Final Technical Report
On
Fortification of Salt

Prepared for the
Micronutrient Initiative

Levente L. Diosady Ph.D., P.Eng.
Professor of Food Engineering
Department of Chemical Engineering and Applied chemistry
University of Toronto

2003

Approved Luis Lafaye

November 20, 2003

Background

As a result of an initiative by Mr. Venkatesh Mannar, then a UNICEF salt consultant the micronutrient Initiative undertook a program to test the concept of double fortification of salt, and eventually to develop a process for double fortification with iron and iodine. All of the research and much of the development work on this program has been carried out by the University of Toronto.

The program's goal is to develop technology for the double fortification of salt with iron and iodine, and to triple fortify with iodine, iron and Vitamin A.

At this point in time, we have developed techniques for agglomeration and encapsulation of premixes containing iodine, iron, Vitamin A, and combined premixes for iodine and Vitamin A. Triple premixes, combinations of iron with either iodine or Vitamin A were unstable.

We have developed techniques for colour masking ferrous fumarate premixes. Thus we have satisfied the need for providing a stable chemical platform, assured blending of the premixes with salt without segregation during manufacture distribution and retail, and minimized the visual impact of iron fortification in normal; household salt.

The technology for double fortification works well with normally iodated salt, in highly refined household salt, using microencapsulated ferrous fumarate as the iron source. With unrefined, coarser salt larger premix particles must be made, and microencapsulated iodine premix is also required, to ensure iodine stability.

Triple fortification was shown to be technically feasible. The stability of Vitamin A in the original formulations is relatively low, resulting in losses of some 50% in 3-6 mooths of storage at high temperature and humidity.

Field tests in Kenya and Nigeria confirmed the laboratory results on DFS and TFS stability. A number of formulations have been successfully field tested.

Scale-up of microencapsulated iodine was completed to small commercial scale, while microencapsulated iron was produced at full commercial scale, and is ready for an initial commercial production run.

A greatly simplified process, using a cement mixer for both agglomeration and encapsulation (coating) was technically feasible, though highly manpower intensive, as much material had to be reworked.

The results of this work have been presented at several international congresses, and in numerous publications, some still in press. A list has been provided earlier.

More technical details have been reported earlier, and also in the following chapters.

1. Introduction

Iodine and iron deficiency impacts over 2 billion people around the world. Insufficient intake of iodine in the diet causes a myriad of health problems collectively known as Iodine Deficiency Disorders (IDD). The consequences of IDD include mental retardation, goiter, growth retardation and increased neonatal and post-natal mortality (Venkatesh Mannar, 1987).

Nutritional iron deficiency is also a major problem in developing countries, often affecting the same population group. Lack of sufficient iron intake can lead to increased maternal mortality, compromised development of motor skills and learning capacity, lethargy and reduced immunity to disease. The combined impact of these deficiencies severely impedes economic and social development in developing countries. (Clydesdale & Weimer, 1985).

The quantity and cost of micronutrients required to combat these deficiency diseases seem trivial – only a few milligrams or micrograms per day costing only a few cents per person annually – yet the logistics of regularly providing these micronutrients pose serious social and technical challenges.

Salt has been successfully fortified with iodine and this has virtually eliminated IDD in developed nations. It seems logical to utilize the infrastructure and experience established with salt iodization in providing iron to combat widespread anemia is. Salt is an excellent carrier for essential micronutrients in view of its almost universal and uniform consumption. Since salt is processed in relatively few production centres, its processing is easier to coordinate. (Narasingha Rao & Vijaya Sarathy, 1975; Zoller et al., 1980, Diosady et al, 1998)

Adding iron and iodine concurrently to salt has its challenges. There are several factors that make direct addition of iron and iodine compounds to salt impractical. Iron and iodine compounds tend to react with each other and impurities normally present in salt and this leads to conversion of iodide or iodate to iodine in its elemental state (I_2). . The free iodine easily sublimes and is lost into the atmosphere.The oxidation of iodide or reduction of iodate to elemental iodine is accelerated by t metal ions and reducing and oxidizing agents, especially in the presence of moisture and sunlight, Iron compounds also undergo reactions that lead to discoloration of the salt and amplified metallic taste (Diosady, et al 1998; Yusufali, 2001).

In the past two decades, there have been progressive attempts to produce double fortified salt. Indian scientists have used ferrous sulphate and sodium hexametaphosphate together with potassium iodide/iodate (Narasingha Rao, 1990; Venkatesh Mannar, Jayapal, & Pandav, 1989). This approach, although stable at low humidity and temperature, is not commercially viable due to the high levels of additives required. When tested under high humidity and high

temperatures typical of tropical countries, the double fortified salt showed poor stability. (Diosady et al, 2001)

The stability of double fortified salt can be greatly enhanced by microencapsulation of the fortifying ingredients. Diosady et al. 2001 proved that double fortified salt prepared by microencapsulation of potassium iodide by spray drying within a dextrin matrix and ferrous fumarate added in dry powder form was stable for up to six months. Spray drying is limited in its application since the particles were very small in the range of 50-100μm. Typical salt particles are 300-1500μm in diameter The spray dried premix particles would readily segregate in the salt and thus the salt was milled to a similar particle size range in order to ensure adequate mixing. This was undesirable from the consumers' point of view and would also increase the cost of producing double fortified salt.

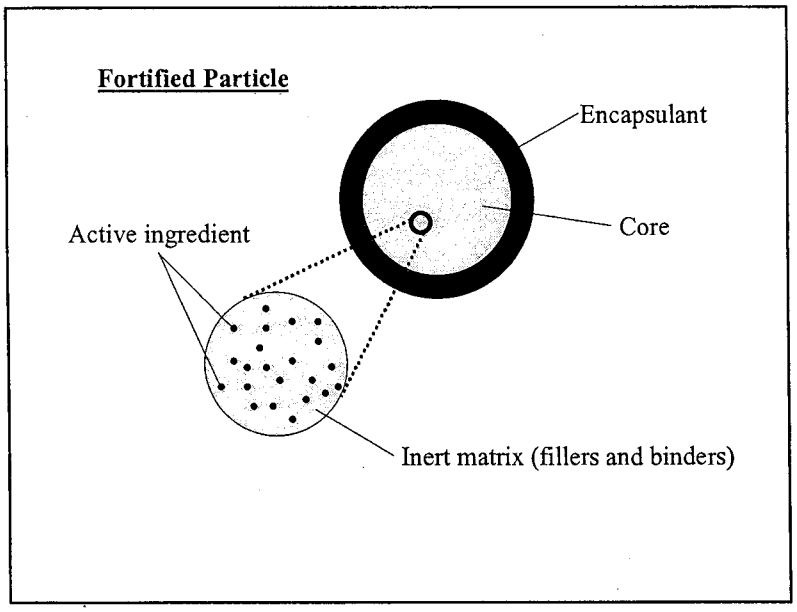

Figure 1 Cross sectional view of an ideal encapsulated particle

An alternate strategy for producing encapsulated iron and iodine premixes for double fortification of salt is presented here. The important criteria were particle size, cost of production, simplicity of process and ultimately, micronutrient stability.

2. Experimental techniques

2.1. Materials

Non-iodized, high grade evaporated salt (food grade) was obtained from Toronto Salt Chemical Co., Toronto. Laboratory grade potassium iodide, potassium iodate, ferrous fumarate, dichloromethane, and food grade ethanol (100%) were purchased from Sigma-Aldrich Chemical Company, Toronto.

A modified starch, Casco Dextrin 7071, from Casco Inc. Cardinal, ON. Canada, was used as a filler/binder. Fully hydrogenated soy stearine was obtained from CanAmera Foods Inc. of Toronto, ON. Canada.

Analytical grade Polyethylene glycol (PEG 2000 & PEG 8000), palmitic acid, ethylcellulose, polymethyl methacrylate, hydrochloric acid and zein were purchased from BDH Chemicals Limited of Toronto.

Reagent grade 1,10-phenanthroline monohydrate and potassium biphthalate were obtained from Sigma-Aldrich Chemical Co. of Toronto. An iron atomic absorption standard was purchased from SCP Science of Toronto.

2.2. Pan Granulation

Pan agglomeration was carried out in a rotating pan fabricated in our machine shop. The pan was 30 cm in diameter and 6 cm deep and was connected to a variable speed motor. The pan inclination angle could be varied by rotating the motor support shaft. A diagram of the pan is shown in figure 2.

A typical granulation run consisted of blending the dry powder formulation using a 5L ribbon blender (LeRoy Somer-LSTronics, Montreal) or glass jar. The powder was then fed into the pan and the rotation speed and inclination angle was adjusted until a cascading motion of the powder in the pan was achieved. The binding solution consisting of 10% dextrin was then sprayed onto the powder in the pan using a TLC atomizer hooked up to an air supply at ~50 kPa. Once the powder was granulated, the granules were placed in a baking pan and dried in an oven at 50°C for 12 hours. After drying, particles between 300um – 700um were recovered by sieving and spray coated.

2.3. Spray Coating

Spray coating of the particles was also done in the rotating pan. The particles to be encapsulated were fed into the rotating pan and set in motion. The encapsulant was dissolved in either alcohol or dichloromethane and sprayed using an air atomizer onto the particles. It was sometimes necessary to dry the particles in-between spraying. The mass of the particles was measured before and after each run to determine the level of encapsulation. The concentration of the encapsulant in the solvent was varied to decrease its viscosity to enable spraying.

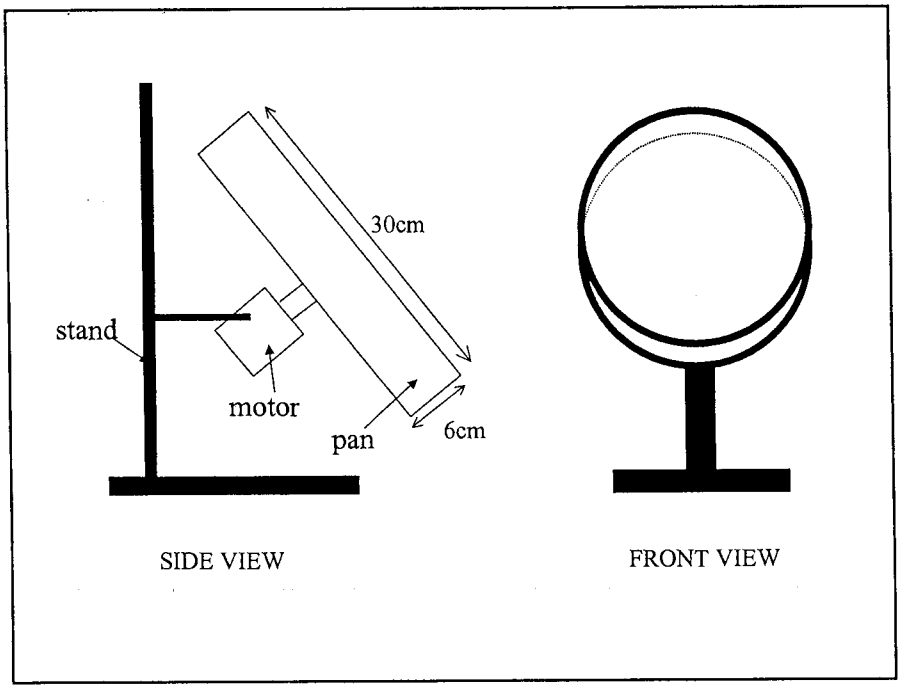

Figure 2 Diagram of rotating pan used for agglomeration and spray coating of particles

2.4. Salt preparation

Seven 50g salt samples in LDPE bags containing 50µg/g iodine and 1000µg/g iron were prepared for each formulation. Separate bags were prepared in order to minimize sampling error normally caused by segregation of particles within the bag. Appropriate amounts of iron and iodine premix particles were weighed into each of the bags.

During analysis, the complete contents of each sample bag was first milled using a Black & Decker coffee grinder and sub-samples from the milled fortified salt were analyzed for iodine and iron content.

2.5. Storage conditions

The packages were stored under elevated temperature and humidity (40°C and ~100%RH) to simulate extreme tropical conditions. The high temperature and humidity was maintained using a controlled temperature oven, in which the air was saturated with moisture by exposure to a tray of water.

2.6. *Analytical methods*

2.6.1 Iodine

Iodine was measured by epithermal neutron activation analysis (ENAA) using a SLOWPOKE nuclear reactor with the help of Professor Ronald Hancock at the Royal Military College in Kingston, Ontario.

In this method, 1-2 g samples of salt were weighed into polyethylene vials. These vials were then shielded with a cadmium shell and irradiated at 10 kW for 3 minutes using a neutron flux of 5.0×10^{11} $cm^{-2}s^{-1}$. The cadmium shell was used to reduce interferences due to the high proportion of chlorine and sodium present in the sample. Following irradiation, there was a time delay of 6 minutes, after which the gamma emissions at 44.3 keV were measured using a hyper pure germanium based gamma ray spectrometer. The concentrations of iodine in the samples were calculated based on a calibration curve obtained from a series of standard samples ranging in iodine concentration from 20 to 2000 ppm.

2.6.2. Iron

Iron was analyzed by spectrophotometry, using the method developed by Harvey, Smart and Edwards (1955). 1,10-phenanthroline complexes are formed with iron(II) and iron(III) and. their absorbances are then measured at 396 nm and 510 nm and correlated to calibration data to determine the total iron and the ferrous iron content of samples simultaneously. The main advantage of this method is that it not only gives the total iron content but also differentiates between ferrous and ferric iron.

2.6.3. Moisture content

The moisture content of samples was determined gravimetrically. Samples weighing not less than 5g were weighed into aluminum boats previously dried in the oven at 110°C to remove moisture. The samples were then dried to constant weight in an oven at 110°C (12-24 hours)

3. Results and Discussion

The ultimate objective of double fortifying salt with iron and iodine is to provide these essential micronutrients to the countless people who's diets are deficient. In order to make these micronutrients available to everyone, the has to be simple, and relatively inexpensive resulting in fortified salt where the micronutrients are stable under typical storage and distribution conditions.

3.1. Size Enlargement and Micronutrient Dosage

In order to add these microencapsulated nutrients to salt, the particle size distribution of the premix had to match that of salt. Because of the differences in particle size between typical commercial salt crystals (300 to 700um) and the iodine and iron compounds (20 to 200 um), segregation of the micronutrients would occur. Since finely powdered salt is not practical for household use under hot, humid conditions, increasing the mean particle size of the premix to match that of typical salt is a more practical approach to ensuring that no segregation

occurs. Pan granulation was chosen as the size enlargement technique because it is a relatively simple and inexpensive compared to other granulation techniques.

The iodine premix was diluted by 50-100 fold. This was necessary because a typical crystal if KI, the size of a salt grain would contain nearly the entire daily iodine requirement. Small errors in mixing would result in over/underdoseing the salt. It was prudent to adjust the concentration of the active micronutrients in order to ensure their safe delivery.

Salt consumption is approximately 10 g per day. The dosage of active nutrients in the salt was set to provide 100% of the recommended daily intake of I and 30% of Fe: 50 ppm iodine and 1000 ppm iron in the salt would provide 0.5 mg iodine and 10 mg iron daily. Studies show that only about one third of the consumed iron is actually absorbed by the body. SO? – did we account for this? Does the RDA take it into account? This may be a red herring that draws criticism unless we explain it away, and may be left out.

For the process to be viable, the amount of premix will have to be limited to ~ 1% of the salt. At lower inclusion levels even mixing is difficult, while higher levels significantly increase the cost of salt, and the pre-mix. To maintain the addition level at 1% of the salt weight the iodine premix consisted of approximately 1% iodine and the iron premix contained approximately 20% iron.

We investigated 3 approaches to producing the premix particles. These are presented in the table below:

	Spray Drying	**Fluidized bed granulation and coating**	**Pan agglomeration and coating**
Particle size distribution	Inappropriate, averaging 50-100um in diameter	Appropriate, process parameters can be adjusted to produce particles averaging 250-700um	Appropriate, process parameters can be adjusted to produce particles averaging 250-700um
Cost	High due to energy requirement and cost of equipment	Capital cost quite high although product yield is good	Cost of equipment relatively low
Yield of particles within the particle size range	Very low, most of the product is too fine	High, but skilled personnel required to control process	Approximately 50% yield, wide distribution of particle size but off sizes can be reworked.

	Process can be controlled by semi-skilled personnel

Fluidized bed processing or pan granulation can both be applied successfully for producing the encapsulated premix; its use would depend on the resources available. We have performed scale-up studies for both approaches with relative ease from 1 to 22 to 60 and finally 225L reactor batch sizes.

We used dextrin as a filler and a binder for granulation runs. Approximately four fifths of the dextrin was combined with ferrous fumarate as a dry powder and fed into the rotating pan. The pan was set into motion and the angle of inclination and the rotation speed was adjusted until the powder flowed in a cascading motion. The binding solution consisting of the remaining dextrin as a 10% aqueous solution was prepared and sprayed onto the powder in order to agglomerate the powder. The spray *rate* ? not volume? was adjusted until sufficient agglomeration took place.

The iodine premix was agglomerated similarly with the exception that only dextrin was fed into the pan and the binding solution consisted of a solution of dextrin, potassium iodide and water (10% solids content).

3.2. Microencapsulation and selection of suitable encapsulants

The agglomerated particles were further protected by a water impervious surface film, by microencapsulation. Encapsulation was achieved by spray coating in the pan granulator. The encapsulant was dissolved in either methylene chloride or ethanol. The spray rate was kept low in order to prevent further granulation.

The criteria used in rating the performance of the microencapsulated nutrients were iodine retention, iron conversion, color and taste.

Salt fortified with uncoated, agglomerated ferrous fumarate and potassium iodate lost all its iodine in 3 months under 40°C and 100%RH. There was 25% conversion of ferrous iron to ferric iron as well and the salt turned blue in color. Salt fortified with agglomerated ferrous fumarate and potassium iodide however, retained almost all the iodine but it had also turned blue. This suggests that there was leakage of iron from the granulated premix which in turn led to the blue color formation, hence there was a need for further protection of the active nutrients. In previous studies by Diosady et al. the blue color of the salt wasn't observed with potassium iodide and ferrous fumarate, indicating that spray-drying, results in a more stable capsule.

From stability results of the unencapsulated, agglomerated premix, it was clear that added protection of the iron and iodine premix was desirable. Encapsulation with a coating agent the would

- form continuous films;
- be impervious to water;
- It should be edible and easily digestible, and
- provide the required protection for the micronutrients.

Several encapsulants were investigated.. The performance of the encapsulant was judged by the retention of iodine after 3 months of storage under 40°C and 100% RH.

KIO_3 particles were agglomerated and subsequently coated with 40% w/w of the coating agent.. Ferrous fumarate was also agglomerated and coated with soy stearine at the 40% level. Each of the encapsulated KIO_3 premixes was used to fortify salt together with ferrous fumarate and encapsulated ferrous fumarate respectively. A total of 14 batches were stored at 40°C and 100% RH for three months and analyzed for iodine content at the end of this time period. Figures 3 and 4 depict the iodine retention of each of the batches after 3 months of storage.

With 40% encapsulant, soy stearine, palmitic acid, and polymethyl methacrylate encapsulated batches showed exceptionally good iodine retention. The 40% encapsulation level was chosen as an upper limit, beyond which further encapsulant addition was impractical, and encapsulant that did not work at this level were not pursued furthere. Following successful identification of three possible encapsulants, soy stearine and was chosen for further optimization studies due to its low cost, availability and acceptability in food regulations.

A factorial experimental design was followed in investigating combinations involving two iodine compounds (KI and KIO_3) and one iron compound (ferrous fumarate) as active nutrients and soy stearine as an encapsulant. We studied soy stearine encapsulation at 0,5%, 10%, 20% and 30% on KI and KIO_3 and at 15% and 30% on ferrous fumarate.

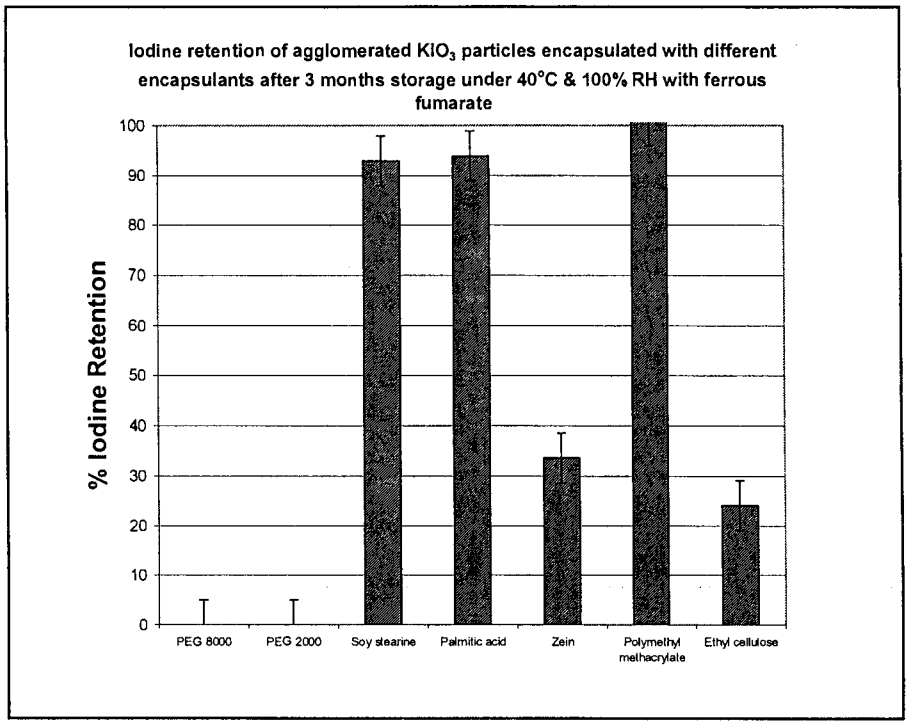

Figure 3 Iodine retention of double fortified batches with KIO_3 premix encapsulated by different encapsulants with unencapsulated ferrous fumarate

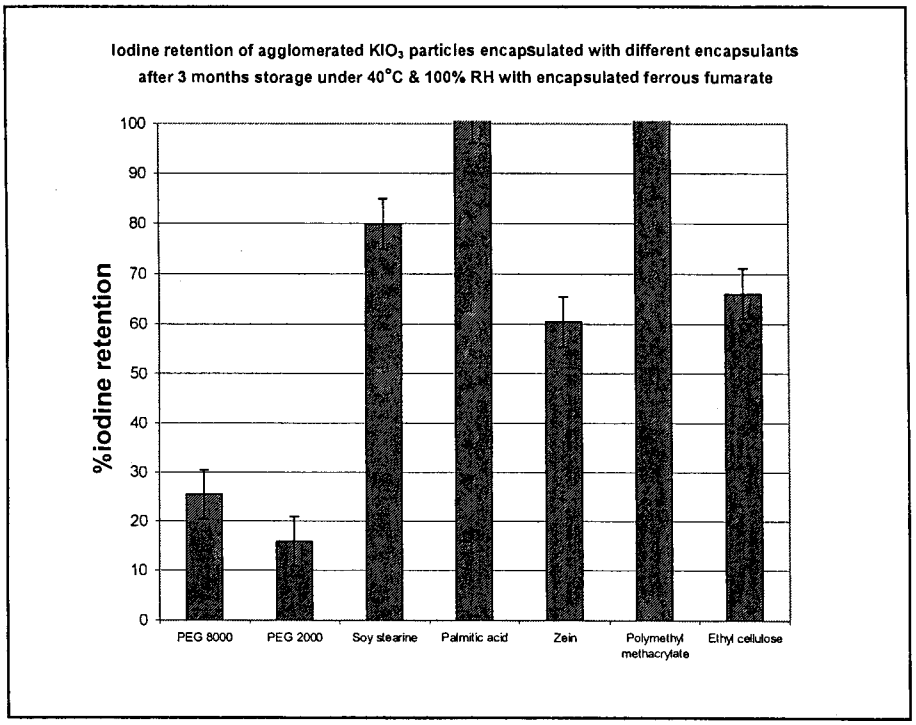

Figure 4 Iodine retention of double fortified batches with KIO_3 premix encapsulated with different encapsulants with encapsulated ferrous fumarate

3.4. *Effect of encapsulation on iodine retention, iron conversion and color*

Potassium iodate and potassium iodide are the most widely used compounds for salt iodization. Potassium iodate is more favored in developing countries and is recommended for salts with higher moisture contents. Potassium iodate however, can be easily reduced in the presence of ferrous salts to free iodine. For optimizing the formulation, both iodine compounds were investigated. You just said thisin the previous section!

3.4.1. Potassium iodide and ferrous fumarate

The iodine retention in formulations containing potassium iodide was generally higher than those containing iodate. The encapsulation level on potassium iodide premix or iron premix did not have a significant effect on iodine stability; the retention of iodine was more than 90% in all combinations. The level of coating did have a significant effect on iron conversion and the color of the salt. The amount of ferrous iron converted to ferric iron dropped from about 35% to 5% as the level of encapsulation on the iodine premix was increased from 0% to 30%. At the higher coating levels the color of the salt remained completely white.

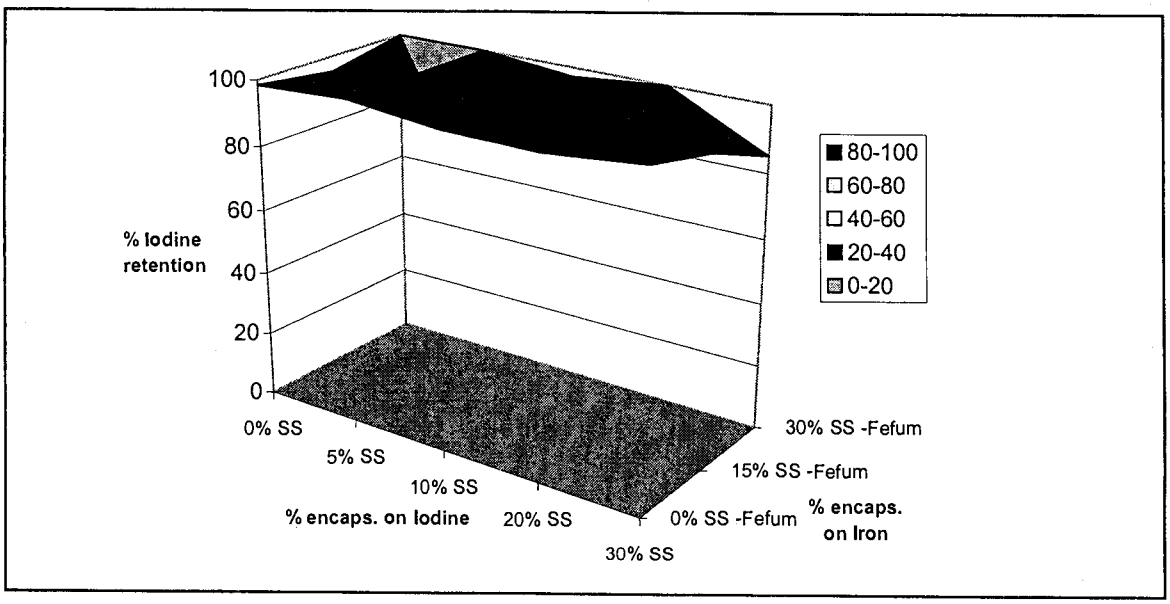

Figure 5 Retention of iodine in batches double fortified with soy stearine encapsulated KI premix and soy stearine encapsulated ferrous fumarate premix after 3 months of storage at 40°C and 100%RH.

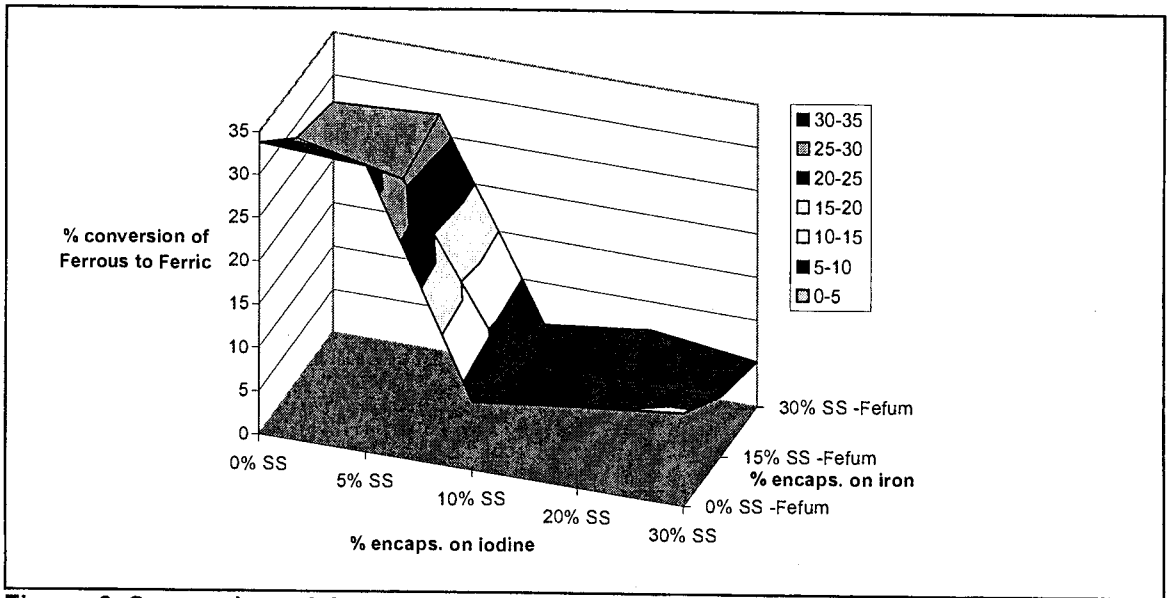

Figure 6 Conversion of ferrous iron to ferric iron in batches double fortified with soy stearine encapsulated KI premix and soy stearine encapsulated ferrous fumarate premix after 3 months of storage at 40°C and 100%RH.

Iron conversion generally correlated with iodine retention and color. Greater iron conversion was noticed in batches with lower iodine retention. The iron conversion also corresponded directly with the color of the salt. Batches which turned blue or green had higher conversion of ferrous iron to ferric iron, thus decreasing its bioavailability. Visual observations of color could therefore be used to qualitatively assess iron conversion.

3.4.2 Potassium iodate and ferrous fumarate

For batches containing potassium iodate, the level of soy stearine coating on the iron premix had a very significant impact on iodine retention. The iodine retention when the ferrous fumarate was not coated was close to 0% and when the ferrous fumarate was coated with 30% soy stearine the iodine retention was around 80%.

This suggests that there is significant leakage of iron from the microparticles. The ferrous iron, if not adequately contained, leaches out and comes into contact with the iodate ion resulting in a reaction which leads to reduction of iodate to iodide and oxidation of ferrous to ferric ion.

The level of soy stearine coating on the potassium iodate did not have a very significant effect on iodine retention. This was expected, as the salt was highly purified, and potassium iodate is stable to heat and light in the absence of impurities.

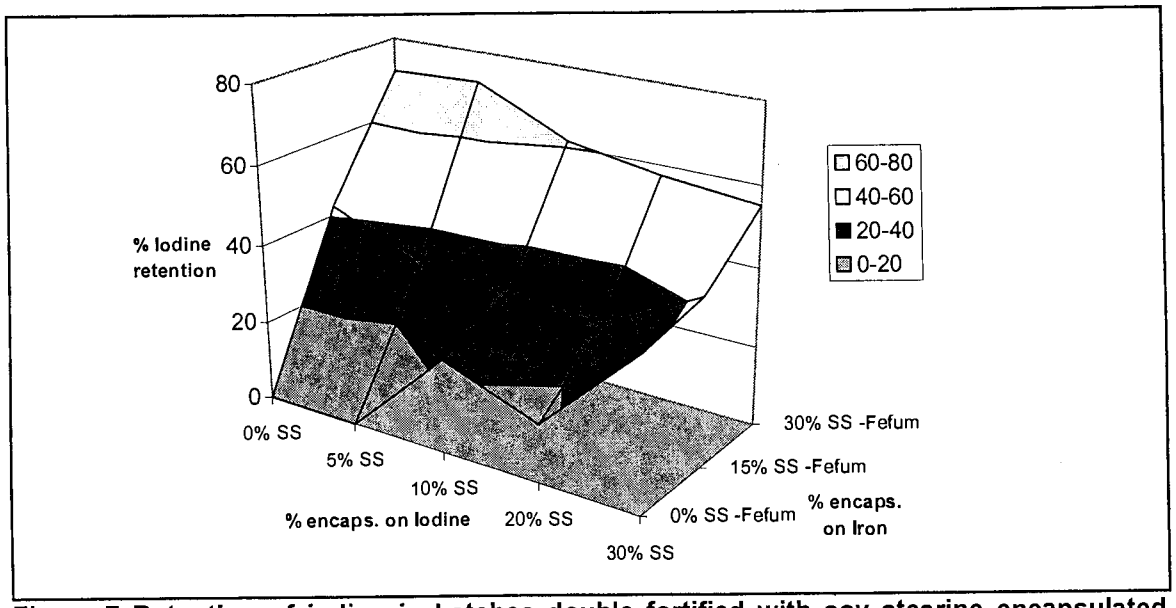

Figure 7 Retention of iodine in batches double fortified with soy stearine encapsulated KIO_3 premix and soy stearine encapsulated ferrous fumarate premix after 3 months of storage at 40°C and 100%RH.

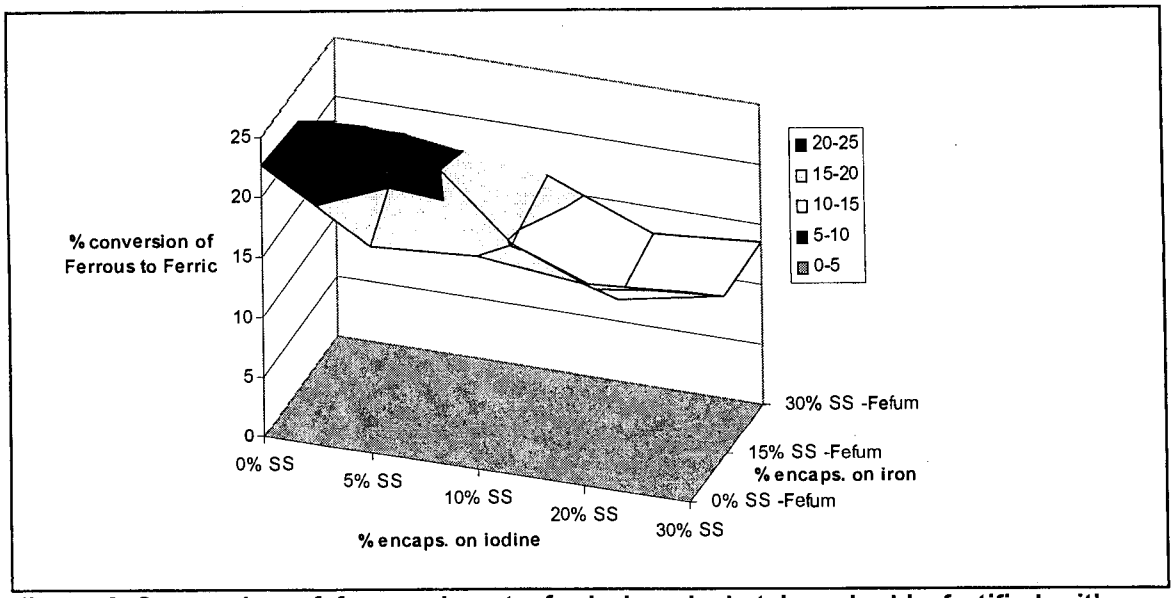

Figure 8 Conversion of ferrous iron to ferric iron in batches double fortified with soy stearine encapsulated KIO_3 premix and soy stearine encapsulated ferrous fumarate premix after 3 months of storage at 40°C and 100%RH.

Protecting the iron compound is therefore necessary in systems where potassium iodate and ferrous fumarate are used. When Potassium iodide is used with ferrous fumarate the level of protection for iron is not critical since both iodide and ferrous are in their reduced state.

4. Conclusions

The highest prevalence of iodine and iron deficiency is found in developing countries. In majority of these countries, the salt that is available is not refined and contains many impurities, including iron compounds. The presence of these impurities and the fact that the climate in most developing countries is warm and humid presents a challenge for double fortification of salt.

We have shown that microencapsulation can be used to protect the micronutrients from losses due to unwanted reactions. Encapsulation provides a physical barrier that prevents contact between the active nutrients and the surroundings and hence increases stability. Microencapsulation through spray drying was successfully applied by Diosady et al. but it had its limitation is terms of particle size and cost. We have demonstrated than agglomeration followed by encapsulation by using a rotating pan is possible. Successful initial scale-up trials using a larger rotating pan were done at Weetabix Canada Limited in Cobourg, ON, Canada.

This simple industrial process can cost-effectively produce premix for double fortified salt. Further research and development aimed towards better encapsulating agents and making the reddish-brown color of the ferrous fumarate premix more cosmetically appealing is desired. We are currently pursuing different processes and formulations aimed at improving the functional characteristics of double fortified salt.

5. Triple Fortification

5.1 Vitamin A Physical and Chemical Properties

Vitamin A is a fat-soluble vitamin that is essential for the health of human beings. It comprises of a family of molecules containing a 20 carbon structure with a methyl substituted cyclohexenyl ring and a tetraene side chain with a hydroxyl group (retinol), aldehyde group (retinal), carboxylic acid group (retinoic acid), or ester group (retinyl ester) at carbon 15.

The term vitamin A also includes provitamin A carotenoids that are dietary precursors of retinol. The term retinoids refers to retinol, its metabolites and synthetic analogues that have a similar structure.

Preformed vitamin A is a yellow crystalline powder or oil. Carotenoids are reddish-brown to deep violet crystalline powders. Preformed vitamin A is found only in animal-derived food products (e.g. liver, dairy products, eggs and fish), whereas dietary carotenoids are present primarily in oils, fruits and vegetables.

Vitamin A is extremely sensitive to oxygen (undergoes oxidation), light (promotes *cis-trans* isomerization), heat (catalyzes *trans* to *cis* isomerization), halogens (forms mixture of *cis-trans* isomers, particularly in the presence of light and high temperature), and is unstable in acidic environment (undergoes rearrangements of the double bonds and dehydrates).

In all fat-soluble vitamins, esters are significantly more stable than alcohols as the free hydroxyl group of their alcohol forms is highly sensitive to oxidation.

5.2 Iron

Iron can exist in oxidation states ranging from -2 to +6. In biological systems, these oxidation states occur primarily as ferrous (+2), ferric (+3), and ferryl (+4) states. The following physical-chemical properties should be taken into consideration when choosing the source of iron fortificant:

- Ferrous salts can be utilized/bioabsorbed more efficiently than ferric salts
- Ferrous salts are more soluble than ferric salts
- Ferrous salts are more reactive than ferric salts in food systems
- Ferric iron generally has a greater tendency to form complexes than ferrous iron; the formation of complexes will greatly reduce iron bioavailability

Ferrous salts are easily oxidized to ferric salts, which are usually orange or dark brown in colour.

Fe^{2+} (green) $\longrightarrow$ Fe^{3+} (orange/red) + e^-

This reaction is promoted in alkaline and high humidity conditions, in the presence of oxidizing agents and common salt impurities such as magnesium chloride or sulphate. Salt containing oxidized iron, especially ferric sulphate, exhibits metallic taste and discoloration. [10]

Electrolytic iron usually contains more than 96% iron. It is almost insoluble in water and has lower bioavailability than soluble iron salts. Other disadvantages are the dark grey colour and high density which could lead to organoleptic problems and segregation.

Ferrous fumarate is a reddish orange to reddish brown powder that is odourless and almost tasteless. As for ferrous sulphate it is only slightly soluble in water. Ferrous sulphate is the cheapest and most widely used iron source in food fortification. It is used in two forms: heptahydrate and anhydrous ferrous sulphate. The anhydrous ferrous sulphate is grayish white in colour; it has a metallic and astringent taste. In contact with water it hydrates to form the light green heptahydrate, which is sparingly soluble.

Ferric sodium EDTA is a pale yellow water-soluble powder with a high stability constant. [11]

Many researchers indicate that while iron absorption from simple iron salts such as ferrous sulphate is very sensitive to inhibiting substances in food, the absorption from ferric NaEDTA is apparently not. [12, 13] While both ferric iron and iron complexes are typically poorly available, the literature seems consistent in considering ferric EDTA to be bioavailable.

5.3 Iodine

Iodine is a dark purple crystalline non-metallic element, which is insoluble in water, but soluble in many organic solvents. In human beings it is a constituent of the thyroid hormones, thyroxine T_3 and T_4 produced by thyroid gland.

Two compounds of iodine are currently used for iodization: potassium iodate and iodide. The Codex Alimentarius standard for food grade salt permits the use of their sodium and potassium salts in the iodization program.

Iodides are readily oxidized when in contact with oxygen in a high humidity environment resulting in the formation and sublimation of free iodine. This process is accelerated in the presence of acidic salt impurities, high temperature and sunlight. [10]

Oxidation: $2I^- \rightarrow I_2 + 2e^-$

Iodates are already oxidized thus they are more stable in tropical and subtropical climates. However, presence of salt impurities also promotes reduction of iodate to form free iodine. [10]

Reduction: $2I^{5+} + 10\ e^- \rightarrow I_2$

A major consideration in the choice of the two compounds is the purity of the salt. The iodides are more readily degraded in the presence of impurities and high humidity environment, whereas the iodates remain stable in salt of lower quality.

Potassium iodate (KIO_3) and potassium iodide (KI) were used for this study. Potassium iodide is highly soluble in water (1440 g/L at 20°C) and the iodine content is 75%. Most of the salt used in the affected areas is unrefined, thus the more stable KIO_3 is commonly used there for iodization. Solubility of KIO_3 in water is very low (81.3 g/L at 20°C) and its iodine content is 59.5%. [15]

5.4 Recommended daily intakes, and target fortification levels

In 1965, a Joint FAO/WHO Expert Group set a recommended daily intake of retinol as **750 µg RE/day**. Vitamin A is a fat-soluble vitamin and can be therefore stored by human bodies.

In general, iron requirements for adult males amount to **0.7 – 1.2 mg/day** (about 14 µg/kg). Requirements for adult females are between **1.2 – 2.5 mg/day** (20 to 40 µg/kg). The greater range is due to the variability of menstrual blood loss.

The Recommended Daily Intake (RDI) for adult humans is **100 - 200 µg/day** and the tolerable Upper Intake Level for adults is 1,100 µg/day. [16]

The iodized salt is becoming the most common source of iodine, with about 70% of the salt consumed being now iodized. The level of fortification that has been used ranges from 15-200 ppm. Numerous regional factors have to be considered when designing iodized salt, including per capita salt consumption, degree of iodine deficiency, required shelf life, and climatic conditions.

Accordingly our TFS fortifications were set at 50 µg/g iodine, 1000 µg/g iron and 150µg/g retinol

5.5 Vitamin A and Iron Interactions

A direct correlation between haemoglobin and serum retinol concentration has been observed. Intervention studies among Indonesian girls have demonstrated that

combining vitamin A with iron supplementation was more effective in increasing haemoglobin concentrations than iron alone. Various studies suggest that VAD impairs iron mobilization from body stores and therefore vitamin A supplementation improves haemoglobin concentrations. [21, 22, 23]

However, ferrous salts are easily oxidized by iodates resulting in formation and sublimation of free iodine.

$$IO_3^- + 6Fe^{2+} + 6H^+ \rightarrow I^- + 6Fe^{3+} + 3H_2O$$

$$IO_3^- + I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

Ferric salts can be reduced by iodides:

$$2I^- + 6Fe^{3+} \rightarrow I_2 + 2Fe^{2+}$$

The rate of these reactions depends mainly on the nature of the food and the environment. The reactions are accelerated in the high humidity environment.

5.6 Designing Triple Fortified Salt

The basic idea behind the design of a premix is to physically separate the active ingredients from each other or a harmful environment. This has been done with iron and iodine in the preparation of double fortified salt (DFS). The DFS prepared by the food engineering group of the University of Toronto was stable for 12 months at 40°C 100% RH. [33]

The premix microcapsule consists of small particles that contain an active nutrient and binding and, if needed, diluting agents surrounded by a moisture resistant coating or shell composed of materials such as polymers, carbohydrates, fats and waxes. Microencapsulation is a useful tool for protecting the integrity of pharmaceuticals and food ingredients such as vitamins, salts or flavours from oxygen, water and light.

Figure 8 is a simplified drawing of an ideal premix particle, containing a micronutrient, binders, fillers and a layer of coating.

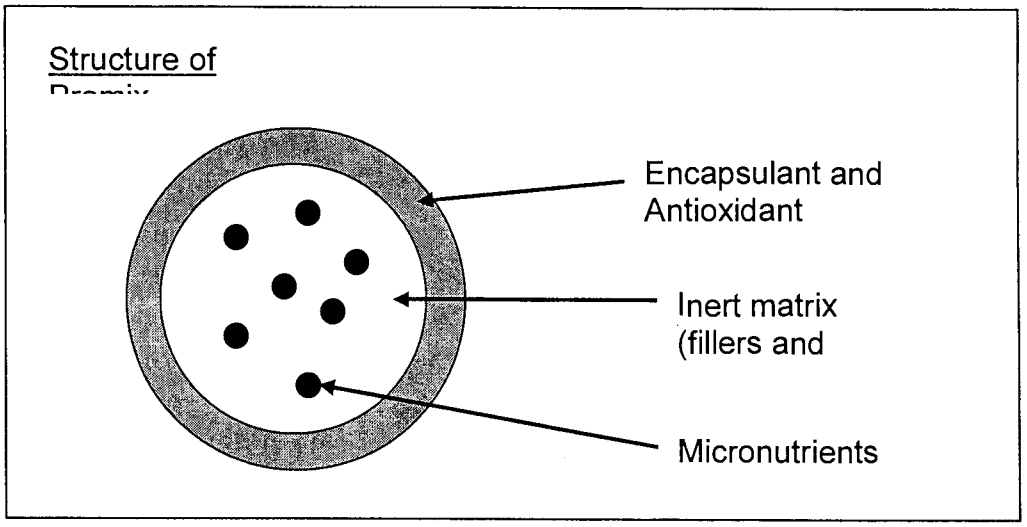

Figure 7: A simplified cross sectional structure of a premix.

The premix must be:

- Non-toxic and all constituents must be approved food additives in all targeted countries
- Physically and chemically stable under the targeted environmental conditions
- Bioavailable
- Uniform, with the same particle size as salt, to avoid segregation
- Sufficiently hard and tensile to avoid breaking during mixing with salt, storage and distribution
- Inexpensive
- be white/off white and tasteless so that the sensory properties of salt are not changed.

Iron compounds used for fortification can be divided into two major groups: water soluble compounds and insoluble compounds. Ferrous sulphate, ferrous fumarate, ferrous ammonium sulphate, ferric ammonium sulphate, ferric ammonium citrate and ferric EDTA are examples of soluble compounds which were previously used for salt fortification. Some insoluble compounds are ferric phosphate, sodium iron pyrophosphate and reduced iron. [11]

A list of common iron compounds that have been previously used by our group in salt fortification, their bioavailability and relative cost is summarized in table 2.3 [10]. The relative costs were calculated on the basis of the iron compound, cost and bioavailability of each compound.

Table 5.1: Iron content and the bioavailability of some iron compounds previously used for salt fortification. [10]

	Iron Compound	% Fe Content	Relative Bioavailability Factor	Relative Cost Factor
	Electrolytic iron	98	0.13-0.90	
	Ferrous Fumarate	33	1.00	1.00
	Ferrous Sulphate x $7H_2O$	20	~1.00	1.67
	Ferric NaEDTA	13	2.50	2.57

The main problem in selecting an iron compound for fortification is that the ones with good relative bioavailability are more likely to be unstable under storage conditions, cause discoloration and changes in flavour or odour. Conversely, the lower the relative bioavailability, the more iron needs to be given to achieve the required effect, thus increasing cost and the probability of inducing undesirable side effects. [11, 53]

5.7 Granulation Technology

Granulation is a process by which a powder mixture is converted to larger free flowing particles called granules. The reasons for granulation are:

- To obtain sufficiently large particles that will not segregate from the salt
- To obtain the desired granule hardness and roughness
- To obtain the desired density

Granulation normally follows initial dry mixing of the necessary powdered ingredients so that a uniform distribution of each ingredient through the premix is achieved. For economic and technical reason premix should be mixed with salt in ~1:100 ratio. Refined salt crystals are between 300 μm and 800 μm in diameter and the size of the premix needs to match the size of salt to avoid segregation.

Excipients are materials used in the manufacturing or the compounding process for the purposes of maintaining the appearance (colour and consistency), protecting the active species (antioxidants), disguising any unpleasant taste/smell (sugar, flavouring agents), changing the dissolution rates of active species (coating, matrix) and manufacturing (fillers/diluents, lubricants and glidants).

Fillers and binders do not have pharmacological activity themselves and are used to dilute the micronutrients and to bind them to form granules of the desired size. The binder has to allow the micronutrient absorption after premix ingestion.

The function of a filler is to increase the bulk volume of the premix so that the final product has the proper volume. The filler has to be inert, compatible, non-hydroscopic, soluble, compactable and inexpensive. The most common fillers are: lactose, sucrose, glucose, mannitol, sorbitol, calcium phosphate, calcium carbonate, and cellulose. Lactose is the most widely used filler in the food industry. It offers good stability and fair protection for micronutrients sensitive to moisture.

The function of a binder is to ensure that granules can be formed with required mechanical strength. Binders may be added to the mixture prior to the granulation process as a dry powder, or in the solution that is used for agglomeration in wet granulation processes

The most common binders are:

- Wet/Solution binders: acacia, gelatin, cellulose, cellulose derivatives, polyvinyl pyrrolidone, starch, sucrose, polyethylene glycol.

- Dry binders: cellulose, methyl cellulose, polyvinyl pyrrolidone, polyethylene glycol

- Some compounds, e.g. sodium hexametaphosphate (SHMP) can be used as both a binder and a stabilizer.

Starch, a complex carbohydrate, $(C_6H_{10}O_5)_x$, is abundant in the seeds of cereal plants. It is an inexpensive dilutent/binding agent widely used by commercial vitamin A producers. The disadvantage of starch is that it has a tendency to absorb moisture.

Industrial dextrin is granular starch with lower molecular weight. Its molecules are reorganized by roasting or by chemical modifications, causing the granules to be cold water-soluble. Depending on the degree of roasting, dextrins are categorized as white and yellow dextrins and British gum.

Both, starch and yellow dextrin, were used in the vitamin A, iron and iodine premix preparations.

Three granulation techniques were used in this program:

Wet granulation – wet mixing of a powder mixture with liquid adhesives followed by wet screening or granulation. The granulation liquid (usually water) may be used alone or, more usually, as a solvent containing a dissolved adhesive (also referred to as a binder or binding agent) that is used to ensure particle adhesion once the granule is dry.

Fluidized bed granulation – is a single step process of mixing the powders and developing granules. This process requires only one piece of machinery that mixes the powder and granules on a bed of air. It is becoming a commonly used method because of the high quality of its products, convenience and the availability of suitable equipment.

Spray drying - is the most widely used industrial process involving particle formation and drying. It is highly suited for the continuous production of dry solids in either powder, granulate or agglomerate form from solutions, emulsions or pumpable suspensions.

Table 2.4 shows the advantages and disadvantages of the methods used for agglomeration.

Table 5.2: The advantages and disadvantages of the methods used for agglomeration. [34]

Process	Advantages	Disadvantages
Wet Granulation	Most reliable Highest probability of meeting the requirements for making a successful particle	Need to prepare a uniform granulation Labor intensive Time consuming Cannot be used for water sensitive nutrients
Dry Granulation	Useful for water and heat sensitive active ingredients Fewer steps	Requires excipients with cohesive properties
Direct Compress	Can prepare a premix from powdered mixture without granulation Faster	Requires materials that can be directly compressed Excipients must have good flow properties

Process	Advantages	Disadvantages
	Useful for crystalline premixes Useful for ingredients that are moisture sensitive Limited number of excipients - optimal bioavailability	Can get non-uniform granules Limited size range of particles
Fluidized Bed Granulation	Requires only one piece of machinery (saves labor cost, transfer losses and time) Process can be automated after initial process optimization	Expensive equipment The optimization of process requires extensive research (there are many apparatus, process and product variables which influence the granulation)

The method and conditions of granulation affect intergranular and intragranular pore structure by changing the degree of packing within the granules. A simple bonding holds precompressed granules, consisting of compressed micronutrients and binder particles, together during compaction. Granules prepared by wet massing consist of intact active ingredients held together in a sponge-like matrix of binder. Fluidized-bed granules are similar to those prepared by the wet massing process, but possess greater porosity and a film of binding agent covers the granule surface. With spray-dried systems the granules consist of spherical particles composed of an outer shell and an inner core of particles. [34]

Common equipment used in the industry for granulation is listed in table 2.5.

Table 5.3: Common equipment used for granulation.

Wet Granulation	Dry Granulation
Shear granulators	Sluggers
High speed/ mixer granulators	Roller compactors
Fluidized bed granulators	
Spray-driers	
Spheronizers/ pelletizers	
Extrusion / Spheronizes	
Tumblers	

Tumblers

The particles of a granular mass will cohere when they are tumbled and sprayed lightly with a liquid binder. The growth may be due to agglomeration of small particles or layering of material evaporated from the sprayed solution. Rotary kilns of the kind used for drying or chemical reaction (cement or lime burning, for instance) are adapted to size enlarging service. Usually the tumbling action is not very intense, only enough to expose the material to sprays. The sprays are fine and are applied to the surface of the bed of particles. The tumbling action then distributes the liquid uniformly through the mass. [36]

Spray Dryers

Spray drying involves the atomization of a liquid feedstock into a spray of droplets and contacting the droplets with hot air in a drying chamber. The sprays are produced by either rotary or nozzle atomizers. Evaporation of moisture from the droplets and formation of dry particles proceeds under controlled temperature and airflow conditions. Powder is discharged continuously from the drying chamber. Operating conditions and dryer design are selected according to the drying characteristics of the product and powder specifications.

Pan Granulators

The pan granulator consists of a tilted pan (inclined 30° to 60°) mounted on motor drive unit. The powder mixture is fed into the pan and the gravitational and centrifugal forces force the particles to move within the pan. Standard spray nozzle is employed to deliver small quantities of binding solution to create a basic adhesion between the particles. The granulation is done by the snowballing effect of the rotating disc. The rotation speed and angle depend on the size of the mixture. Too high speed

will force the mass to the pan rim due to centrifugal forces; with too slow rotation sliding with no mixing will occur.

The required disc-rotation speed is given in terms of the critical speed, i.e., the speed at which a single particle is held stationary on the rim of the disc due to centripetal forces. The critical speed N_c is given by:

$$N_c = [(g \sin \varphi)/ (2\pi^2 D)]^{1/2}$$

Where g is the gravitational acceleration, φ is the angle of the disc to the horizontal, and D is the disc diameter. The typical operating range for discs is 50 to 75% of critical speed, with angles φ of 45–55°. [37]

Figure 8 shows a diagram of rotating pan used for both agglomeration and pan coating.

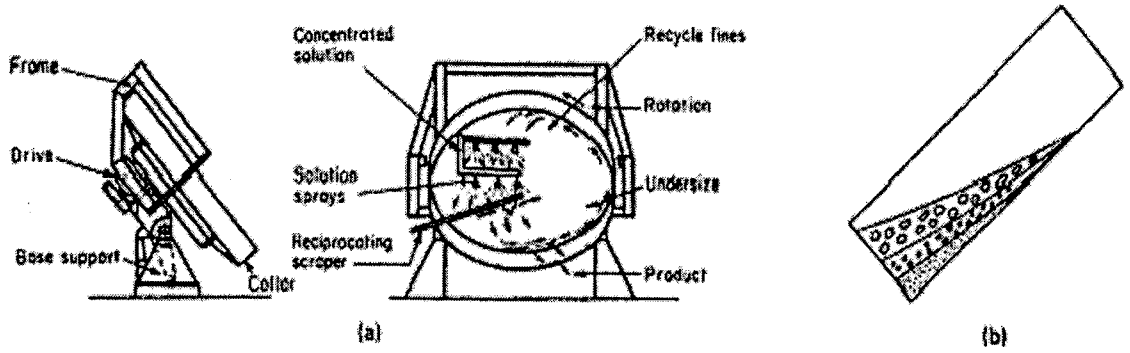

Figure 8: (a) Edge and face view of a pan granulator; (b) Stratification of particle size during rotation [36]

During agglomeration, the finer particles settle to the bottom and the largest remain at the top. Because of the size stratification, the product of disk granulation is more uniform in size than of drum granulators, which discharge a mixed product.

Fluidized Bed or Würster Granulators

The fluidized bed granulation process (also known as agglomeration) involves suspending particulates in an air stream and spraying a liquid from the top down onto the fluidized bed (figure 2.6). Particles in the path become tacky, collide with other particles and adhere to them to form granules.

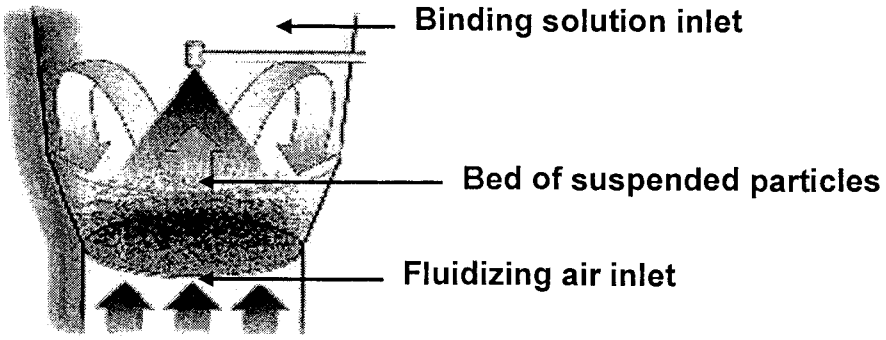

Figure 9. Fluidized Bed [38]

There are two different modes of fluidized bed granulating: dry stage and wet stage. In dry stage granulation, the particles only require a slight wetting to become tacky and stick to each other. The granulating solution is applied at a rate less than or equal to the evaporation rate. Thus the particles remain "dry" through the entire process. In wet stage granulation, the particles require significant wetting before they become tacky enough to stick to each other. The granulating solution is applied at a rate higher than the evaporation rate until the particles build up enough moisture to granulate. The characteristics of the particles when wet and the type of granulating solution that is being used will determine which mode of granulating is most appropriate. While dry stage is more common, wet stage granulating allows for denser products. [36, 39]

5.8 Microencapsulation Technology

Microencapsulation is done in order to protect the micronutrients against deterioration by environmental factors such as sunlight, temperature variations, moisture etc., to maintain physical and chemical integrity of premix and to enhance product acceptance and appearance.

The choice of the coating material often depends on the purpose of microencapsulation and it is often crucial to achieving these goals. There are over a hundred substances suited (and already used) to form microcapsule films.

They can be put into roughly five categories as follows: [40]

- Polysaccharides/hydrocolloids, such as starch, algin/alginate, agar/agarose, pectin/polypectate, carrageenan, and other gums.
- Proteins such as gelatin, casein, zein, soy, and albumin.
- Fats and fatty acids such as mono-, di- and triglycerides, and lauric, capric, palmitic and stearic acid and their salts.

- Cellulosic derivatives such as methyl- and ethyl-cellulose.
- Hydrophilic and lipophilic waxes such as shellac, PEG (polyethylene glycol), or carnauba wax or beeswax.

In several cases, mixtures of these compounds can be used to obtain better results.

Methocel, ethocel, shellac and soy stearine are commonly used coating agents which werealso used for the premix preparations.

Methocel (methyl-cellulose, MC) is soluble in cold water, GI fluids and organic solvents.

Ethocel (ethyl-cellulose, EC) cannot be used alone because it is totally insoluble in water and GI fluids. Its main purpose is to toughen the film. EC is soluble in ethanol and other organic solvents.

Shellac is pH dependent, soluble in water (at pH > 7) and some organic solvents (isopropanol, benzalcohol) and insoluble in GI fluids.

Soy stearine is fully hydrogenated soy oil with melting point ~ 60°C. It is insoluble in water, soluble in organic solvents such as hexane, ether and dichloromethane.

The coating of solid particles can be done in a rotating pan and a fluidized bed.

6. Experimental Techniques and Analytical Methods

6.1 Materials Used

All chemicals used, their suppliers and grades are listed in table 6.1.

Table 6.1: List of materials used

Material	Producer	Grade
1,10 Phenantroline	Sigma Chemical Co., St. Louis, USA	Laboratory
Butylated hydroxyanisole, BHA	Sigma Chemical Co., St. Louis, USA	Food
Butylated hydroxytoluene, BHT	Sigma Chemical Co., St. Louis, USA	Food
z	Casco, Toronto, ON	Food

Material	Producer	Grade
Dichloromethane	EM Sciences, Gibbstown, NJ	Laboratory
Electrolytic iron, reduced	EM Sciences, Gibbstown, NJ	ACS
Ethanol, 95%		Commercial
Ethocell	DOW Chemical Co., Midland, Michigan	Drug
Ferric NaEDTA	Aldrich Chemical Co Inc., Milwaukee, USA	ACS
Ferrous Fumarate	Sigma Chemical Co., St. Louis, USA	ACS
Ferrous Sulphate	Sigma Chemical Co., St. Louis, USA	ACS
Hexane	EM Sciences, Gibbstown, NJ	Commercial, b.p. 60 - 68°C
Hydrochloric acid	BDH Inc., Toronto, ON	Laboratory
Isopropanol	Aldrich Chemical Co Inc., Milwaukee, USA	Spectral
Methocell	DOW Chemical Co., Midland, Michigan	Drug
Potassium Biphtalate	Sigma Chemical Co., St. Louis, USA	Laboratory
Potassium Hydroxide	Aldrich Chemical Co Inc., Milwaukee, USA	Laboratory
Potassium Iodate	Aldrich Chemical Co Inc., Milwaukee, USA	ACS
Potassium Iodide	Aldrich Chemical Co Inc., Milwaukee, USA	ACS
Pyrogallic Acid	Aldrich Chemical Co Inc., Milwaukee, USA	ACS
Salt, iodized	Sifto Canada Inc, Mississauga, ON	Food
Salt, non-iodized	Cargill Food Inc., Mineapolis, USA	Top-flo®

Material	Producer	Grade
		uniodized salt
Shellac	A.S. Paterson Co. Ltd, Toronto, ON	Drug
Sodium Tiosulphate	BDH Inc., Toronto, ON	ACS
Soy Stearine	Casco, Toronto, ON	Fully hydrogenated soy oil
Starch, white	Casco, Toronto, ON	Food
Sulphuric acid	BDH Inc., Toronto, ON	Laboratory
Tert-Butyl Hydroquinone, TBHQ	Sigma Chemical Co., St. Louis, USA	Food
Titanium Dioxide	EM Sciences, Gibbstown, NJ	Food
Vitamin A acetate	BASF Corporation, New Jersey, USA	Food
Vitamin A palmitate	BASF Corporation, New Jersey, USA	Food
Vitamin A palmitate	Watson Food Co., Inc., West Haven, USA	Food
Vitamin A palmitate	Roche Vitamins Inc., Parsippany, USA	Food
Vitamin A, USP standard	USP Rockville MD, USA	Analytical

For vitamin A premix, nine commercial vitamin A forms in two different forms (palmitate and acetate) were tested. Six of them are produced by BASF Corporation, two by Roche Vitamins Inc. and one by Watson Foods Co., Inc. The vitamin A forms differ in the composition of their matrix, bioactivity and the type of antioxidant they are stabilized with. Detailed description can be found in table 3.2.

Table 6.2: Commercial vitamin A forms tested

Vitamin A	Bioactivity [I.U./g]	Matrix	Product Number

Vitamin A	Bioactivity [I.U./g]	Matrix	Product Number
BASF Dry VAP 250 Food	250 000	E 414, sucrose, starch, E 307, E 341	Prod:303081 Lot:200719YO & 483111UO
BASF Dry VAP 250 CK CWD	250 000	E 414, sucrose, starch, BHT, E 301, E 341	Prod:311740 Lot:739795TO
BASF Dry VA Acetate 325 GFP	325 000	E 414, sucrose, starch, E 307, E 341	Prod:30569401 Lot:029119YO
BASF Dry VAP 250 CWD	250 000	gelatin, sucrose, starch, BHT, E 554	Prod:306325 Lot:11344182
BASF Dry VAP 500	500 000	gelatin, sucrose, modified starch, BHT, E554	Prod:30306901 Lot:183027TO
BASF Dry VA Acetate 500	500 000	gelatin, sucrose, modified starch, BHT, E 554	Prod:303064 Lot:20830174
Roche Dry VAP 500	500 000	corn starch, gelatin, sucrose, BHT,	Prod: 0483834 004 Lot:WB00104197
Roche Dry VAP 250 S/N	250 000	modified starch, sucrose, fractionated coconut oil, E 301, sodium benzoate, sorbic acid, silicon dioxide	Prod:50 00718004 Lot:WB11104541
Watson VAP 250 Powder	250 000	modified starch, coconut oil, BHT, silica flour	Prod:F240114 Lot:01542

Note: E 301-sodium ascorbate (antioxidant), E 307-DL-alpha-tocopherol (USP/FCC, antioxidant), E 341- tricalcium phosphate (anti-caking agents), E 414 - gum Arabic, E554- sodium aluminium silicate (anti-caking agents)

Products 1, 2, 3 and 4 are dispersible even in cold water (10°C) and form a stable milky emulsion. [44]. Products 5 and 6 are dispersible in warm water (35-40°C) and form a milky emulsion. [44]. Roche Dry VAP 500 is insoluble in water. [45]

Roche Dry VAP 250 S/N disperses quickly and completely in cold water. High concentrations produce cloudy dispersions, which, however, remain uniform for relatively long periods. [46]

Vitamin A palmitate from Watson dissolves in cold water resulting in a cloudy dispersion. [57]

Four forms of iron used in this study were electrolytic iron, ferrous fumarate, ferrous sulphate and ferric NaEDTA. These sources were chosen based on their positive stability results in double and triple fortified salt, previously studied by our group.

Potassium iodate and potassium iodide are most commonly used for salt iodization and were also used in this study.

White starch and yellow dextrin were used as binders to granulate the micronutrients.

The ability of three hydrophilic (Methocel, Ethocel and Shellac) and one hydrophobic (soy stearine) coating agents to protect the active ingredients from the high humidity environment was also studied.

BHA, BHT and TBHQ are fat soluble antioxidants which were used to stabilize the vitamin A premix.

6.2 Equipment

Two stainless steel rotating pans were used for both wet agglomeration and spray coating. The pans were 26 cm and 13 cm in diameters and 6 cm deep. A 250 mL hand sprayer bottle from VWR was used as an atomizer to spray the binding and coating solutions.

Blending of both, solid powders prior to granulation and salt with premixes, was done in a glass or plastic jar by shaking for 10 minutes on a shaking apparatus (Burrell wrist action shaker model #75, Burrell Corp., Pittsburg, PA, USA). Grinding was done using a Braun coffee grinder.

UV-Vis spectroscopy was done with a UV-Vis Spectrophotometer, Beckman Coutler, model DU-7U. The fluorescent emission was measured with a Perkin Elmer Luminiscence Spectrophotometer, model LS 50B.

6.3 Experimental Procedures

6.3.1 Pan Agglomeration

Wet agglomeration was used to granulate the premix. Steps involved in granulation were

- blending
- adding liquid
- granulating

- drying
- screening

Well blended material consisting of fillers and active ingredients was placed in a rotating pan. The pan was angled at approximately 45 degrees and rotated at about 50 rpm. The speed depended on the size of the pan used and the amount of material granulated. A hand sprayer was used to spray the binding solution, which was usually water or water- ethanol solution. Once the material in the pan was free flowing and the size of the particles increased sufficiently, the material was dried in a Blue M oven from Blue M Electric Company, Blue Island, IL, model number OV-490A-3 at 60°C. After drying, the material was sieved using the Ro-Tap sieve shaker. Two Tyler standard US Mesh sieves with sizes 300 μm and 710 μm were used. Particles falling into this range were used in the coating process.

6.3.2 Microencapsulation

The microencapsulation was also done in the pan agglomerator. The granulated and screened particles were placed into the rotating pan and the angle and rotation speed were set to approximately the same values as for the agglomeration. The granules were coated with coating agents dissolved in hot solvents (either ethanol-water solution or dichloromethane) using the hand sprayer. The air inlet was set at approximately 3 ~ 5 psig. The concentration of the coating solution was dependent on the coating agent used. In case of soy stearine, 20% w/v solution was needed. For other coating agents, 30-40% solutions were sufficient.

Dichloromethane evaporated practically immediately and no additional drying step was needed. When ethanol-water solution was used as solvent, an additional drying step was required (~15 min at 60°C). The level of encapsulation was determined by weighing the particles before and after each run.

6.4 Analytical Methods

6.4.1 The Sampling Method

The sampling technique relied on the concept of random sampling. A set of nine fractions was built from a bulk of sample so that each fraction contained all the characteristics, including particle size distribution, as the bulk from which they all came.

Each sample was built from these fractions by a process of multiple additions so that a statistically representative image of the bulk existed in each of the newly created sample fractions.

6.4.2 Vitamin A Analysis

A standard fluorometric method [47] was used for vitamin A analysis. This method is based on measurement of fluorescence of retinol and retinol esters in hexane after appropriate cleanup which separates the vitamin A from other lipids in the sample matrix. The hexane extract is excited by radiation at 330 nm and the fluorescent emission is measured at 480 nm. The fluorescence of retinol is a linear function of concentration from zero to 0.25 μg/mL. At higher concentrations self-absorption becomes significant. [48]

The method consists of saponification (also called alkaline hydrolysis), extraction and measurement of fluorescence.

During saponification, 70% potassium hydroxide solution and 1% ethanolic pyrogallol are used to free the vitamin A from fat and to convert the retinol esters into retinol (vitamin A alcohol). After saponification, most of the saponifiable material becomes soluble in polar solvents. Vitamin A can be then extracted with hexane.

The fluorescence was measured with a Perkin-Elmer Luminescence Spectrometer, Model LS 50B, PE Shelton, Connecticut. The details can be found in Appendix 9.1.1.

6.4.3 Iron Analysis

Spectrophotometric determination of iron developed by Harvey, Smart and Edwards (1955) was used [49]. When 1, 10-phenantroline is added to a solution containing both ferrous and ferric irons, a reddish orange ferrous complex and a yellow ferric complex form immediately. The iron (II) complex has an absorbance maximum at 512 nm. The two complexes have identical extinction coefficients at 396 nm. The absorbance was determined with a Beckman Coulter model DU-7U, UV Spectrometer. The details are presented in Appendix 9.1.2.

6.4.4 Iodine Analysis

6.4.4.1 Determination of Potassium Iodide

The iodine content was determined using epithermal neutron activation analysis (ENAA), using the slowpoke reactor at the Royal Military College in Kingston, Ontario.

1 to 2 g of sample was weighed into a polyethylene vial. The sample vials were then shielded in cadmium and irradiated at 1 kW for 3 minutes using a neutron flux of 5.0×10^{11} $cm^{-2}sec^{-1}$. The samples were rested for 6 minutes and the gamma emissions were measured at 443 keV using a hyperpure germanium based gamma ray spectrometer. The concentration of iodine was calculated from the calibration curve obtained using series of standards covering a range of 0 to 1000 ppm. [50]

6.4.4.2 Determination of Potassium Iodate

Iodate was determined by iodometric titration using AOAC standard method 33.149 [51]. This method is based on reduction of iodate to free iodine, which forms a deep blue color with starch. The free iodine is then titrated with standardized solution of sodium tiosulphate. The details are presented in Appendix 9.1.3.

6.4.5 Moisture Content Determination

Moisture content was determined gravimetrically. Approximately 20g samples (w_i) were dried at 105°C for 24 hours. After cooling down the samples were weighed (w_f). The weight difference is assumed to be the moisture content.

$$\text{Moisture [\%]} = (w_i - w_f) * 100\ \% / w_i$$

w_i – initial weight, before drying [g]

w_f – final weight [g]

6.4.6 Particle Size Distribution

The analysis was performed using the Ro-Tap sieve shaker (figure 3.1). A stack of three Tyler standard US Mesh sieves with sizes 300 μm, 500 μm and 710 μm was used. Approximately 50g samples were shaken for 10 – 15 minutes. The separated parts were weighed and the fraction (% by weight) of the particles accumulated between two neighboring sieves was determined.

Mean particle diameter retained by a screen is the sum of the aperture of the screen on which the material is retained, plus the aperture of the next largest screen, divided by 2. Mean particle diameter of a sample is the sum of the mass fractions retained on each screen multiplied by the mean diameter of particles retained by that screen.

6.5 Packaging, Storage Conditions and Sample Handling

Each sample formulation was prepared in **six** 150g **replicates**, packed in polyethylene "Ziploc" bags, 18 x 15 cm. The samples were stored under **two conditions** for **three months** (hence the replicates, table 3.3):

- high temperature, high humidity (40°C, 100% RH)
- high temperature, medium humidity (40°C, 60% RH)

The 40°C and 60% RH setting is a good estimate of the average conditions in tropical environment. A relative humidity of 100% was chosen as the extreme condition that TFS is expected to encounter during storage, transportation and distribution.

The high temperature and high humidity conditions were maintained using controlled temperature oven. The approximately 100% relative humidity was achieved by placing a tray of water into the oven thus obtaining approximately saturated air.

The high temperature and medium humidity conditions were maintained in an environmental chamber type ZH-8-1-H/AC, manufactured by Cincinati Sub-Zero Co., Cincinnati, OH, USA. The stability of the micronutrients was determined monthly.

7. Results and Discussions

Figure 4.1 shows a microscopic image of salt fortified with all three micronutrients (60 x magnification). The micronutrients were granulated with dextrin to match the size range of salt crystals and coated with soy stearine to protect them from a high temperature (40°C) and high humidity (60% RH or ~100% RH) environment. The vitamin A and iodine premixes were slightly yellow in colour, the ferric NaEDTA was off-white.

7.1 Premix Formulations

From the nine commercial vitamin A sources, 36 premixes were prepared and tested both alone and after being mixed with the salt. The targeted vitamin A concentration in each of them was 25 000 IU/g of premix which would give the desired 250 IU/g salt at 1% addition level.

Three main types of vitamin the A premix were produced:

1. Premix containing only vitamin A as the active ingredient.

2. Premix containing vitamin A and iodine (either as iodide or as iodate).

3. Premix containing all three micronutrients in one particle.

The premix containing source vitamin A and iron in one particle was studied previously by our group [56], and the retention of vitamin A was between 80% (ferric NaEDTA) and 20% (ferrous sulphate) after two months of storage in 40°C 60% RH.

All vitamin A sources were granulated with either Casco Dextrin CAS: 9004-53-9 (yellow) or Casco Starch CAS: 9005-25-8 (white) and encapsulated with Soy Stearine (30% or 40%), Shellac (40%) or Methyl Cellulose (MC) and Hexaethyl Cellulose (HEC). The antioxidants used to stabilize the vitamin A premix were either BHA and BHT (in 1:1 ratio) or TBHQ.

The composition of all the premixes used in this study is shown in Tables 7.1 to 7.5. Table 7.1 shows the premix formulations which were coated with 40% soy stearine. These vitamin A premixes were mixed with potassium iodate or iodized salt and one of the four iron premixes to form the TFS. Vitamin A premixes coated with Shellac can be found in Table 7.2. Premixes containing all three micronutrients in one particle are listed in Table 7.3. The composition of the vitamin A premixes encapsulated with MC/HEC (level of encapsulation is 40%) are shown in Table 4.4. Lastly, Table 4.5 lists the composition of various premix and TFS formulations: (a) TFS formed with vitamin A premix mixed with KI and iron premixes, (b) vitamin A granulated with a source of iodine, and (c) vitamin A coated with 30% of soy stearine.

Table 7.1: Composition of the vitamin A premix formulations coated with 40% SS

Sample #	Vitamin A	Binder	Antioxidant	Encapsulant
KR20-1	BASF Dry VAP 250 Food Lot:200719YO	Dextrin	BHA/BHT	40%SS
KR20-2	BASF Dry VAP 250 Food Lot:200719YO	Dextrin	TBHQ	40%SS
KR21	BASF Dry VAP 250 Food Lot:200719YO	Starch	TBHQ	40%SS
KR22	BASF Dry VAP 250 CK CWD	Dextrin	TBHQ	40%SS
KR23-1	BASF Dry VAP 250 CK CWD	Starch	BHA/BHT	40%SS
KR23-2	BASF Dry VAP 250 CK CWD	Starch	TBHQ	40%SS
KR24-1	BASF Dry VA Acetate 325 GFP	Dextrin	BHA/BHT	40%SS
KR24-2	BASF Dry VA Acetate 325 GFP	Dextrin	TBHQ	40%SS
KR25-1	BASF Dry VAP 250 Food Lot:483111UO	Dextrin	BHA/BHT	40%SS
KR25-2	BASF Dry VAP 250 Food Lot:483111UO	Dextrin	TBHQ	40%SS
KR26-1	BASF Dry VAP 250 CWD	Dextrin	BHA/BHT	40%SS
KR26-2	BASF Dry VAP 250 CWD	Dextrin	TBHQ	40%SS
KR27-1	BASF Dry VAP 500	Dextrin	BHA/BHT	40%SS
KR27-2	BASF Dry VAP 500	Dextrin	TBHQ	40%SS
KR28-1	BASF Dry VA Acetate 500	Dextrin	BHA/BHT	40%SS
KR28-2	BASF Dry VA Acetate 500	Dextrin	TBHQ	40%SS
KR29-1	Roche Dry VAP 500	Dextrin	BHA/BHT	40%SS
KR29-2	Roche Dry VAP 500	Dextrin	TBHQ	40%SS
KR30-1	Roche Dry VAP 250 S/N	Dextrin	BHA/BHT	40%SS

Sample #	Vitamin A	Binder	Antioxidant	Encapsulant
KR30-2	Roche Dry VAP 250 S/N	Dextrin	TBHQ	40%SS
KR31-1	Watson VAP 250,000 IU/g Powder	Dextrin	BHA/BHT	40%SS
KR31-2	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	40%SS

Table 7.2: Composition of the vitamin A premixes encapsulated with Shellac

Sample	Vitamin A	Binder	Antioxidant	Encapsulant
KR11	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	40% Shellac
KR12	BASF Dry VAP 250 Food	Dextrin	TBHQ	40% Shellac

Table 7.3: Composition of the premixes containing all three micronutrients in one particle

Sample	Vitamin A	Binder	Antioxidant	Encapsulant	note
KR13-1	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	40%SS	with FeFum and KIO_3 in one particle
KR13-2	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	40%SS	with Fe NaEDTA and KIO_3 in one particle
KR14-1	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	30%SS	with FeFum and KIO_3 in one particle
KR14-2	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	30%SS	with Fe NaEDTA and KIO_3 in one particle

Table 7.4: Composition of the vitamin A premixes encapsulated with MC/HEC (level of encapsulation was 40%)

Sample	Vitamin A	Binder	Antioxidant	Encapsulant
KR15	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	30% MC/ 10 %HEC
KR16	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	20% MC/ 20 %HEC

Table 7.5: Composition of the premixes and TFS samples containing various sources of iodine. Components of premixes coated with 30% SS.

Sample	Vitamin A	Binder	Antioxidant	Encapsulant	Source of Iodine in TFS
KR17	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	40%SS	KI premix
KR18-1	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	40%SS	KIO_3 and vit A in one particle
KR18-2	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	40%SS	KI and vit A in one particle
KR19-1	Watson VAP 250,000 IU/g Powder	Dextrin	TBHQ	30%SS	KIO_3 premix
KR19-2	BASF Dry VAP 250 Food	Dextrin	TBHQ	30%SS	KIO_3 premix

The coated vitamin A premixes were slightly yellow in colour. These premixes were mixed with an iron premix and an iodine premix or iodized salt. Total of 120 samples were prepared for each test condition (40°C 60% RH and 40°C ~100% RH). When iodine premix or the vitamin A premix containing source of iodine (either in the form of potassium iodide or potassium iodate) was used, the non-iodized salt was used for sample preparation.

Four iron premixes were prepared using one of the following iron compounds:

- Electrolytic iron, reduced

- Ferrous Fumarate
- Ferrous Sulphate, heptahydrate
- Ferric Sodium EDTA

Both iron and iodine premixes were granulated with dextrin and coated with 40% soy stearine. The colour of the iodine premixes was yellowish. Elemental iron gave grey premix, ferrous fumarate gave white premix with brown spots (showing defects in coating), ferrous sulphate was off white and the premix containing Fe NaEDTA was sandy in colour. Figure 4.2 shows the appearance of typical vitamin A, iron and iodine premixes.

7.3 Stability of Vitamin A

The results (Figures 4.3. and 4.4) show that overall vitamin A is highly unstable under the studied conditions. The average retention of vitamin A in TFS after three months at 40°C 60% RH and 40°C ~100% RH was 39 ± 1% and 31 ± 1%, respectively. Although there is a wide range within both RH studies, seven of the commercially available forms of vitamin A in 40°C 60% RH and five in 40°C ~100% RH demonstrated above average stability. These included:

- BASF Dry VAP 250 Food (premix KR20 in both RH and KR25 in 40°C 60% RH)
- BASF Dry VAP 250 CK CWD (premix KR22 in 40°C and both RH)
- BASF Dry VA Acetate 325 GFP (premix KR24 in 40°C 60% RH)
- BASF Dry VAP 500 (premix KR27 in 40°C and both RH)
- Roche Dry VAP 500 (premix KR29 in 40°C and both RH)
- Roche Dry VAP 250 S/N (premix KR30 in 40°C 60% RH)
- Watson VAP 250,000 IU/g Powder (premix KR 31 in 40°C ~100% RH)

The instability of some vitamin A samples in particular demonstrates the importance of choosing an appropriate source of commercial vitamin A. It is clear that the processes used for commercial stabilization of vitamin A have a major effect on the vitamin's stability in the triple fortified salt.

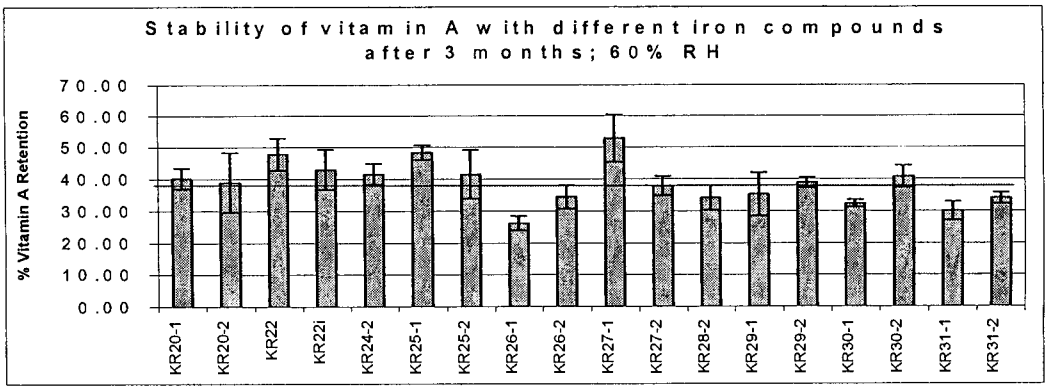

Figure 10: Stability of vitamin A premixes, granulated with dextrin, coated with 40% SS and stabilized with either BHA/BHT (marked with number 1) or TBHQ (marked with number 2), in the presence of iron and potassium iodate premix. The sample containing KR22i premix was prepared with iodized table salt. The TFS samples were stored for three months at 40°C 60% RH.

In 40°C 60% RH vitamin A was most stable when ferric NaEDTA was used. In contrast, the lowest stability was observed under these conditions when ferrous fumarate was used. The same trends were not observed at 40°C ~100% RH. At 40°C ~100% RH the reverse was true: the best stability of vitamin A was achieved with ferrous fumarate and the worst with ferric NaEDTA. Because the difference between these results is less than 5%, the source of iron was not a major factor affecting the stability of vitamin A. Nonetheless, as shown in the previous research done by our group [56], iron is a major contributor to the instability of TFS.

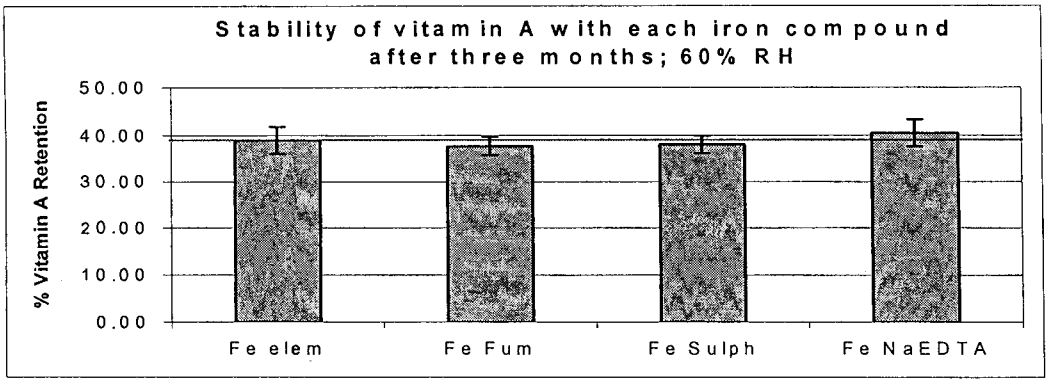

Figure 11: Stability of vitamin A premixes, granulated with dextrin, coated with 40% SS and stabilized with either BHA/BHT or TBHQ, in the presence of different iron compounds and potassium iodate premix. The TFS samples were stored for three months at 40°C 60% RH.

The decrease in vitamin A in TFS as a function of time was found to be consistent with a first order reaction rate. The first order reaction rate constants (k) were calculated to be (Figure 4.7) 0.15/month ($5.8x10^{-8}$ s^{-1}) at 40°C 60% RH and 0.3/month ($1.2 x10^{-7}$ s^{-1}) at ~100% RH for sample containing KR 27-1 premix (BASF Dry VAP 500) and ferric NaEDTA (R^2 (RSQ) 0.94 and 0.97, respectively). The results confirm that, as expected, the rate of degradation of vitamin A was slower in the lower humidity environment.

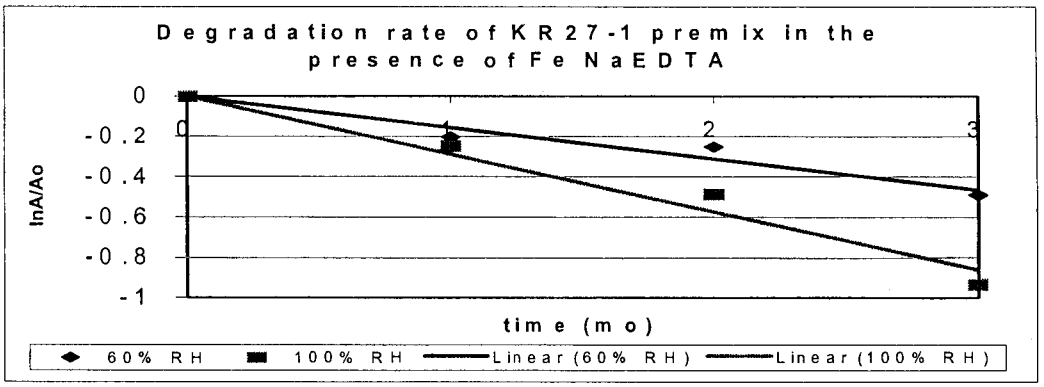

Figure 12: Degradation rate of vitamin A premix KR 27-1 (BASF Dry VAP 500 granulated with dextrin, coated with 40% SS and stabilized with BHA/BHT) in TFS sample containing ferric NaEDTA. The TFS sample was stored for three months at 40°C 60% RH and ~100% RH.

Five premixes showed stability higher than 48% after three months at 40°C, 60% RH. The highest stability of 55 ± 2% was achieved in the sample which contained elemental iron and vitamin A stabilized with BHA/BHT.

BASF Dry VAP 500 is recommended by the manufacturer particularly for products with high moisture content, and for preparations that are to be stored in climates with high relative humidity. The stability of this vitamin A in the dry powder is supposed to be very good even in the presence of minerals. The product contains ~ 500,000 I.U./g of vitamin A.

This vitamin A product was granulated with dextrin, coated with 40% soy stearine and stabilized with 27-1: BHA/BHT (1:1 ratio) or 27-2: TBHQ. Reasonable stability was observed in both RH environments.

The samples containing elemental iron showed high vitamin A retention, with 65 ± 2% retained (sample Fe elem-1) at 40°C 60% RH and as much as 44 ± 6% (sample Fe elem-2) at 40°C ~100% RH. Fe NaEDTA-1 sample retained 61 ± 5% of vitamin A at 40°C 60% RH and just under 40% at 40°C ~100% RH.

Overall, four out of the eight TFS samples containing KR22 premix (BASF Dry VAP 250 CK CWD) gave vitamin A retentions greater than 50% after two months of

storage at 40°C 60% RH. In addition, two samples retained more than 40% of this vitamin A at 40°C ~100% RH after the same period of time.

In the case of premix KR25 (BASF Dry VAP 250 Food), three TFS samples showed retention greater than 50% at 40°C 60% RH, but none of them retained more than 40% of vitamin A at 40°C ~100% RH after three months of storage.

TFS samples containing premix KR27 (BASF Dry VAP 500) showed very good stability under both RH conditions. At 40°C 60% RH, two samples retained more than 60% of vitamin A and at 40°C ~100% RH three samples showed retention higher than 40% after three months of storage under these conditions.

All three vitamins (BASF Dry VAP 250 CK CWD, BASF Dry VAP 250 Food and BASF Dry VAP 500) should be investigated further as they show promising stability in TFS samples. Altering the composition of their matrix and coating agents could promote their stability even more.

Vitamin A Acetate vs. Palmitate

The effect of different vitamin A esters on the vitamin's stability in TFS was evaluated by comparing the stability of vitamin A acetate and palmitate. In general, vitamin A palmitate showed better stability than the acetate form under all experimental conditions. This is most likely due to the differences in chemical structure between the acetate and palmitate forms.

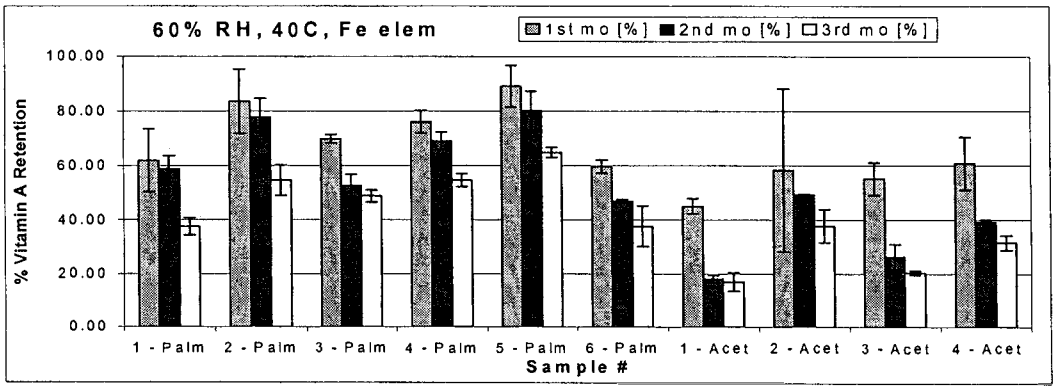

Figure 12: Stability of vitamin A acetate and palmitate premixes (granulated with dextrin, coated with 40% SS and stabilized with either BHA/BHT or TBHQ), in the presence of elemental iron and potassium iodate premix. The sample containing KR22i premix (1-Palm) was mixed with iodized salt. The TFS samples were stored for three months at 40°C 60% RH.

Effect of Iron Source on Vitamin A Stability

The influence of iron source on vitamin A stability was tested using the three most stable premixes, KR22 (BASF Dry VAP 250 CK CWD), KR25 (BASF Dry VAP 250 Food) and KR27 (BASF Dry VAP 500). Only TFS samples containing potassium iodate

and vitamin A stabilized with TBHQ were studied. Ferric EDTA was marginally more stable than the other iron sources.

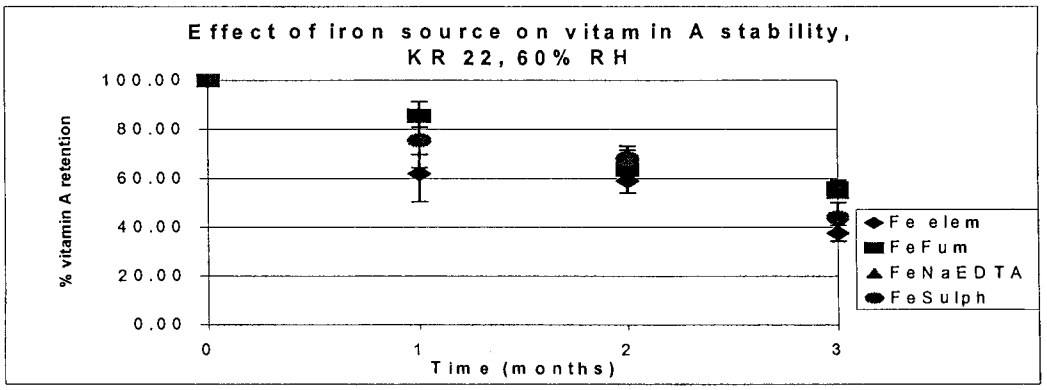

Figure 13: Effect of iron source on the stability of vitamin A in premix KR 22 (BASF Dry VAP 250 CK CWD, granulated with dextrin, coated with 40% SS and stabilized with TBHQ), in the presence of different iron compounds and potassium iodate premix. The TFS samples were stored for three months at 40°C 60% RH.