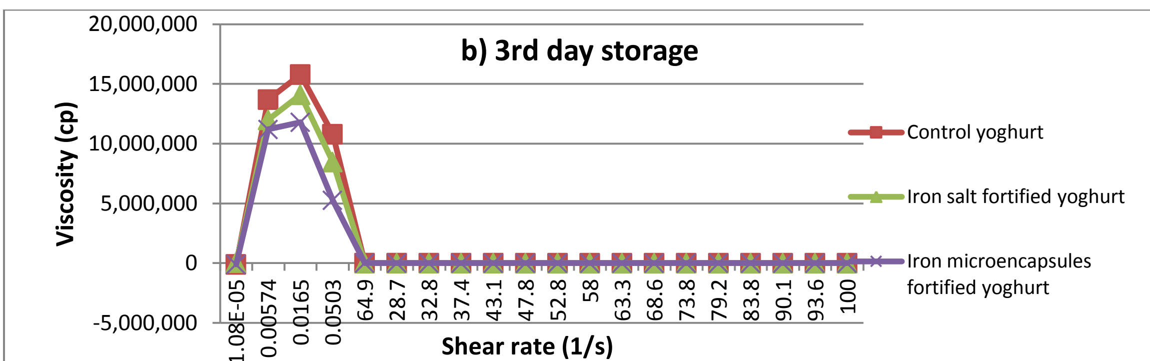
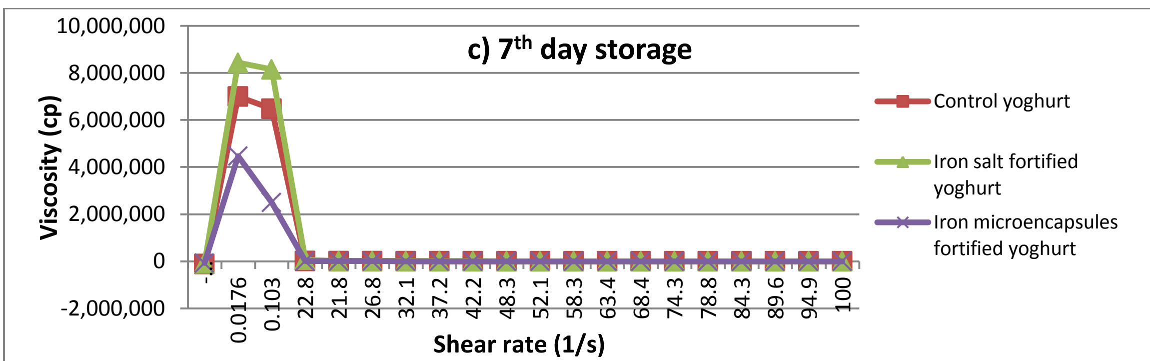



**Figure 4.46: Flow behaviour curve of control (unfortified), iron salt and iron microencapsules fortified yoghurt during storage a) 0th day, b) 3rd day, c) 7th day, d) 10th day and e) 15th day**



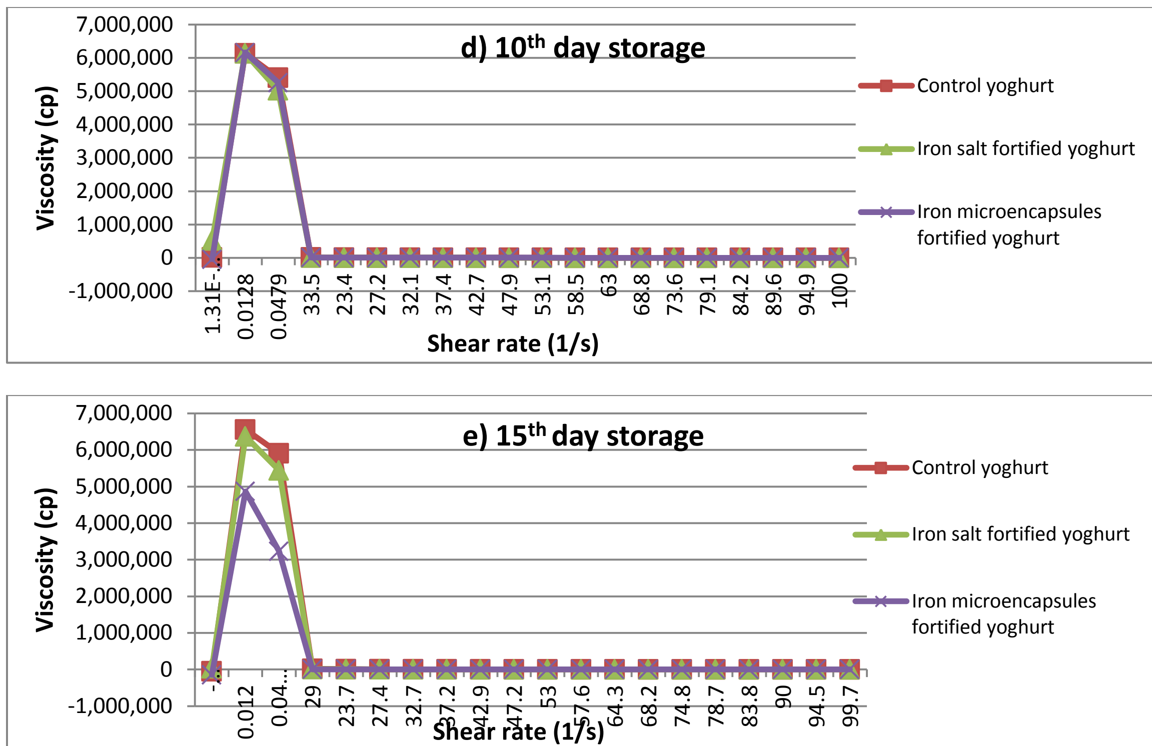



**Figure 4.47: Viscosity of control (unfortified), iron salt and iron microencapsules fortified yoghurt as affected by different shear stress during storage a) 0$^{th}$ day, b) 3$^{rd}$ day, c) 7$^{th}$ day, d) 10$^{th}$ day and e) 15$^{th}$ day**

Viscosity of iron salt fortified yoghurt was highest on 7$^{th}$ day of storage followed by control (unfortified) and iron microencapsules fortified yoghurt. On 10$^{th}$ day of storage, viscosities of the samples were similar to each other.

### 4.6.4 Microbial parameters

#### 4.6.4.1 *Streptococcus thermophilus* count

*Streptococcus thermophilus* count of iron microencapsules fortified yoghurt was significantly lower ($p<0.05$) than the iron salt fortified yoghurt on 0$^{th}$ day of storage (Table 4.52 and Figure 4.48). On storage, *Streptococcus thermophilus* count of all the samples did not differ significantly different ($p>0.05$) from each other. *Streptococcus thermophilus* count increased for the first 3 days of storage and then gradually decreased for the entire duration of storage of 15 days.

**Table 4.52: Effect of iron salt and iron microencapsules fortification on *Streptococcus thermophilus* count ($10^8$ cfu/g)**

| Sample → / Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | 448.67 ± 34.353$^{ABc}$ | 473.00 ± 24.007$^{Bc}$ | 428.67 ± 30.661$^{Ac}$ |
| 3rd day | 483.67 ± 39.976$^{Ad}$ | 497.00 ± 51.098$^{Ac}$ | 467.00 ± 52.548$^{Ad}$ |
| 7th day | 439.00 ± 25.325$^{Ac}$ | 458.67 ± 21.942$^{Ac}$ | 423.67 ± 24.969$^{Ac}$ |
| 10th day | 387.67 ± 15.909$^{Ab}$ | 392.00 ± 11.930$^{Ab}$ | 368.33 ± 29.801$^{Ab}$ |
| 15th day | 342.33 ± 19.675$^{Aa}$ | 355.67 ± 14.621$^{Aa}$ | 324.33 ± 14.746$^{Aa}$ |

Data are presented as means±SEM (n=3)
$^{A-B}$Means within row with different uppercase superscript are significantly different (p<0.05) from each other.
$^{a-b}$Means within column with different lowercase superscript are significantly different (p<0.05) from each other.

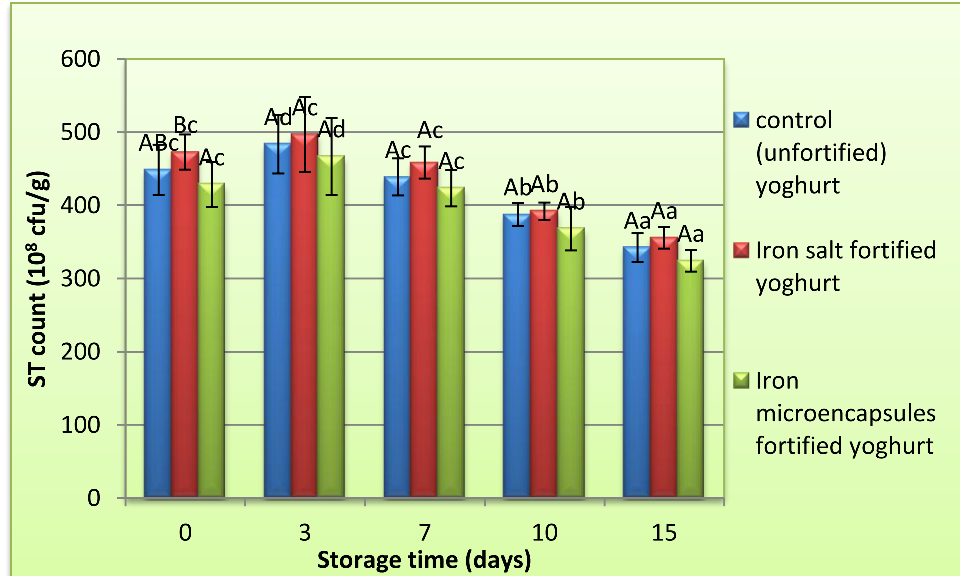



**Figure 4.48: Effect of iron salt and iron microencapsules fortification on *Streptococcus thermophilus* count ($10^8$ cfu/g)**

$^{A-B}$Samples represented with different letters are significantly different (p<0.05) from each other.
$^{a-b}$Samples represented with different letters are significantly different (p<0.05) from each other.
Error bars show the variations of three determinations in terms of standard error of mean.

### 4.6.4.2 *Lactobacillus bulgaricus* count

*Lactobacillus bulgaricus* count of all the yoghurt samples did not differ significantly (p>0.05) from each other (Table 4.53 and Figure 4.49). *Lactobacillus bulgaricus* count increased for the first 3 days of storage and then gradually decreased for the entire duration of storage of 15 days.

**Table 4.53: Effect of iron salt and iron microencapsules fortification on *Lactobacillus bulgaricus* count ($10^8$ cfu/g)**

| Sample → <br> Storage time ↓ | Control (Unfortified) | Iron salt fortified | Iron microencapsules fortified |
|---|---|---|---|
| 0th day | $437.67 \pm 44.243^{Ac}$ | $445.33 \pm 27.906^{Ac}$ | $417.00 \pm 19.296^{Ac}$ |
| 3rd day | $468.00 \pm 31.182^{Ad}$ | $483.33 \pm 47.977^{Bd}$ | $450.33 \pm 45.816^{Ad}$ |
| 7th day | $428.33 \pm 14.333^{Ac}$ | $436.67 \pm 17.910^{Ac}$ | $411.00 \pm 22.941^{Ac}$ |
| 10th day | $379.33 \pm 14.170^{Ab}$ | $379.33 \pm 9.821^{Ab}$ | $351.67 \pm 14.881^{Ab}$ |
| 15th day | $336.67 \pm 15.300^{Aa}$ | $345.67 \pm 11.566^{Aa}$ | $317.33 \pm 9.684^{Aa}$ |

Data are presented as means±SEM (n=3)

$^{A-B}$Means within row with different uppercase superscript are significantly different ($p<0.05$) from each other.

$^{a-b}$Means within column with different lowercase superscript are significantly different ($p<0.05$) from each other.

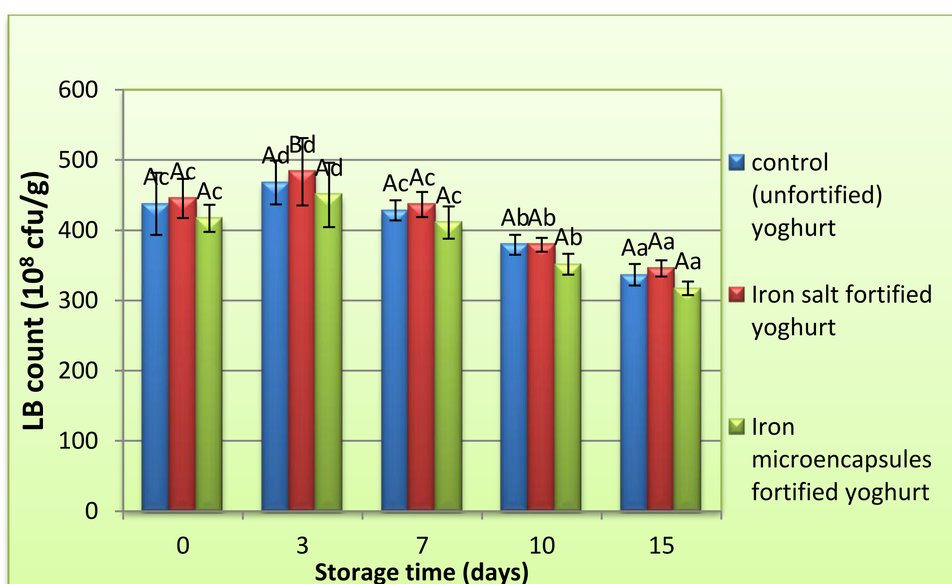



**Figure 4.49: Effect of iron salt and iron microencapsules fortification on *Lactobacillus bulgaricus* count ($10^8$ cfu/g)**

$^{A-B}$Samples represented with different letters are significantly different ($p<0.05$) from each other.

$^{a-b}$Samples represented with different letters are significantly different ($p<0.05$) from each other.

Error bars show the variations of three determinations in terms of standard error of mean.

Our results were in accordance with El-Behairy and Mohamed (2010) who reported higher lactic acid count in yoghurt prepared from iron fortified milk (ferric chloride and ferrous sulphate). However, Simova *et al.* (2008) reported that iron fortification of milk with ferrous lactate @ 8, 15 and 27 ppm iron had no effect on the incubation time required for the yoghurt mixes to reach pH 4.5±0.1 and it did not affect

significantly the growth and viability of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* during milk fermentation and storage of yoghurt. Lactic acid bacteria are reported to be exception among living organisms, as they show no iron requirements (Bruyneel *et al*. 1989; Cervaux *et al*. 2000). However, there are reports which indicate that some *Lactobacillus* species have the ability to bind ferric hydroxide at their cell surface, rendering it unavailable to pathogenic microorganisms.

Hekmat and McMahon (1997) also reported that counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* after 1 day of storage in iron-fortified skim yoghurts (casein chelated iron and whey protein-chelated iron @ 20 ppm) were not significantly different from numbers in unfortified yoghurts. Iron fortification had no effect on the incubation time required for the yoghurt mixes to reach pH 4.3. El-Kholy *et al*. (2011) showed that fortification of yoghurt with different iron salts had no effect on the total lactic acid bacteria in all treatments. Ocak and Köse (2010) found that iron fortification with ferric nitrate (@ 0.3 and 0.6 ppm) had no effect on the incubation time required for the yoghurt mixes to reach pH 4.5 ± 0.1.

**4.6.4.3 Coliform counts**

No coliform growth was observed in any of the samples during the storage

**4.6.4.4 Yeast and mould counts**

No yeast and mould growth was observed in any of the samples during storage.

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# CHAPTER –5

# Summary and Conclusion

# SUMMARY AND CONCLUSION

Micronutrient malnutrition is a serious threat to health and productivity of billions of people worldwide. Globally, micronutrient deficiencies of greatest public health significance are those of iron, vitamin A and iodine. Among micronutrients, iron is an essential trace element in animal and human diets. Lack of this trace element is the most common nutritional deficiency around the world. Iron deficiency anaemia affects almost two billion people worldwide, producing physical and psychical diseases, reducing productivity and affecting health budget. It is estimated that 20-45% of productive age women and 70% of children are anaemic in developing countries. Dairy products could be considered as an appropriate food vehicle for iron delivery to correct the dietary deficiency of these nutrients as it is centrally processed, widely distributed and regularly consumed by all sections of the society in predictable amount. Milk is relatively poor in iron (0.2-0.4 mg/l). RDA of iron is 17 mg/d for adult man and 21 mg/d for adult women. Fortification of dairy products with iron is the only remedy to overcome this nutritional deficiency of dairy products. Among dairy products, yoghurt is preferred since it is widely consumed throughout the world for its nutritional benefits. Yoghurt is believed to be effective in both the prevention and treatment of various illnesses, viz., gastrointestinal disorders, heart diseases and tumor development in man as well as animals. Fortification with iron is not an easy task as it may result in metallic taste, unacceptable flavours and undesirable colour changes. Microencapsulation could be a suitable method for adding iron to yoghurt for preventing these negative effects.

Microencapsules were prepared using same core material ferrous sulphate heptahydrate and different coating materials i.e. phosphatidylcholine, PGMS, SA, blend of SA and pectin, blend of SA and MS, blend of GA, MD and MS.

1. Iron microencapsules using phosphatidylcholine were prepared by liposome method. Egg and soy phosphatidylcholine were both used to produce liposome. Soy phosphatidylcholine showed very low EE and therefore was rejected. After sensory evaluation, it was found that iron microencapsules prepared using egg phosphatidylcholine were not suitable for fortification in milk and milk products even at low concentration of iron.

2. Iron microencapsules using PGMS were prepared by spray cooling method. These microencapsules showed very low EE, and hence were not utilized further.
3. Iron microencapsules using SA, blend of SA and pectin, blend of SA and MS were prepared by spray cooling method. The effect of different concentration of $CaCl_2$ on the EE of these microencapsules was studied and it was observed that spraying in 0.1 M $CaCl_2$ solution gave highest EE of microencapsules. Among the different microencapsules prepared SA + MS (0.1 M $CaCl_2$) showed highest EE. On sensory evaluation, it was found that these microencapsules were difficult to dissolve in milk and formed sediment at the bottom of the vessel.
4. Iron microencapsules prepared using SA was also prepared by deemulsification method and these showed high EE. On sensory evaluation, it was found that these microencapsules were not suitable for fortification in yoghurt.
5. Iron microencapsules using a blend of GA, MD and MS were prepared by two methods i.e. spray cooling method and using alcohol as dehydrating media. Process was optimized for preparation of microencapsules by both the methods and three microencapsules (GA 300 T60 ST60, GA 300 1:10 and GA 500 1:10) with maximum EE were selected. On sensory evaluation, it was found that all the three microencapsules were suitable for fortification in yoghurt
6. These microencapsules were further analysed by SEM and it was observed that GA 300 1:10 and GA 500 1:10 had spherical structures, whereas GA 300 T60 ST60 was not spherical. These microencapsules were used in the manufacture of iron fortified yoghurt. Yoghurt containing these microencapsules (GA 300 1:10 and GA 500 1:10) showed non significant difference in TBA, therefore GA 500 1:10 was finally selected for fortification.
7. Average particle size of microencapsules was 15.54 μm (range 6.84 – 33.42 μm).
8. Level of addition of microencapsules in yoghurt was optimized by fortification of yoghurt with 25 and 30 ppm iron and evaluated by sensory analysis. No significant difference in sensory scores was observed between yoghurt

containing 25 and 30 ppm iron, therefore 30 ppm iron was selected for fortification of yoghurt.

9. Method was standardized for the estimation of iron content in both microencapsules and yoghurt and it was observed that 8 hrs ashing time and acombination of dry and wet digestion of the sample were most suited analytical conditions for estimation of iron content.
10. Yoghurt fortified with GA 500 1:10 @30 ppm iron was analysed for effect of fortification on sensory, physicochemical, rheological and microbiological properties of yoghurt. Sensory analysis revealed that iron microencapsules fortified yoghurt was similar to control (unfortified) yoghurt upto 7 days of storage. TBA value of iron microencapsules fortified yoghurt was significantly lower from iron salt fortified yoghurt upto 5$^{th}$ day, whereas induction time was significantly higher. These results showed that iron microencapsules released the iron slowly after fortification in yoghurt.
11. Control (unfortified), iron salt fortified, iron microencapsules fortified yoghurt did not differ significantly in pH, acidity, setting time, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* counts. However, significant difference was observed in acetaldehyde content, iron microencapsules fortified yoghurt showed the highest value followed by control (unfortified) and iron salt fortified yoghurt.
12. Textural properties and water holding capacity of all the yoghurt samples were significantly different from each other. Iron microencapsules fortified yoghurt showed highest value followed by iron salt and control (unfortified) yoghurt. Maximum shear rate and viscosity was observed for all the samples on 3$^{rd}$ day of storage.
13. Yoghurt is a high moisture milk product and microencapsules were readily soluble in water, leading to lower stability of these microencapsules. These microencapsules may be more suitable for fortification in low or medium moisture foods.

# Bibliography

# BIBLIOGRAPHY


Abbasi, S. and Azari, S. (2011). Efficiency of novel iron microencapsulation techniques: fortification of milk. *International Journal of Food Science and Technology,* **46:** 1927-1933

Abd-Rabou, N. M. S.; Hofi, M. A. and Abbas, H. M. (1999). Effect of milk enrichment with different sources of zinc on the properties of yoghurt. *Mansoura University Journal of Agricultural Sciences*, **24 (2):** 837-845

ACC/SCN (2000). United Nations Administrative Committee on Coordination, Sub-Committee on Nutrition. Nutrition throughout the lifecycle. 4th report on the world nutrition situation, Geneva: ACC/SCN in collaboration with IFPRI

Achanta, K.; Aryana, K. J. and Boeneke, C. A. (2007). Fat free plain set yogurts fortified with various minerals. *LWT-Food Science and Technology*, **40 (3):** 424-429

Allen, L.; Benoist, B. D.; Dary, O. and Hurrell, R. (2006). Guidelines on food fortification with micronutrients. World Health Organization and Food and Agriculture Organization of the United Nations (WHO/FAO)

Amatayakul, T.; Halmos, A. L.; Sherkat, F. and Shah, N. P. (2006). Physical characteristics of yoghurts made using exopolysaccharide producing starter cultures and varying casein to whey protein ratios. *International Dairy Journal*, **16 (1):** 40-51

Andersson, M.; Thankachan, P.; Muthayya, S.; Goud, R. B.; Kurpad, A. V.; Hurrell, R. F. and Zimmermann, M. B. (2008). Dual fortification of salt with iodine and iron: a randomized, doubleblind, controlled trial of micronized ferric pyrophosphate and encapsulated ferrous fumarate in Sothern India. *American Journal of Clinical Nutrition*, **88 (5):** 1378-1387

AOAC (2005). Official methods of analysis. The Association of Official Analytical Chemists. 18th Edition. 481. North Fredrick Avenue Gaithersburg, Maryland, USA

Azzam, M. A. (2009). Effect of fortification with iron-whey protein complex on quality of yoghurt. *Egyptian Journal of Dairy Science*, **37 (1):** 55-63

Babu, U. S. and Calvo, M. S. (2010). Modern India and the vitamin D dilemma: Evidence for the need of a national food fortification program. *Molecular Nutrition and Food Research,* **54 (8):** 1134-1147

Badham, J.; Zimmermann, M. B. and Kraemer, K. (2007). The Guidebook: Nutritional Anaemia. Sight and Life Press, Basel, Switzerland

Baldwin, R. E.; Shelley, D. S. and Marshall, R. T. (1982). Flavor of milk one week after addition of iron complex. *Journal of Dairy Science*, **65 (8):** 1390-1393

Ballot, D.; Baynes, R. D.; Bothwell T. H.; Gillooly, M.; MacFarlane, B. J; MacPhail, A. P.; Lyons, G.; Derman, D. P.; Bezwoda, W. R. and Torrance, J. D. (1987). The effects of fruit juices and fruits on the absorption of iron from a rice meal. *British Journal of Nutrition*, **57 (3):** 331-343

Ballot, D. E.; McPhail, A. P. and Bothwell, T. H. (1989). Fortification of curry powder with NaFe(III)EDTA: report of a controlled iron fortification trial. *American Journal of Clinical Nutrition*, **49 (1):** 162-169

Bandgar, T. R. and Shah, N. S. (2010). Vitamin D and hip fractures: Indian scenario. *The Journal of the Association of Physicians of India*, **58:** 535-537

Beshkova, D.; Simova, E.; Frengova, G. and Simov, Z. (1998). Production of flavour compounds by yogurt starter cultures. *Journal of Industrial Microbiology and Biotechnology*, **20 (3-4):** 180-186

Beyer, P. (2002). Golden Rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *Journal of Nutrition,* **132:** 506S–510S

Bhattacharya, S.; Narasimha, H. V. and Bhattacharya S. (2003). Effect of gum arabic on the rheology of corn flour doughs and fried product quality. *Journal of Texture Studies,* **34 (4):** 421-436

Boccio, J. R.; Zubillaga, M. B.; Caro, R. A.; Gotelli, C. A.; Gotelli, M. J. and Weill, R. (1996). Bioavailability and stability of microencapsulated ferrous sulphate in fluid milk: studies in mice. *Journal of Nutrition Science and Vitaminology*, **42 (3):** 233-239

Boccio, J. R.; Zubillaga, M. B.; Caro, R. A.; Gotelli, C. A.; Gotelli, M. J. and Weill, R. (1997). A new procedure to fortify fluid milk and dairy products with high-bioavailable ferrous sulfate. *Nutrition Reviews*, **55 (6):** 240-246

Bothwell, T. H.; Charlton, R. W.; Cook, J. D. and Finch, C. A. (1979). Iron Metabolism in Man. Blackwell Scientific Publications, London

Bottazzi, V.; Battistotti, B. and Montescani, G. (1973). Influence des souches seules et associe´es de *L. bulgaricus* et *Str. thermophilus* ainsi que des traitements du lait sur la production d'aldehyde ace´tique dans le yaourt. *Lait*, **53:** 295-308

Brasil, Ministerio da Agricultura, Pecuaria e Abastecimento. (2007). Instrucao Normativa n 46 de 23 de outubro de. 2007. Available at www.agricultura.gov.br.

Bruyneel, B.; Vande-Woestyne, M. and Verstraete, W. (1989). Lactic acid bacteria: micro-organisms able to grow in the absence of available iron and copper. *Biotechnology Letters*, **11 (6):** 401-406

Burgi, H.; Supersaxo, Z. and Selz, B. (1990). Iodine deficiency diseases in Switzerland one hundred years after Theodor Kocher's survey: a historical review with some new goitre prevalence data. *Acta Endocrinologica*, **123 (6):** 577-590

Calleros, C. L.; Hernández, M.C.; Zambrano, C.C.; Castilla, O.S.; Romero, S. M.; Orozco, Y. H. and Carter J. V. (2007). Microencapsulation of *Lactobacillus casei* 81 lyo using sodium alginate blended with amidated low-methoxyl pectin of modified starch as wall materials: viability in yoghurt. *Acta Microscopica,* **16:** 1-2

Carpenter, C. E. and Mahoney, A. W. (1992). Contributions of heme and nonheme iron to human nutrition. *Critical Reviews in Food Science and Nutrition*, **31 (4):** 333-367

Cervaux, C.; Ehrlich, S. D. and Maguin, E. (2000). Physiological study of *Lactobacillus delbruekii* subsp. *bulgaricus* in a novel chemically defined medium. *Applied and Environmental Microbiology*, **66 (12):** 5306-5311

Champange, C. P. and Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. *Current Opinion in Biotechnology,* **18:** 184-190

Chen, Z. and Oldwage, W. (2002). Food fortification to prevent and control iron deficiency. *African Journal of Food, Agriculture, Nutrition and Development*, **2:** 436-457

Cho, Y.H. and Park, J. (2003). Evaluation of process parameters in the O/W/O multiple emulsion method for flavor encapsulation. *Journal of food science,* **64 (2):** 534-538

Codex Alimentarius Commission. *Codex Standard Follow-up Formula CODEX STAN 156-1987 (amended 1989).* Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, 1987 (http://www.codexalimentarius.net/download/standards/293/CXS_156e.pdf, accessed 7 march 2012)

Conrad. M. E. and Schade, S. G. (1968). Ascorbic acid chelates in iron absorption: a role for hydrochloric acid and bile. *Gastroenterology*, **55 (1):** 35-45

Cook, J. D. and Monsen, E. R. (1976). Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on non-heme iron absorption. *American Journal of Clinical Nutrition*, **29 (8):** 859-867

Cook, J. D. and Monsen E. R. (1977). Vitamin C, the common cold and iron absorption. *American Journal of Clinical Nutrition*, **30 (2):** 235-241

Cook, J. D. and Reusser, M. E. (1983). Iron fortification: an update. *American Journal of Clinical Nutrition*, **38 (4):** 648-659

Cook, J. D. (1990). Adaptation in iron metabolism. *American Journal of Clinical Nutrition*, **51 (2):** 301-308

Dallman, P. R. (1989). Upper limits of iron in infant formulas. *The Journal of Nutrition*, **119 (12):** 1852-1855

Danesh, J. and Appleby, P. (1999). Coronary heart disease and iron status: meta-analyses of prospective studies. *Circulation*, **99 (7):** 852-854

Darnton-Hill, I. and Nalubola, R. (2002). Fortification strategies to meet micronutrient needs: successes and failures. *Proceedings of the Nutrition Society*, **61 (2):** 231-241

Davidsson, L.; Galan, P.; Cherouvrier, F.; Kastenmayer, P.; Juillerat. M. A.; Hercberg, S. and Hurrell, R. F. (1997). Bioavailability in infants of iron from infant cereals: effect of dephytinization. *American Journal of Clinical Nutrition*, **65 (4):** 916-920

Demott, B. J. and Park, J. R. (1973). Effect of processing milk upon association of added iron with different protein fractions. *Journal of Dairy Science*, **57 (1):** 121-123

Demott, B. J. and Dincer, B. (1976). Binding added iron to various milk proteins. *Journal of Dairy Science*, **59 (9):** 1557-1559

Derbyshire, E.; Brennan, C. S.; Li, W. and Bokhari, F. (2010). Iron deficiency – is there role for the food industry?. *International Journal of Food Science and Technology,* **11:** 2443-2448

Disler, P. B.; Lynch, S. R.; Charton, R. W.; Bothwell, T. H.; Walker, R. B. and Mayet, F. (1975a). Studies on the fortification of cane sugar with iron and ascorbic acid. *British Journal of Nutrition*, **34 (1):** 141-152

Disler, P. B.; Lynch, S. R.; Torrance, J. D.; Sayers, M. H.; Bothwell, T. H. and Charlton, R. W. (1975b). The mechanism of the inhibition of iron absorption by tea. *South-African Journal of Medical Sciences*, **40 (4):** 109-116

Douglas, F. W.; Rainey, N. H.; Wong, N. P.; Edmonson, L. F. and LaCroix, D. E. (1981). Color, flavor and bioavailability in iron-fortified chocolate milk. *Journal of Dairy Science*, **64 (9):** 1785-1793

Downey, W. K. (1969). Lipid oxidation as a source of off-flavour development during the storage of dairy products. *Journal of Society of Dairy Technology*, **22 (3):** 154-161

Dumont, J. and Adda, J. (1973). Me´thode rapide [d'e´tude] des compose´s tre`s volatils de l'arome des produits laitiers. Application au yoghourt. *Lait*, **53:** 12-22

Dziezak, J. D. (1988). Microencapsulation and encapsulated ingredients. *Food Technology*, **42:** 136-139

Edmonson, L. F.; Douglas, F. W. and Avants, J. K. (1971). Enrichment of pasteurised whole milk with iron. *Journal of Dairy Science*, **54 (10):** 1422-1426

El-Behairy, S. A. and Mohamed, E. A. (2010). Properties of yoghurt made from fortified buffalo's milk with iron and zinc salts. *World Journal of Dairy and Food Sciences,* **5(2):** 207-213

El-Kholy, A. M.; Osman, M.; Gouda, A. and Ghareeb, W. A. (2011). Fortification of yoghurt with iron. *World Journal of Dairy and Food Sciences*, **6 (2):** 159-165

El-samragy, Y. A.; Mahran, G. A.; Khorishid, M. A. and Abd-Rabou, N. S. (1995). Chemical composition, organoleptic properties and nutritive value of white soft cheese as affected by iron fortification. In 6th *Egyptian Conference for Dairy Science and Technology,* Le Caire 4–6 november

FAO/WHO (1992). International Conference on Nutrition World Declaration and Plan of Action for Nutrition

Fomon, S. J.; Ziegler, E. E.; Serfass, R. E.; Nelson, S. E. and Frantz, J. A. (1997). Erythrocyte incorporation of iron is similar in infants fed formulas fortified with 12 mg/L or 8 mg/L of iron. *Journal of Nutrition*, **127 (1):** 83-88

FSSAI. (2011). Akalank's food safety and standards Act, rules and regulation. Akalank publication, pp-224

Foy, H. (1976). Fortification of salt with iron. *American Journal of Clinical Nutrition*, **29 (9):** 935-936

Gallardo-Escamilla, F. J.; Kelley, A. L. and Delahunty, C. M. (2005). Influence of starter culture on flavor and headspace volatile profiles of fermented whey and whey produced from fermented milk. *Journal of Dairy Science,* **88 (11):** 3745-3753

Garby, L. and Areekul, S. (1974). Iron supplementation Thai fish sauce. *Annals of Tropical Medicine and Parasitology*, **68 (4):** 467-476

Gaucheron, F.; Le Graet, Y.; Boyaval, E. and Piot, M. (1997). Binding of cations to casein molecules: importance of physicochemical conditions. *Milchwissenchaft,* **52 (6):** 322-327

Gaucheron, F. (2000). Iron fortification in dairy industry. *Trends in Food Science and Technology,* **11:** 403-409

Gbassi, G. K.; Vandamme, T.; Ennahar, S. and Marchioni, E. (2009). Microencapsulation of *Lactobacillus plantarum* spp in an alginate matrix coated with whey proteins. *International Journal of Food Microbiology*, **129:** 103–105

Gibson, R. S.; Ferguson, E. L. and Lehrfeld, J. (1998). Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. *European Journal of Clinical Nutrition*, **52 (10):** 764-770

Gillooly, M.; Bothwell, T. H.; Torrance, J. D.; MacPhail, A. P.; Derman, D. P.; Bezwoda, W. R.; Mills, W. and Charlton, R. W. (1983). The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *British Journal of Nutrition*, **49:** 331-342

Gillooly, M.; Bothwell, T. H.; Charlton, R. W.; Torrance, J. D.; Bezwoda, W. R.; MacPhail, A. P.; Derman, D. P.; Novelli, L.; Morral, P. and Mayet, F. (1984). Factors affecting the absorption of iron from cereals. *British Journal of Nutrition*, **51:** 37-46

Gragnolati, M.; Shekar, M.; Das Gupta, M.; Bredenkamp, C.; Lee, Y. K. (2005). India's undernourished children. A call for reform and action, World Bank Report.

Green, R.; Charlton, R.; Sefte, H.; Bothwell, T.; Mayet, F.; Adams, B.; Finch, C. and Layrissem M. (1968). Body iron excretion in man: a collaborative study. *American Journal of Medicine*, **45 (3):** 336-353

Guzun-Cojocaru, T.; Cayot, P.; Loupiac, C. and Cases, E. (2010). Effect of iron chelates on oil–water interface, stabilized by milk proteins: the role of phosphate groups and pH. Prediction of iron transfer from aqueous phase toward fat globule surface by changes of interfacial properties. *Food Hydrocolloids*, **24 (4):** 364-373

Hallberg, L. (1981). Bioavailability of dietary iron in man. *Annual Review of Nutrition*, **1:** 123-147

Hallberg, L.; Brune, M. and Rossander-Hulten, L. (1986). Low availability of carbonyl iron in man: studies on iron fortification of wheat flour. *American Journal of Clinical Nutrition*, **43 (1):** 59-67

Hallberg, L. (1987). Wheat fiber, phytates and iron absorption. *Scandinavian Journal of Gastroenterology* (Supplement), **129:** 73-79

Hallberg, L. and Rossander-Hulten, L. (1991). Iron requirements in menstruating women. *American Journal of Clinical Nutrition*, **54 (6):** 1047-1058

Hallberg, L.; Brune, M.; Erlandsson, M.; Sandberg, A. S. and Rossander-Hulten, L. (1991). Calcium: effect of different amounts on non-heme and heme-iron absorption in humans. *American Journal of Clinical Nutrition*, **53 (1):** 112-115

Hallberg, L.; Hulthen, L. and Garby, L. (1998). Iron stores in man in relation to diet and iron requirements. *European Journal of Clinical Nutrition*, **52 (9):** 623-31

Harinarayan, C. V.; Ramalakshmi, T.; Prasad, U. V.; Sudhakar, D.; Srinivasrao, P. V. L. N.; Sarma, K. V. S. and Kumar, E. G. T. (2007). High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. *American Journal of Clinical Nutrition*, **85 (4):** 1062-1067

Hazell, T. (1985). Minerals in foods: dietary sources, chemical forms, interactions, bioavailability. *World Review of Nutrition and Dietetics*, **46:** 1-123

Hegenauer, J.; Saltman, P.; Ludwig, D.; Ripley, L. and Bajo, P. (1979). Effects of supplemental iron and copper on lipid oxidation in milk. 1. Comparison of metal complexes in emulsified and homogenized milk. *Journal of agricultural and food chemistry,* **27 (4):** 860-867

Hekmat, S. and McMahon, D. J. (1997). Manufacture and quality of iron fortified yoghurt. *Journal of Dairy Science*, **80 (12):** 3114-3122

Herbert (1987). Recommended dietary intake (RDI) of iron in humans. *American Journal of Clinical Nutrition*, **45 (4):** 679-686

Hertrampf, E. (2002). Iron fortification in the Americas. *Nutrition Review*, **60 (7 Part 2):** S22-S25

Hinnai, M.; Rehman, S.; Huma, N. and Rafiq, F. (2000). Studies on wheat atta fortified with elemental iron used for chapatti production. *Pakistan Journal of Food Science*, **10 (3-4):** 5-7

Homayouni, A.; Ehsani, M. R.; Azizi, A.; Yarmand, M. S. and Razavi, S. H. (2007). Effect of lecithin and calcium chloride solution on the microencapsulation process yield of calcium alginate beads. *Iranian Polymer Journal,* **16 (9):** 597-606

Horton, S. (1999). Opportunities for investments in nutrition in low-income asia. *Asian Development Review,* **17 (1-2):** 246-273

Horton, S. and Ross, J. (2003). The economics of iron deficiency. *Food Policy*, **28 (1):** 51-75

Hulten, L.; Gramatkovski, E.; Gleerup, A. and Hallberg, L. (1995). Iron absorption from the whole diet. Relation to meal composition, iron requirements and iron stores. *European Journal of Clinical Nutrition*, **49 (11):** 794-808

Huma, N.; Rehman, S. U.; Anjum, F. M. and Murtaza, M. A. and Sheikh, M. A. (2007). Food fortification strategy-preventing iron deficiency anemia: a review. *Critical Reviews in Food Science and Nutrition*, **47 (3):** 259-265

Hunnell, J. W.; Yasumatsu, K. and Moritaka, S. (1985). Iron enrichment of rice. In: Iron Fortification of Foods. Academic Press, London

Hurrell, R. F.; Lynch, S. R.; Trinidad, T. P.; Dassenko, S. A. and Cook, J. D. (1988). Iron absorption in humans: bovine serum albumin compared with beef muscle and egg white. *American Journal of Clinical Nutrition*, **47 (1):** 102-107

Hurrell, R. F. (1989). Iron fortification of infant cereals. *Bibiliotheca Nutrition et Dieta*, **44**: 114-122

Hurrell, R. F. and Cook J. D. (1990). Strategies of iron fortification of foods. *Trends in Food Science and Technology,* **1:** 56-61

Hurrell, R. F.; Reddy, M. B.; Dassenko, S. A.; Cook, J. D. and Shepherd, D. (1991). Ferrous fumarate fortification of a chocolate drink powder. *British Journal of Nutrition*, **65:** 271-283

Hurrell, R. F.; Juillerai, M. A.; Reddy, M. B.; Lynch, S. R.; Dassenko, S. A. and Cook, J. D. (1992). Soy protein, phytate and iron absorption in man. *American Journal of Clinical Nutrition,* **56 (3):** 573-578

Hurrell, R. F. (1992). Prospects of improving the iron fortification of foods, In: Nutritional Anemias, Fomon, S. and Zlotokin, S. (Editors), Raven Press Ltd. New York, pp. 193-208

Hurrell, R. F. (1997). Preventing iron deficiency through food fortification, *Nutrition Reviews*, **55 (6):** 210-222

Hurrell, R. F. (1999). Iron. The Mineral Fortification of Food. Leatherhead, Surrey, Leatherhead Publishing, pp. 93-95

Hurrell, R. F.; Reddy, M. B.; Burri, J. and Cook, J. D. (2000). An evaluation of EDTA compounds for iron fortification of cereal based foods. *British Journal of Nutrition*, **84 (6):** 903-910

Hurrell, R. F. (2002). How to ensure adequate iron absorption from iron-fortified food. *Nutrition Reviews*, **60 (2):** S7-S15

IDF 117B (2003). International Dairy Federation Standards. Yoghurt: enumeration of characteristic microorganisms colony count technique at 37°C. Brussels, Belgium, pp. 6-8

Indian Council of Medical Research (ICMR). (2009). Nutrient requirements and recommended dietary allowances for Indians (A Report of Expert Group of ICMR)

International Nutritional Anemia Consultative Group (INACG) (1993). Iron EDTA for food fortification. International Life Sciences Institute, Washington, DC

IS: 10083 (1992). Method of test for determination of SNF (Solid-not-fat) in milk by the use of lactometer. Indian Standard Institution, Manak Bhawan, New Delhi

IS: 7768 (1975). Methods for sensory evaluation of milk. Indian Standard Institution, Manak Bhawan, New Delhi

IS: 1224 (1977). Determination of fat by Gerber Method. (Part 1). Milk (First Revision). Indian Standard Institution, Manak Bhawan, New Delhi

IS: SP: 18 (Part XI) (1981). Handbook of Food Analysis. Dairy Products. Bureau of Indian Standards, New Delhi

Jackson, L. S. and Lee, K. (1992a). The effect of dairy products on iron availability. *Critical Reviews in Food Science and Nutrition,* **31 (4):** 259–270

Jackson, L.S. and Lee K (1992b). Fortification of Cheese with Microencapsulated Iron. *Cultured Dairy Products Journal,* **27:** 4–7

Jain, V.; Gupta, N.; Kalaivani, M.; Jain, A.; Sinha, A. and Aggarwal, R. (2011). Vitamin D deficiency in healthy breastfed term infants at 3 months and their mothers in India: Seasonal variation and determinants. *Indian Journal of Medical Research*, **133:** 267-273

Jankowski, T.; Zielinska, M. and Wysakowska A. (1997). Encapsulation of lactic acid bacteria with alginate/starch capsules. *Biotechnology Techniques,* **1 (1):** 31-34

Jayalalitha, V.; Balasundaram, B.; Dorai, P. and Kumar, N. C. (2012). Fortification of encapsulated iron in probiotic yoghurt. *International Journal of Agriculture: Research and Review*, **2 (2):** 80-84

Johnson, P. E. and Evans G. W. (1977). Coffee as a low calorie vehicle for iron fortification. *Nutrition Reports International*, **16 (1):** 89-92

Joint FAO/WHO (1999). Expert committee on Food Additives (JECFA). Fifty third meeting, Rome (http:/www.who.int/pcs/jecfa/summery53revised.pdf)

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT- Food Science and Technology*, **39:** 1221–1227

Kanakdande, D.; Bhosale, R. and Singhal, R.S. (2007). Stability of cumin oleoresin microencapsulated in different combination of gum arabic, maltodextrin and modified starch. *Carbohydrate Polymers,* **67 (4):** 536-541

Kane, A. P. and Miller, D. D. (1984). *In vitro* estimation of the effects of selected proteins on iron bioavilability. *American Journal of Clinical Nutrition*, **39 (3):** 393-401

Kapil, U. and Jain, K. (2011). Magnitude of zinc deficiency amongst under five children in India. *Indian Journal of Pediatrics*, **78 (9):** 1069-1072

Kehagias, C. and Radma L. (1973). *Netherlands Milk and Dairy Journal*, **27:** 379-382

Khosroyar, S.; Akbarzade, A.; Arjoman, M.; Safekordi, A. A. and Mortazavi, S. A. (2012). Ferric – saccharate capsulation with alginate coating using the emulsification method. *African Journal of Microbiology Research,* **6 (10):** 2455-2461.

Kim, S. J.; Ahn, J.; Seok, J. S. and Kwak, H. S. (2003). Microencapsulated Iron for Drink Yogurt Fortification. *Asian-Australian Journal of Animal Sciences,* **16 (4):** 581-587

Kim, S. J.; Cho, S.Y.; Kim, S.H.; Song, O.J.; Shin, I. S.; Cha, D.S. and Park, H. J. (2008). Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121. *LWT- Food Science and Technology*, **41:** 493-500

Klug, S. L.; Patrizio, F. J. and Einstman, W. J. (1973). Iron fortified soluble coffee method for preparing the same. US Patent 4006, pp. 263

Kinder, F. W.; Muller, S. and Mitchell, H. S. (1942). The availability of the iron of cocoa and of iron fortified cocoa mixtures. *Journal of Dairy Science*, **25:** 401-405

Kotecha, P. V. (2008). Micronutrient malnutrition in India: Let us say "no" to it now. *Indian Journal of Community Medicine*, **33 (1):** 9-10

Krishnan, S.; Bhosale, R. and Singhal, R. S. (2005). Microencapsulation of cardamom oleoresin: Evaluation of blends of gum arabic, maltodextrin and a modified starch as wall materials. *Carbohydrate Polymers,* **61 (1):** 95–102

Krishnaveni, G. V.; Hill, J. C.; Veena, S. R.; Bhat. D. S.; Wills, A. K.; Karat, C. L.; Yajnik, C. S. and Fall, C. H. (2009). Low plasma vitamin $B_{12}$ in pregnancy is associated with gestational 'diabesity' and later diabetes. *Diabetologia*, **52 (11):** 2350-2358

Kumar, P. and Mishra, H. N. (2004). Mango soy fortified set yoghurt: effect of stabilizer addition on physicochemical, sensory and textural properties. *Food chemistry,* **87 (4):** 501-507

Kurtz, F. E.; Tamsma, A. and Pallansch, M. J. (1973). Effect of fortification with iron on susceptibility of skim milk and non fat dry milk to oxidation. *Journal of Dairy Science*, **56 (9):** 1139-1143

Kwak, H. S., Ihm, M. R. and Ahn, J. (2001). Microencapsulation of β-galactosidase with fatty acid esters. *Journal of Dairy Science.* **84:** 1576-1582

Kwak, H. S.; Yang, K. M. and Ahn, S. (2003a). Microencapsulated iron for milk fortification. *Journal of Food Chemistry*, **51 (26):** 7770-7774

Kwak, H. S.; Ju, Y. S.; Ahn, H. J.; Ahn, J. and Lee, S. (2003b). Microencapsulated iron fortification and flavor development in cheddar cheese. *Asian-Australian Journal of Animal Sciences,* **16 (8):** 1205-1211

Laine, P.; Lampi, A. M.; Tenkanen, M. and Jouppila, K. (2008). Miroencapsulation with gum arabic and spruce galactmannan. *XVIth International Conference on Bioencapsulation, Dublin, Ireland.* 4-6 September

Law, B. (1981). The formation of aroma and flavour compounds in fermented dairy products. *Dairy Science Abstracts*, **43:** 143

Lea, C. H. and Swoboda, P. A. T. (1962). Simple vacuum distillation procedure for determination of the volatile carbonyl content of autoxidising edible fats. *Journal of Science of Food and Agriculture,* **13 (3):** 148-158

Lee, K. Y. and Heo, T. R. (2000). Survival of *Bifidobacterium longum* immobilized in calcium alginate beads in simulated gastric juices and bile salt solution. *Applied and Environmental Microbology,* **66 (2):** 869-873

Lieberman, E.; Ryan, K. J.; Monson, R. R. and Schoenbaum, S. C. (1988). Association of maternal hematocrit with premature labor. *American Journal of Obstetrics and Gynecology*, **159:** 107–114

Lonnerdal, B. and Hernell, O. (1994). Iron, zinc, copper and selenium status of breast feed infant and infants feed trace element fortified milk based infant formula. *Acta Paediatrica,* **83 (4)**: 367-373

Lotfi, M.; Mannar, M. G. V.; Merx, R. H. J. M.; Naber-van, D. and Heuvel, P. (1996). Micronutrient fortification of foods: current practices, research and opportunities. Ottawa: The Micronutrient Initiative, International Agriculture Centre, Wagneningen, Netherlands

Lucca, P.; Hurrell, R. and Potrykus. (2002). Fighting iron deficiency anemia with iron-rich rice. *Journal of the American College of Nutrition*, **21** (**3 Suppl**): 184S–190S

Lund, E. K.; Wharf, S. G.; Fairweather-Tait, S. J. and Johnson, I. T. (1999). Oral ferrous sulfate supplements increase the free radical generating capacity of feces from healthy volunteers. *American Journal of Clinical Nutrition*, **69 (2):** 250-255

Lynch, S. R.; Hurrell, R. F.; Dassenko, S. A. and Cook, J. D. (1989). The effect of dietary proteins on iron bioavailability in man. *Advances in Experimental Medicine and Biology*, **249:** 117-132

Lynch, S. R.; Hurrell, R. F.; Bothwell, T. H. and MacPhail, A. P. (1993). Iron EDTA for food fortification. International Nutritional Anemia. Consultative Group/ILSI Press. Washington, DC

Madziva, H.; Kailasapathy, K. and Phillips, M. (2005). Alginate–pectin microcapsules as a potential for folic acid delivery in foods. *Journal of microencapsulation.* **22 (4):** 343-351

Mandal, S. (2006). Microencapsulation of probiotics and their application in manufacture of biodynamic milk chocolate. Ph.D. Thesis, National Dairy Research Institute (Deemed University), Karnal, India

Mandal, S., Puniya, A. K. and Singh, K. (2006). Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC 298. *International Dairy Journal.* **16:** 1190-1195

Marine, D. and Kimball, O. P. (1920). Prevention of simple goitre in man. *Archives of Internal Medicine*, **25:** 661-672

Marwaha, R. K.; Tandon, N.; Reddy, D. R.; Aggarwal, R.; Singh, R.; Sawhney, R. C.; Saluja, B.; Ganie, M. A. and Singh, S. (2005). Vitamin D and bone mineral density status of healthy schoolchildren in northern India. *American Journal of Clinical Nutrition*, **82 (2):** 477-482

Mehanna, N. M.; Saleh, T.M.; Mehanna, A.S. and El-Asfory, S. M. A. (2000). Composition and some properties of Zabady made from iron-fortified buffalo's milk. *Egyptian Journal of Dairy Science*, **28 (2):** 183-194

Mehansho, H. (2006). Iron fortification technology development: new approaches. *The journal of nutrition,* **136:** 1059-1063

MIF (1959). Laboratory Manual- Method of analysis of milk and milk products. By Milk Industry Foundation, Washington 6, DC

Morris, E. R. and Ellis, R. (1982). Phytate, wheat bran and bioavailability of dietary iron. In: Kies, C. (Editor). Nutritional Bioavailability of Iron (ACS Symposium Series 203). Washington DC: American Chemical Society, pp. 121

Moy, R. J. D. (2000). Iron fortification of infant formula. *Nutrition Research Reviews*, **13**: 215-227

Nadiger, H. A.; Krishnamachari, K. A. V. R. and Nadaminu, N. A. (1980). The use of common salt (sodium chloride) fortified with iron to control anaemia: results of prliminary study. *British Journal of Nutrition*, **43 (1)**: 45-51

National Family Health Survey (NFHS-3) (2007). National Report (2005-2006), Ministry of Health and Family Welfare, Government of India

National Nutrition Monitoring Bureau (NNMB), (2003) Reports, National Institute of Nutrition, Hyderabad

National Nutrition Policy (NNP) (1993). Government of India, Department of Women and Child development, Ministry of Human Resource Development, New Delhi, (www.wed.nic.in/nnp.pdf)

Navarrete, N. M.; Camacho, M. M.; Lahuerta, J. M.; Monzo, J. M. and Fito, P. (2002). Iron deficiency and iron fortified foods - a review. *Food Research International*, **35 (2-3):** 225-231

Nestel, P. (2003). Complementary food supplements to achieve micronutrient adequacy for infants and young children. *Journal of Pediatric Gastroenterology and Nutrition*, **36:** 316–328

Ocak, E. and Köse, Ş. (2010). The effects of fortifying milk with Cu, Fe and Zn minerals on the production and texture of yoghurt. *Journal of Food, Agriculture and Environment*, **8 (1):** 122-125

Olivares, M.; Pizzaro, F.; Pienda, Q.; Jose, J.; Hertampf, E. and Tonas, W. (1997). Milk inhibits and ascorbic acid favors ferrous bis-glycine chelate bioavailability in human. *American Society for Nutritional Sciences,* **127 (7):** 1407-1411

Osei, A.; Houser, R.; Bulusu, S.; Joshi, T. and Hamer, D. (2010). Nutritional status of primary school children in Garhwali Himalayan villages of India. *Food and Nutrition Bulletin*, **31 (2):** 221-233

Osman, M. M. and Ismail, M. M. (2004). Effect of fortification with zinc; iron and ascorbic acid on the chemical; microbiological and organoleptic properties of buffalo's bio-yoghurt. *Mansoura University Journal of Agricultural Sciences*, **29:** 237-251

Pathak, P.; Kapil, U.; Yajnik, C. S.; Kapoor, S. K.; Dwivedi, S. N. and Singh R. (2007). Iron, folate, and vitamin $B_{12}$ stores among pregnant women in a rural area of Haryana state, India. *Food and Nutrition Bulletin*, December, **28 (4):** 435-438

Petranovic, D.; Batinac, T.; Petranovic, D.; Ruzic, A. and Ruzic, T. (2008). Iron deficiency anaemia influences cognitive functions. *Medical Hypotheses,* **70:** 70–72

Picciano, M. F. (2003). Pregnancy and lactation: physiological adjustments, nutritional requirements and the role of dietary supplements. The Journal of Nutrition, **133 (6):** 1997S-2002S

Puri, S.; Marwaha, R. K.; Agarwal, N.; Tandon, N.; Agarwal, R.; Grewal, K.; Reddy, D. H. and Singh, S. (2008). Vitamin D status of apparently healthy schoolgirls from two different socioeconomic strata in Delhi: relation to nutrition and lifestyle. *British Journal of Nutrition*: **99 (4):** 876-882

Puvanenthiran, A.; Williams, R. P. W. and Augustin, M. A. (2002). Structure and visco-elastic properties of set yoghurt with altered casein to whey protein ratios. *International Dairy Journal,* **12 (4):** 383–391

Rao, D. V. and Ramamurthy, M. K. (1987). Influence of the metal catalysts on the pattern of carbonyl production during the autoxidation of cow milk fat. *Indian Journal of Animal Sciences*, **57 (5):** 475-478

Ravikiran, M. K.; Amma, P. and Sareen, K. N. (1977). Milk fortification with a system containing both iron and ascorbic acid. *Indian Journal of Nutrition and Dietetics*, **14 (9):** 260-266

Rehman, S.; Anjum, S. A. and Anjum, F. M. (2003). Chemical, rheological and storage studies of iron fortified whole wheat flour. *Pakistan Journal of Food Science*, **13:** 73-76

Remeuf, F.; Mohammed, S.; Sodini, L. and Tissler, J. P. (2003). Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yoghurt. *International Dairy Journal*, **13 (9):** 773-782

Rice, W. H. and McMahon, D. J. (1998). Chemical, physical and sensory characteristics of mozzarella cheese fortified using protein-chelated iron or ferric chloride. *Journal of Dairy Science*, **81 (2)**: 318-326

Richmond, F. L. and Moss, R. D. (1983). Vitamin encapsulation. US Patent-4389419, 1-6

Rossander, L. (1987). Effect of dietary fiber on iron absorption in man. *Scandinavian Journal of Gastroenterology* (Supplement), **129:** 68-72

Rossander-Hulthen, L.; Gleerup, A. and Hallberg, L. (1990). Inhibitory effect of oat products on non-haem iron absorption in man. *European Journal of Clinical Nutrition*, **44 (11):** 783-791

Sachan, A.; Gupta, R.; Das, V.; Agarwal, A.; Awasthi, P. K. and Bhatia, V. (2005). High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. *American Journal of Clinical Nutrition*, **81 (5):** 1060-1064

Sadler, A. M.; Lacroix D. E. and Alford J. A. (1973). Iron content of baker's and cottage cheese made from fortified skim milks. *Journal of Dairy Science*, **56 (10):** 1267-1270

Saini, S. P. S.; Jam, S. C. and Bains, G .S. (1987). Effect of iron fortification on flavour of buffalo milk. *Indian Journal of Dairy Science*, **40 (1):** 88-93

Salguiero, J.; Torti, H.; Meseri, E.; Furno, J.; Zubillaga, M.; Janjetic, M.; Barrado, A. and Boccio, J. (2007). Bioavailability studies of stabilized iron (II) sulphate by means of the prophylactic-preventive method. *Biological Trace Element Research*, **120 (1-3):** 110-113

Salvi, V. S. and Damania, K. R. (2005). Neural tube defects in India - time for action. *The Lancet*, **366 (9489):** 871-872

Sandoval-Castilla, O.; Lobato-Calleros, C.; Aguirre-Mandujano, E. and Vernon-Carter, E. J. (2004). Microstructure and texture of yogurt as influenced by fat replacers. *International Dairy Journal*, **14 (2):** 151-159

Shaikh, J.; Bhosale, R. and Singhal, R.S. (2006). Microencapsulation of black pepper oleoresin. *Food Chemistry,* **94:** 105-110

Siegenberg , D.; Baynes, R. D.; Bothwell, T. H.; Macfariane, B. J.; Lamparelli, R. D.; Car, N. G., MacPhail, P.; Schmidt, U.; Tal, A. and Mayet, F. (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *American Journal of Clinical Nutrition*, **53 (2):** 537-541

Simova, E.; Ivanov, G. and Simov, Z. (2008). Growth and activity of Bulgarian yoghurt starter culture in iron-fortified milk. *Journal of Industrial Microbiology and Biotechnology*, **35 (10):** 1109-1115

Simpson, K. M.; Morris, E. R. and Cook, J. D. (1981). The inhibitory effect of bran on iron absorption in man. *American Journal of Clinical Nutrition*, **34:** 1469-1478

Singh, B.; Dheeravathu, S. N. and Usha, K. (2010). Micronutrient deficiency: A global challenge and physiological approach to improve grain productivity under low zinc availability. *Plant Stress*, November **11:** 76-93

Slatkavitz, C. A. and Clydesdale, F. M. (1988). Solubility of inorganic iron as affected by proteolytic digestion. *American Journal of Clinical Nutrition*, **47:** 487-497

Stekel, A.; Olivares, M. and Cayazzo, M.; Chadud, P.; Claguno, S. and Pizaro, F. (1988). Prevention of iron deficiency by milk fortification. II. A field trial with a full fat acidified milk. *American Journal of Clinical Nutrition*, **47:** 265-269

Sultana, K.; Godward, G.; Reynolds, N.; Arumugaswamy, R.; Peiris, P. and Kailasapathy, K. (2000).Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *International Journal of Food Microbiology,* **62:** 47–55

Snedecor, G. W. and Cochran, W. G. (1994). Statistical Methods, 8th Edition. Affiliated East-West Press, Iowa State University Press

Suthar, B. (2008). Microencapsulation of probiotic organisms for food applications. M.Tech. Thesis, National Dairy Research Institute (Deemed University), Karnal, India

Swain, J. H.; Newman, S. M. and Hunt, J. R. (2003). Bioavailability of elemental iron powders to rats is less than bakery-grade ferrous sulfate and predicted by iron solubility and particle surface area. *The Journal of Nutrition*, **133 (11):** 3546-3552

Tan, L. H.; Chan, L. W. and Heng P. W. S. (2009). Alginate/starch composites as wall material to achieve microencapsulation with high oil loading. *Journal of Microencapsulation.* **26 (3):** 263-271

Teotia, S. P. S. and Teotia, M. (2008). Nutritional bone disease in Indian population. *Indian Journal of Medical Research*, **127:** 219-228

Theuer, R. C.; Kemmerer, K. S.; Martin, W. H.; Zoumas, B. L. and Sarett, H. P. (1973). Effect of processing on availability of iron salts in liquid infant formula products. Experimental milk-based formulas. *Journal of Agricultural and Food Chemistry*, **21 (3):** 482-485

Theuer, R. C. (2002). Effect of iron on the color of barley and other cereal porridges. *Journal of Food Science*, **67 (3):** 1208-1211

Tomar, S. K. and Prasad, D. N. (1987). Effect of pre-heat treatment on microstructure of cow whole milk yoghurt as revealed by scanning electron microscopy. *Microbiologie Aliments Nutrition,* **5:** 345-350

Trindade, M. A. and Grosso, C. R. F. (2000). The stability of ascorbic acid microencapsulated in granules of rice starch and in gum arabic. *Journal of Micoencapsulation,* **17 (2):** 169-176

Umbelino, D. C.; Cardello, H. M. A. B. and Rossi, E. A. (2001). Effect of iron salts addition on the sensory characteristics of soy yoghurt. *Archivos Latinoamericanos de Nutricion*, **51 (2):** 199-203

Umbelino, D. C. and Rossi, E. A. (2009). Soy yogurt fortified with iron and calcium: stability during the storage. *Alimentos e Nutrição*, **20 (1):** 7-13

Unnikrishnan, V. and Rao, M. B. (1977). Distribution of added iron in milk of cows and buffaloes and its effect on oxidized flavor development. *Journal of Food Protection*, **40 (2):** 86-89

Chandramouli, V.; Kailasapathy, K.; Peiris, P. and Jones, M. (2004). An improved method of microencapsulation and its evaluation to protect **Lactobacillus spp.** in simulated gastric conditions. *Journal of Microbiological Methods,* **56:** 27– 35

Vaidya, S.; Bhosale, R. and Singhal, R.S. (2006). Microencapsulation of Cinnamon Oleoresin by Spray Drying Using Different Wall Materials. *Drying Technology: An International Journal,* **24 (8):** 983-992

Vijayendra, S. V. N. and Gupta, R. C. (2011). Assessment of probiotic and sensory properties of *dahi* and yoghurt prepared using bulk freeze-dried cultures in buffalo milk. *Annals of Microbiology,* DOI: 10.1007/s13213-011-0331-5

Walter, T.; Pino, P.; Pizarro, F. and Lozoff, B. (1998). Preventing of iron deficiency anemia: comparison of high and low iron formulas in term healthy infants after six months of life. *Journal of Pediatrics*, **132 (4):** 635-640

Wang, C. F. and King, R. L. (1973). Chemical and sensory evaluation of iron fortified milk. *Journal of Food Science*, **38 (6):** 938-940

Whittaker, P. (1998). Iron and zinc interactions in humans. *American Journal of Clinical Nutrition*, **68:** 442-446

WHO (2000). Malnutrition: the global picture. World Health Organisation; (www.who.int/nut/malnutrition_worldwide.htm)

Williams P. A. and Phillips G. O. (2000). Gum Arabic. *Hanbook of Hydrocolloids,* CRC Press, Cambridge, England. pp. 155-168

Woestyne, M. V.; Bruyneel, B.; Mergeay, M. and Verstraeta, W. (1991). The Fe chelator proferrorosamine A is essential for the siderophore-mediated uptake of iron by *Psuedomonas roseus fluorescences*. *Applied and Environmental Microbiology,* **57:** 949-954

www.a2zproject.org/pdf/Food-Fortification-Formulator.pdf, accessed on September 16, 2012

www.copenhagenconsensus.com, accessed August 11, 2012

www.motherchildnutrition.org/india/pdf/mcn-integrating-nutrition-with-health.pdf, accessed on December 06, 2012

www.nutritionimprovement.com, accessed on August 23, 2012

www.fnbnew.com, accessed on November 23, 2012

*www.pg.gda.pl/chem/CEEAM/Dokumenty/CEEAM/chapter13.pdf* ,June 8, 2013

Xia, S. and Xu, S. (2005). Ferrous sulfate liposomes: preparation, stability and application in fluid milk. *Food Research International,* **38:** 289-296

Yajnik, C. S.; Deshpande, S. S.; Jackson, A. A.; Refsum, H.; Rao, S.; Fisher, D. J.; Bhat, D. S.; Naik, S. S.; Coyaji, K. J.; Joglekar; C. V.; Joshi, N.; Lubree, H. G.; Deshpande, V. U.; Rege, S. S. and Fall, C. H. (2008). Vitamin $B_{12}$ and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia,* **51 (1):** 29-38

Yaseen, E. I.; Herald, T. J.; Aramouni, F.M. and Alavi, S. (2005). Rheological properties of selected gum solutions. *Food Research International*. **38:** 111–119

Yu, D.; Qiao, W.; Li, Q. and Pei, G. (2012). Preparation and properties of olive oil microencapsules. *Journal of Fiber Bioengineering and informatics,* **5 (1):** 67-76

Zhang, D. and Mahoney, A. W. (1988). Sensory quality of iron fortified fresh cheddar cheese. *Journal of Dairy Science*, **71 (Supplement 1):** D163-D166

Zhang, D. and Mahoney, A. W. (1989). Effect of iron fortification on quality of cheddar cheese. *Journal of Dairy Science*, **72 (2):** 322-332

Zhang, D. and Mahoney, A. W. (1990). Effect of iron fortification on quality of cheddar cheese-2. Effects of aging and fluorescence light on pilot scale cheeses. *Journal of Dairy Science*, **73 (9):** 2252-2258

Zhang, D. and Mahoney, A. W. (1991). Iron fortification of process cheddar cheese. *Journal of Dairy Science*, **74 (2):** 353-358

Ziegler, E. E and Fomon, S. J. (1996). Strategies for the Prevention of Iron Deficiency: Iron in Infant Formulas and Baby Foods. *Nutrition Reviews,* **54 (11):** 340-354

Zilberboim, R.; Kopelman, I. J. and Talmon, Y. (1986a). Microencapsulation by a dehydrating liquid: Retention of paprika oleoresin and aromatic esters. *Journal of Food science,* **51 (5):** 1301-1306

Zilberboim, R.; Kopelman, I. J. and Talmon, Y. (1986b). Microencapsulation by a dehydrating liquid: A microstructural study by scanning electron microscopy. *Journal of Food science,* **51 (5):** 1307-1310

# Annexures

# Annexure I

## SCORE CARD FOR YOGHURT

**Date:** **Storage time:**

**Name of panelist :**
**Objective : To evaluate yoghurt prepared for sensory attributes**

**A) Assign score for each sample for different characteristics**

| Attribute | Maximum score | Sample score | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Flavour | 45 | | | |
| Body & texture | 30 | | | |
| Acidity | 10 | | | |
| Colour and appearance | 10 | | | |
| Container and closure | 5 | | | |
| Total | 100 | | | |

**B) Indicate the degree of defects such as the following. Encircle the one applicable and deduct from attributes.**

| Attribute | Defect | Intensity of defect | | |
|---|---|---|---|---|
| | | Slight | Definite | Pronounced |
| Flavour | Highly acidic, bitter, metallic, | 7 | 9 | 11 |
| | yeasty, cheesy | 10 | 13 | 16 |
| Body and texture | Grainy, thin body | 2 | 5 | 8 |
| | ropy and wheying off | 4 | 8 | 12 |
| Acidity | Too low, too high | 1 | 3 | 5 |
| Colour and appearance | Unnatural colour, | 1 | 3 | 5 |
| | presence of foreign matter | 2 | 4 | 6 |
| Container and closure | Soiled, improperly covered | 1 | 2 | 3 |

Comments:

Signature