# CHAPTER 2

# LITERATURE REVIEW

*In this chapter a literature review is presented on vitamin A deficiency, fortification and factors that might have an influence on the success of such a program.*

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## 2.1 Introduction

The estimates from the World Health Organisation in the period 1995 - 2005 indicate that 190 million preschool children (~32%) and nearly 20 million pregnant women (~10%) are vitamin A deficient in low income countries (WHO, 2009). An estimated 250 000 to 500 000 vitamin A deficient children become blind every year, half of them dying within 12 months of losing their sight (WHO, 2003).

To successfully combat vitamin A deficiency (VAD), short-term interventions and proper feeding in infancy must be supported by long-term sustainable solutions. The solutions to nutritional well-being include a combination of breastfeeding and vitamin A supplementation, coupled with long-term food-based solutions, such as the promotion of vitamin A-rich diets and food fortification.

Breast milk is the main sources of vitamin A for infants. Poor maternal vitamin A status, and the resultant low breast milk retinol content are risk factors for the early onset of VAD in infants, as is early cessation of breastfeeding (Allen and Gillespie, 2001).

The most widely practised approach to control VAD in high-risk countries is the periodic delivery of Vitamin A supplements. While periodic vitamin A delivery in the community has been shown to reduce the risks of xerophthalmia or night blindness (by ~90%) and mortality (by ~23–30%) in young children, the reasons for the modest and transient effect in raising population serum retinol concentrations remain unclear. Many high-risk countries have also adopted the WHO policy of supplementing mothers with a 200 000 IU oral dose of vitamin A within six weeks after delivery to enrich the vitamin A content of their breast milk, although in practice coverage remains quite low (WHO, 2009).

Fortifying a widely consumed centrally processed food or condiment capitalizes on the production and distribution system of the food market to deliver low doses of vitamin A daily to a large number of people. Food fortification has many advantages: it is generally socially acceptable, it requires minimal changes in food habits, fortified foods usually costs <2% more than the cost of the unfortified food, its delivery system is already in place and it can become sustainable (Dary and Mora, 2002). The success of a fortification program depends among other factors on the mix of micronutrients and the concentration thereof in the fortified products. A number of aspects, including nutrient interactions, the stability of the specific micronutrients added to the food under anticipated conditions of storage and processing can all have an influence on the fortificant concentration.

In this chapter vitamin A deficiency and factors contributing thereto; vitamin A metabolism and absorption; factors influencing the vitamin A concentration in fortified products and measurements of relative bioavailability will be discussed.

## 2.2 Vitamin A and the isomers

Vitamin A is a generic term used for a group of structurally related chemical compounds known as retinoids. Retinoids refer to both naturally occurring and synthetic compounds with, or without, the biological activity of vitamin A (O'Byrne and Blaner, 2005). Figure 2.1 shows the chemical structures of some retinoids. The term vitamin A is often used as a general term for all compounds that exhibit the biological activity of retinol.

In vivo, vitamin A is generally found as the free alcohol form (retinol) or esterified with a fatty acid (retinyl ester). All-*trans*-retinol is by definition vitamin A. The vitamin is available in pure form by chemical synthesis or as vitamin A palmitate or acetate. It is a pale yellow solid, which dissolves freely in oils and fats, but is insoluble in water (Fox and Cameron, 1995).

### 2.2.1 Sensitivity of Vitamin A

Vitamin A is affected by pH, enzymatic activity, light and oxidation associated with the double bond system (DSM/USAID, n.d.b). In Table 2.1 a summary of Vitamin A sensitivities compared to other vitamins is presented. Vitamin A is quite stable when heated to moderate temperatures in the absence of oxygen and light. Overall loss of activity during anaerobic heating may range from 5-50%, depending on time, temperature and nature of the retinoids. In the presence of oxygen and light, there can be extensive loss of vitamin A activity through oxidation. The presence of trace metals accelerates this reaction (Ottaway, P.B. cited Mehansho et al., 2003).

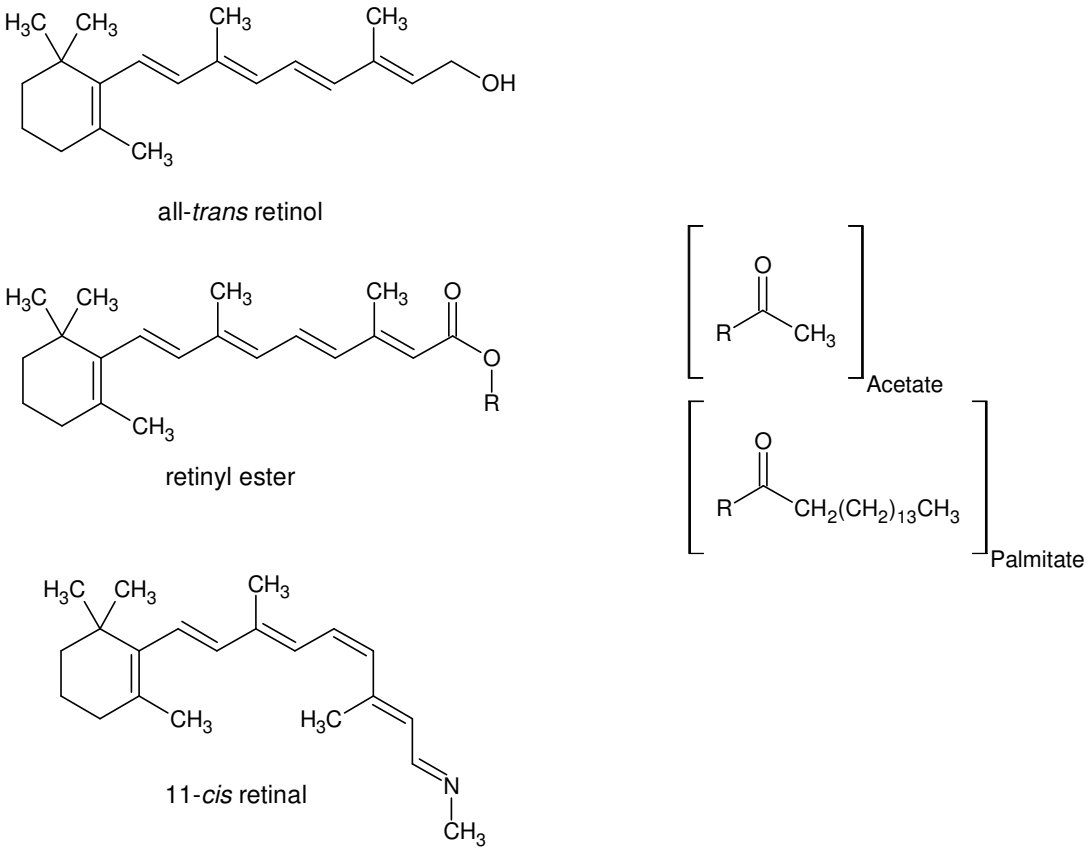


**Figure 2.1:** Chemical structures of different retinoids. All-*trans*-retinol is by definition vitamin A. When a fatty acyl group is esterified to the hydroxyl terminus of all-*trans*-retinol, a storage form of retinol, the retinyl ester is formed. The most abundant retinyl esters are those of palmitic, oleic, stearic and linoleic acids. Retinyl acetate and palmitate are often used as dietary supplements, but do not occur naturally. Retinol can be reversibly oxidized to retinal, which as the 11-*cis* isomer is essential for the visual cycle (O'Byrne and Blaner, 2005)

**Table 2.1:** Sensitivity of vitamin A compared to other vitamins (DSM/USAID, n.d.b)

| | Light | Oxidizing agents | Reducing agents | Heat | Humidity | Acids | Alkalis |
|---|---|---|---|---|---|---|---|
| **Vitamin A** | +++ | +++ | + | ++ | + | ++ | + |
| **Vitamin D** | +++ | +++ | + | ++ | + | ++ | ++ |
| **Vitamin E** | ++ | ++ | + | ++ | + | + | ++ |
| **Vitamin K** | +++ | ++ | + | + | + | + | +++ |
| **Vitamin C** | + | +++ | + | ++ | ++ | ++ | +++ |
| **Thiamine (Vit $B_1$)** | ++ | + | + | +++ | ++ | + | +++ |
| **Riboflavin (Vit $B_2$)** | +++ | + | ++ | + | + | + | +++ |
| **Niacin** | + | + | ++ | + | + | + | + |
| **Pyridoxine (Vit $B_6$)** | ++ | + | + | + | + | ++ | ++ |
| **Cyanocobalamin (Vit $B_{12}$)** | ++ | + | +++ | + | ++ | +++ | +++ |
| **Pantothenic Acid** | + | + | + | ++ | ++ | +++ | +++ |
| **Folic Acid** | ++ | +++ | +++ | + | + | ++ | ++ |
| **Biotin** | + | + | + | + | + | ++ | ++ |

+ Hardly or not sensitive ++ Sensitive +++ Highly sensitive

In dehydrated foods, vitamin A and provitamin A are highly susceptible to loss by oxidation. The extent of this loss depends on the severity of the drying process, protection provided by packaging materials and conditions of storage. Vitamin A in pure form is unstable in the presence of mineral acids but stable in the presence of alkali.

Naturally occurring vitamin A is insoluble in water but soluble in oil. In the natural form the vitamin has limited applicability in fortification. Vitamin A as fortificant are commercially available in a wide range of forms adapted for use under various conditions as presented in Table 2.2.

**Table 2.2:** Commercially available forms of vitamin A, their characteristics and their main applications (WHO, 2006).

| Product | Characteristics | Application(s) |
|---|---|---|
| Oily vitamin A acetate | Retinol ester of acetic acid which may be stabilized with especially antioxidants | Fortification of fat-based foods, margarine and dairy products |
| Oily vitamin A palmitate | Retinol ester of palmitic acid which may be stabilised with antioxidants | Fortification of fat-based foods, especially margarine and dairy products |
| Oily vitamin A palmitate or acetate with vitamin D3 | Retinol ester and cholecalciferol mix, stabilised with antioxidants | Fortification of fat-based foods, especially margarine and dairy products where the combination of both vitamins is required |
| Dry vitamin A palmitate or acetate | Vitamin A embedded in a water-soluble matrix (e.g. gelatin, gum acacia, starch) and stabilised with antioxidants | Fortification of dry food products, (i.e. flour and dry milk, beverage powders) and fortification of water-based foods |
| Dry vitamin A palmitate or acetate with vitamin $D_3$ | Vitamin A and vitamin $D_3$ embedded in a water-soluble matrix (e.g. gelatin, gum acacia, starch) and stabilised with antioxidants | Fortification of dry food products, (i.e. flour and dry milk, beverage powders) and fortification of water-based foods |

For use in fat or oil based foods such as margarines, oils and dairy products, vitamin A, in the acetate or palmitate form, has been used. These forms are stabilised with a mixture of phenolic antioxidants or with tocopherols. For mixing with dry products, a dry form of the fortificant is required with the appropriate size and density. Encapsulation of the vitamin in a more hydrophilic coat is commonly practised in order to achieve a more water dispersible product. Some materials used in encapsulation are gum acacia, starch and gelatin. These dry forms of the vitamin are also stabilised using tocopherols or phenolic antioxidants (Clarke, 1995; WHO, 2006). Vitamin A compounds needed for fortification of dry matrixes (e.g. flour and sugar) are at least four times more expensive than the oily forms, and their stability is inferior (Dary and Mora, 2002).

According to the South African fortification regulations a protected, stabilized vitamin A palmitate containing 75 000 µgRE activity per gram fortification mix must be used (Department of Health, 2003).

## 2.3 Vitamin A Metabolism and Deficiency

### 2.3.1 The role of Vitamin A in human metabolic processes

Although an essential nutrient needed in only small amounts, vitamin A is necessary for normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function and reproduction. Vitamin deficiency disorders occur when body reserves are depleted to the limit at which physiological functions are impaired. Vitamin A in the diets of most human communities comes from a very wide variety of plant and animal sources (FAO, 2001). In the more industrialised countries over two-thirds of dietary vitamin A is derived from animal sources as preformed vitamin A, whereas in developing countries, communities depend primarily on provitamin A carotenoids from plant sources (Ahmed and Darnton-Hill, 2004). In an effort to satisfy energy needs, poor populations may have chosen diets of lesser quality and variety, which would increase the risk of multiple micronutrient deficiencies (West and Sucheta, 2010).

Provitamin A carotenoids is the collective term for all the carotenoids that can be converted to retinoids by humans and some animals (O'Byrne and Blaner, 2005). However, the conversion and bioavailability of the provitamin A carotenoids are much less efficient than retinol (Van Lieshout and West, 2004).

Preformed vitamin A in animal foods occurs as retinyl esters of fatty acids in association with membrane-bound cellular lipid and fat-containing storage cells. Normal digestive processes free vitamin A from embedding food matrices. Vitamin A is absorbed more efficient from animal products than from vegetable tissues. Retinyl esters are hydrolysed and the retinol is incorporated into lipid-containing, water-miscible micellar solutions. Products of fat digestion (e.g., fatty acids, monoglycerides, cholesterol, and phospholipids) and secretions in bile (e.g., bile salts and hydrolytic enzymes) are essential for the efficient solubilisation of retinol. Retinol is trapped intracellularly by re-esterification or binding to specific intracellular binding proteins (O'Byrne and Blaner, 2005). Retinyl esters together with other lipids are incorporated into chylomicrons, excreted into intestinal lymphatic channels and delivered to the blood through the thoracic duct. If not immediately needed, retinol is re-esterified and retained in the fat-storing cells of the liver (FAO, 2001).

Vitamin A functions at two levels in the body. The first is in the visual cycle in the retina of the eye; the second is in all body tissues systemically to maintain growth and the soundness of cells. The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency (VAD). The immune system is also compromised by direct interference with production of some types of protective secretions and cells (FAO, 2001).

### 2.3.2 Bioavailability of vitamin A

The amount of a nutrient absorbed from the gut which becomes available to tissues is referred to as bioavailability (Van Lieshout and West, 2004). Preformed vitamin A is absorbed in the small intestine. The bioavailability of retinol is generally high;

ranging from 70 – 90% (Dary and Mora, 2002; Otten, Hellwig and Meyers, 2006), while that of carotenoids is lower and is affected by various factors (Castenmiller et al., 1999; Van het Hof et al., 2000). Different carotenoids have different levels of vitamin A activity depending upon the efficiency of their absorption and the rate of their conversion to vitamin A. Whereas 1 retinol equivalent (RE) is equal to 1 mg of all-*trans* retinol, the same level of vitamin A activity requires 6 mg of beta-carotene and 12 mg of other carotenoids with vitamin A activity (West, Eilander and Van Lieshout, 2002). By the late 1990's, conversion factors for estimating vitamin A obtained from plant foods were revised from 6:1 to 12:1 (µg ß-carotene:retinol activity equivalent (RAE)) by the US Institute of Medicine (IOM). De Pee, West and colleagues proposed a conversion factor of 21:1 for a mixed diet (12:1 for fruits and 26:1 for vegetables (De Pee et al., 1998; IVACG, n.d.). The cost factor in using carotenoids as the source of vitamin A activity in fortification is generally considered prohibitive (Clarke, 1995).

When vitamin A intake is adequate, more than 90% of total body vitamin A is located in the liver, which releases the nutrient into the circulation. Factors such as dietary fat, intestinal infections, the food matrix, and food processing can affect the absorption of vitamin A by the body. Dietary fat appears to enhance absorption, whereas absorption is reduced in individuals with diarrhoea, intestinal infections and infestations (Blomhoff, 1994; Herrero-Barbudo, et al., 2006; Edem, 2009).

### 2.3.3 Dietary Requirements and Toxicity

#### *2.3.3.1 Definitions of Recommended Dietary Allowance, Recommended Safe Intake and Daily Reference Intake*

The mean requirement for an individual is defined by the FAO as the minimum daily intake of vitamin A as presented in Table 2.3 to prevent xerophthalmia in the absence of clinical or sub-clinical infection. This intake should account for proportionate bioavailability of preformed vitamin A (about 90%) and pro-vitamin A carotenoids from a diet that contains sufficient fat (e.g., at least 5–10g). The required level of intake is set to prevent clinical signs of deficiency, allow for normal growth, and reduce the risk of vitamin A–related severe morbidity and mortality on a population basis. It does not allow for frequent or prolonged periods of infections or other stresses (FAO, 2001). The safe level of intake for an individual is defined as the average continuing intake of vitamin A required to permit adequate growth and other vitamin A–dependent functions and to maintain an acceptable total body reserve of the vitamin. This reserve helps offset periods of low intake or increased need resulting from infections and other stresses. Estimates for the requirements and recommended safe intakes of all age groups are estimates derived from vitamin A requirements/body weight/day for late infancy (FAO, 2001).

Recommended dietary allowances (RDA) as defined by the FAO and WHO are set to meet the needs of almost all (97-98%) individuals in a group. For healthy breastfed infants, the adequate intake (AI) is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all individuals in the group, but a lack of data prevents being able to specify with confidence the percentage of individuals covered by this intake (FAO, 2001).

**Table 2.3:** FAO estimated mean requirement and safe level of intake for vitamin A (FAO, 2001)

| Age Group | Mean Requirement (µgRE/day) | Recommended Safe Intake (µgRE/day) |
|---|---|---|
| **Infants** | | |
| 0-6 months | 180 | 375 |
| 7-12 Months | 190 | 400 |
| **Children** | | |
| 1-3 years | 200 | 400 |
| 4-6 years | 200 | 450 |
| 7-9 years | 250 | 500 |
| **Adolescents** | | |
| 10-18 years | 330 – 400 | 600 |
| **Adults** | | |
| Females: 19-65 years | 270 | 500 |
| Males: 19-65 years | 300 | 600 |
| **Elderly** | | |
| 65+ years | 300 | 600 |
| **Pregnant Women** | 370 | 800 |
| **Lactating Women** | 450 | 850 |

The Food and Nutrition Board of the National Academies' Institute of Medicine (IOM), with support from the US and Canadian Governments developed a new, broader set of dietary reference values known as the Dietary Reference Intakes (DRIs). See Table 2.4. The DRIs expand upon and replace the RDAs with four categories of values intended to help individuals optimise their health, prevent disease and avoid consuming too much of a nutrient. The reference values include the estimated average requirement (EAR), the recommended dietary allowance (RDA), the adequate intake (AI) and the tolerable upper intake level or upper limit (UL). The following definitions and criteria are used:

- The estimated average requirement (EAR) is the average daily nutrient intake level that is estimated to meet the nutrient needs of half of the healthy individuals in a life stage or gender group. Metabolic weight ($kg^{0.75}$) ratio method was used to extrapolate the data from adults (IOM, 2001).
- The definition of the RDA is the same as described above.

- The adequate intake (AI) is a recommended average daily nutrient intake level based on observed or experimentally determined approximates of nutrient intake by a group of apparently healthy people who are assumed to be maintaining an adequate nutritional state (IOM, 2001).
- The upper limit (UL) is the maximum level of daily nutrient intake that is likely to pose no risk of adverse effects. Unless otherwise specified, the UL represents total intake from food, water, and supplements. The UL for vitamin A applies only to preformed vitamin A. It does not apply to vitamin A derived from carotenoids (IOM, 2001).

**Table 2.4:** Dietary Reference intakes (DRIs) for Vitamin A by life stage group (IOM, 2001)

| Age Group | EAR (µg/day) | RDA (µg/day) | AI (µg/day) | UL (µg/day) |
|---|---|---|---|---|
| **Infants** | | | | |
| 0-6 months | | | 400* | 600 |
| 7-12 Months | | | 500* | 600 |
| **Children** | | | | |
| 1-3 years | 210 | 300 | | 600 |
| 4-8 years | 275 | 400 | | 900 |
| 9-13 years | 445 | 600 | | 1 700 |
| **Males** | | | | |
| 14-18 years | 630 | 900 | | 2 800 |
| 19-50 year | 625 | 900 | | 3 000 |
| >50 years | 625 | 900 | | 3 000 |
| **Females** | | | | |
| 14-18 years | 485 | 700 | | 2 800 |
| 19-50 year | 500 | 700 | | 3 000 |
| >50 years | 500 | 700 | | 3 000 |
| **Pregnant** | | | | |
| **Women** | 530 | 750 | | 2 800 |
| ≤18 years | 550 | 770 | | 3 000 |
| 19-50 years | | | | |
| **Lactating** | | | | |
| **Women** | 885 | 1 200 | | 2 800 |
| ≤18 years | 900 | 1 300 | | 3 000 |
| 19-50 years | | | | |

*For healthy breastfed infants, the AI is the mean intake.

As seen in Table 2.3 and Table 2.4, lactating women require the highest vitamin A intake. The mean requirement estimated by the FAO (Table 2.3) is generally lower than the EAR (Table 2.4) as determined by the IOM; with the most significant difference for pregnant and lactating women. The IOM also determines RDA values that are generally higher than the Recommended Safe Intakes (RSI) estimated by the FAO. The main reason for the higher EAR and DRI values from the IOM is the fact that the estimates was made on metabolic weight and not on total body weight as was used by the FAO.

#### *2.3.3.2 Toxicity*

Because vitamin A is fat soluble and can be stored, primarily in the liver, routine consumption of large amounts of vitamin A over a period of time can result in toxic symptoms. A review of the latest available information by a WHO Expert Group recommended that daily intakes in excess of 3 000 µg (10 000 IU) or weekly intakes in excess of 7 500 µg (25 000 IU), should not be taken (FAO, 2001). Vitamin A fortification of foods in dosages not exceeding the RDA does not cause toxic effects (Lotfi et al., 1996).

## 2.4 Vitamin A Deficiency

Vitamin A deficiency (VAD) is, after protein-energy malnutrition and iron deficiency anaemia, the nutritional health problem of highest public health significance in developing countries. Globally, more than 200 million children are vitamin A deficient, and VAD is still the leading cause of blindness in children. Women in developing

countries are also at risk of VAD, especially during pregnancy and lactation (Ahmed and Darnton-Hill, 2004).

In South Africa, 1 in 3 preschool children has a marginal vitamin A status (serum vitamin A concentration <0.7 µmol/L) (SAVACG, 1996). Normal serum vitamin A concentration for pre-school children is between 0.63 – 1.75 µmol/L (WHO, 1996.). 55–68% of children aged 1–9 years consume <50% of the recommended dietary intake of vitamin A (700 µg retinol equivalents) (NFCS, 2000). Figure 2.2 shows the prevalence of VAD in the nine different provinces in South Africa. Children living in rural areas are the most affected (SAVACG, 1996; NFCS, 2000). VAD is caused by a habitual diet that provides too little bioavailable vitamin A to meet physiological needs.

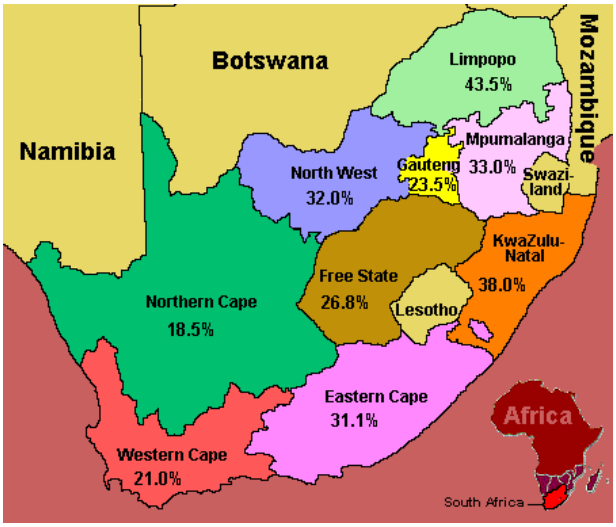


**Figure 2.2:** Prevalence of Vitamin A Deficiency in 6-71 month old children in South-Africa in 1994 as determined by the SAVACG study (SAVACG; 1996)

VAD is associated with a higher risk of death in preschool-age children, presumably because of vitamin A's role in the immune function and in maintaining the integrity of epithelial tissue. Supplementation with vitamin A reduces the risk of child mortality and may reduce maternal mortality. It also reduces the risk of severe diarrhoea and

measles, both of which are important and sometimes serious illnesses in developing countries. Because the vitamin A content of breast milk is often low in vitamin A-depleted women, infants of these women are at greater risk of becoming VAD early in life. If left untreated, this can result in a vicious cycle of deficiency that is not resolved (Van Lieshout and West, 2005).

Malnutrition causes the loss of about 140 million disability adjusted life years. The Disability Life Year (DALY) is the only quantitative indicator of burden of disease that reflects the total amount of healthy life lost, to all causes, whether from premature birth mortality or from some degree of disability over a period of time. DALY's are a quantitative way to compare the effect of various diseases on societies. Figure 2.3 shows that almost 25 million DALY's are lost due to VAD worldwide. In Africa alone, VAD causes the loss of almost 17 million DALY's (WHO, 2002); and in South Africa between 86 388 and 136 009 DALY's are lost (Nojilana et al., 2007).

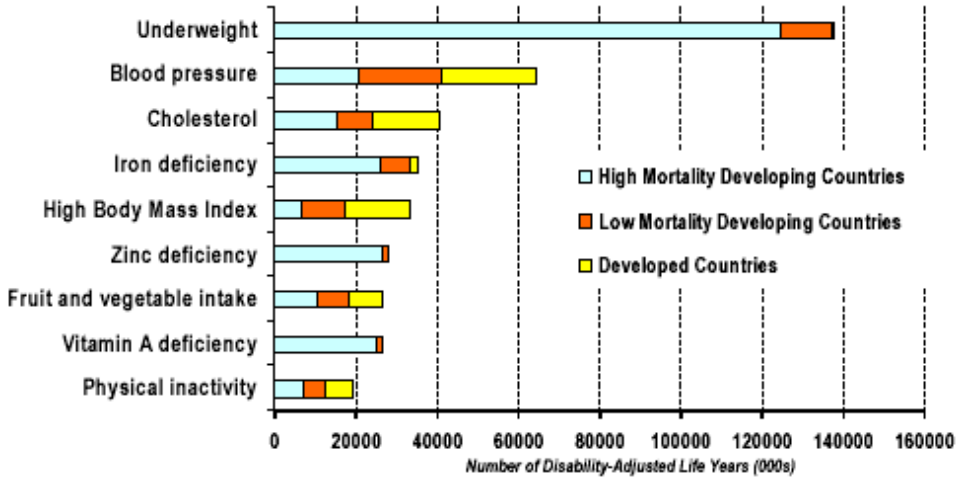


**Figure 2.3:** Disease burden (DALY's) in 2000 attributable to undernutrition and diet-related risks and physical inactivity (WHO, 2002)

It is understood that food consist of many nutrients, and that when communities are at risk for vitamin A deficiency, they may be at risk of other nutrient deficiencies as

well. Correcting VAD in populations at risk of deficiency is an investment to improve human development.

### 2.4.1 Strategies for controlling Vitamin A deficiencies

Increasing the efficacious nutrient supply, by using food-based approaches or by using pharmanutrient approaches, can control deficiency of vitamin A, as well as deficiency of other micronutrients. Another strategy for controlling VAD is to reduce the nutrient requirements, by for example, controlling infection (Van Lieshout and West, 2004).

Food-based interventions are viewed as those most likely to be sustained, provided the culture and ecology of the vitamin A-containing foods is addressed in programs based in agriculture, food processing, social marketing and public health education (Blum et al., 1997). When considering food approaches for combating vitamin A deficiency, it is necessary to take into account whether the efficacious nutrient supply can be met. Figure 2.4 illustrates the balance between the supply of nutrients and the requirements thereof. The efficacious nutrient supply depends on:

- Amount of foods containing vitamin A or provitamin A carotenoids consumed
- Vitamin A or provitamin A carotenoid content of each food consumed, and
- Bioefficacy of vitamin A or provitamin A carotenoids in the food consumed (Van Lieshout and West, 2004).

By far the most efficient food based approach for increasing the nutritional status of the nation is through fortification of widely consumed and accessible staple foods (Randall, 2001).

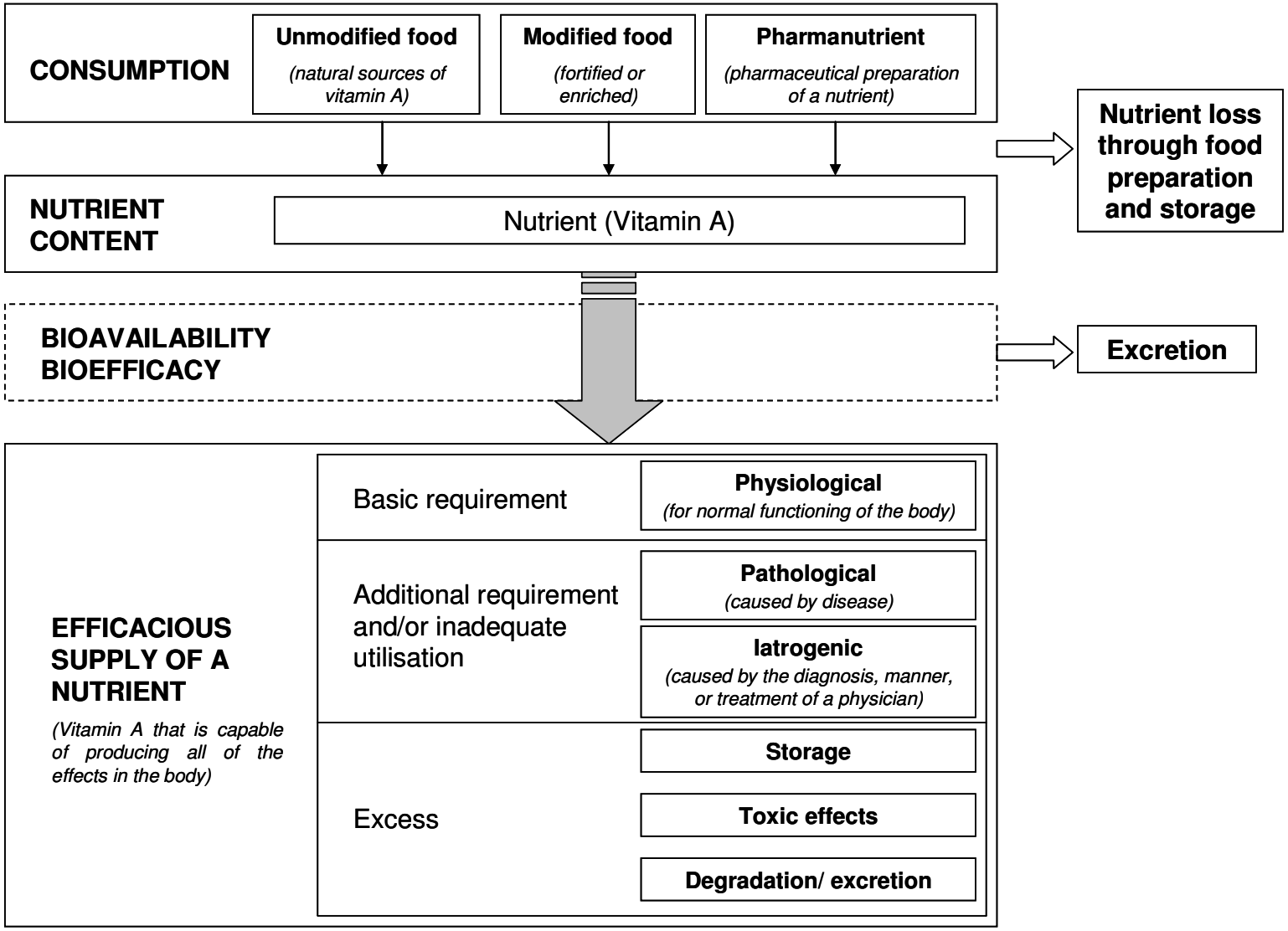


**Figure 2.4:** Balance between the supply of nutrients and requirements (Van Lieshout and West, 2005)

## 2.5 Fortification of staple foods with Vitamin A

Food fortification refers to the addition of micronutrients to foods during the production process. If fortified foods are consumed on a regular basis they will maintain body stores of nutrients more efficiently and more effectively than intermittent supplements. Fortification generally aims to supply micronutrients in amounts that approximate to those provided by a good, well-balanced diet. Consequently, fortified staple foods will contain “natural” or near natural levels of micronutrients, which may not necessarily be the case with supplements (WHO, 2006). Fortification of widely distributed and widely consumed foods has the potential for improving the nutritional status of a large proportion of the population. Fortification of food with vitamin A and its distribution are most feasible where the processed food industry is well-developed and supported. That may not be the case in resource-poor areas where vitamin A is lacking in the diet, deficiency is most extreme and various barriers exist for the most vulnerable to access fortified food (Trowbridge et al., 1993). An example in South Africa is food produced on farms which probably is used as in lieu of monetary payment that escapes mandatory fortification. This contributes to the finding that children on commercial farms are the worst fed as was found by the NFSC (NFSC, 2000).

To have a sustained impact on VAD, policy makers and program planners in agriculture and health must understand the nature of the fortificant (vitamin A), the food that is to be fortified (maize meal), methods of preparation and conservation of the food. Such knowledge will improve the dietary quality and quantity of vitamin A in fortification programs.

### 2.5.1 Maize meal as a vehicle for micronutrient fortification

The NFCS recommends maize meal (super, special and sifted) as one of the suitable vehicles for mandatory multiple micronutrient fortification (NFCS, 2000):

- Maize meal offers the best potential to deliver micronutrients to the widest spectrum of South Africans;
- Consumption among children is high, especially among 1-3 year-olds;
- 96% of maize meal is purchased from retailers;
- Production is relatively centralised with seven major companies dominating South Africa's maize milling industry, and contributing to about 90% of the domestic maize meal market. The three kinds of maize meal produced (from the most highly to the least processed) are: super, with a low extraction rate and high price; special, with an intermediate extraction rate and an intermediate price; and sifted maize with a very high extraction rate and low price (Bekker, 2004).

During the industrialised milling process many of the micronutrients concentrated in the outer layers of the maize kernel are removed (DSM/USAID, n.d.a), resulting in a highly refined product that is practically nothing more than pure starch. Refer to addendum A for the nutrient content of unfortified white maize meal. Refined white maize meal is the main staple food due to consumer demand (Shopo 1985 cited Smale and Jayne, 2003).

### 2.5.2 Vitamin A as a fortificant

Several forms of vitamin A are available for food fortification. These include retinyl acetate, retinyl palmitate, and provitamin A (ß-carotene). ß-carotene has an intense

orange colour that makes it unsuitable as a fortificant for many foods, but can be used to give an orange-yellow colour to margarines and beverages. The retinyl esters are available in an oil-soluble form (for fortification of oils and fats), spray-dried (for flours and powdered milk) and as water-dispersible beadlets (for fortification of sugar and other water soluble foods). A special coated, protected form of retinyl palmitate, often generically referred to as SD250, is the recommended form of vitamin A for flour fortification because it is considered to be the most stable in this application. This product contains encapsulates and antioxidants that differ between manufacturers, making it impractical to specify its exact composition. The stability of vitamin A in these commodities was found to be surprisingly good, with over 95% retained after nine months. There were additional losses during milling and baking, so that about 80% of the added vitamin A is actually consumed. Lower retentions, as low as 50%, can occur in non-bread baked products and maize meal (Johnson, Mannar, and Ranum, 2004).

### 2.5.3 Factors that affect nutrient delivery in fortification of maize meal

The success of a fortification program depends on a number of technical aspects, including nutrient interactions, the stability of micronutrients added to the food under anticipated conditions of storage and processing (food preparation at the household level) and bioavailability of the nutrients. Prior to selecting the fortificant(s), it is important to consider the factors affecting its/their stability (Figure 2.5). Physical and chemical factors include high temperatures, moisture, exposure to air or light, and acid or alkaline environments. The exposure of the fortificant to any of these factors during food processing, distribution, or storage affects its stability.

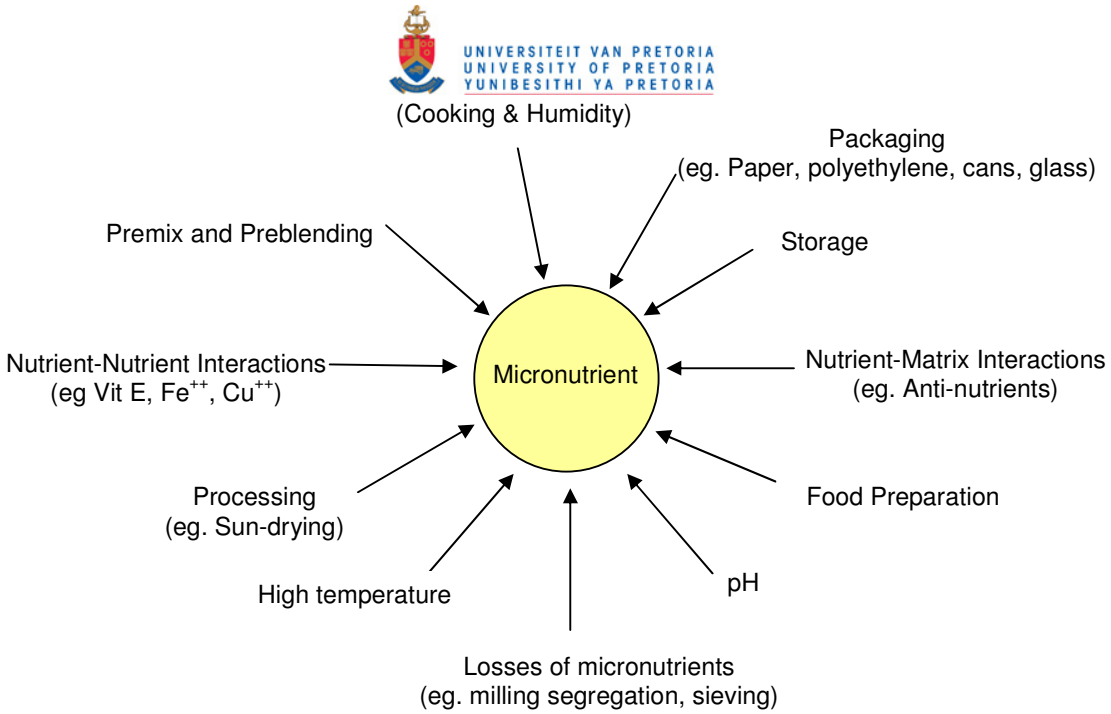


**Figure 2.5:** Physical and chemical factors influencing the stability of nutrients.

### *2.5.3.1 Nutrient-nutrient interactions*

When more than one fortificant is being added to a particular vehicle, consideration must be given to the interactions, both positive and negative tat may occur. The presence of vitamin E has been shown to increase the bioavailability of vitamin A. One explanation for this is that tocopherol as a lipid phase antioxidant, stabilises vitamin A in the gastrointestinal tract (Clarke, 1995). On the other hand, the degradation (autoxidation) of vitamin A is accelerated by the presence of bioavailable iron as fortificant (Mehansho, et al., 2003; WHO, 2006), as well as other trace elements such as copper (Wirakartakusumah and Hariyadi, 1998). In South Africa “electrolytic iron" as elemental iron powder is included in the fortification premix as regulated by the Foodstuffs, Cosmetics and Disinfectants Act (Department of Health, 2003).

### *2.5.3.2 Nutrient-matrix interactions*

Besides nutrient - nutrient interactions, other components of the food matrix may also affect the functionality of the fortificant. Selection of the vehicle in fortification programs must be such as to avoid reduced bioavailability of nutrients due to the presence of anti-nutritional compounds (Clarke, 1995). The naturally occurring anti-nutrients in maize meal such as fibres and phytates inhibit the absorption of trace metals (iron and zinc) (Welch, 1997). Dietary fibre reduces the bioavailability of vitamin A if consumed within the same meal (Lotfi et al., 1996). Vitamin A prevents the inhibitory effect of phytates and polyphenols on iron absorption (Garćia-Casal et el., 1998). The effect of phytates on vitamin A absorption and/or stability is not indicated in the literature and needs further investigation.

### *2.5.3.3 Moisture*

Moisture contents in excess of about 7-8% in a food are known to adversely affect the stability of vitamin A. Beyond the critical moisture content, there is a rapid increase in water activity, which permits various deteriorative reactions to occur (Clarke, 1995). The moisture level of South African unfortified super maize meal, unfortified special maize meal and unfortified sifted maize meal is 12.0%, 11.6% and 11.9% respectively (Wolmarans, Danster and Chetty, 2005). A 6.5% moisture content of maize grits showed hardly any loss, whereas 11.4% moisture resulted in a loss of one-fifth of vitamin A (Cort et al., 1976, Lotfi et al., 1996).

### *2.5.3.4 Temperature*

Vitamin A is stable under an inert atmosphere; however, it rapidly loses its activity when heated in the presence of oxygen, especially at higher temperatures (Lešková, 2006). Temperature may have an effect during storage as well as food preparation and is discussed under these headings (See 2.5.3.10 and 2.5.3.11).

### *2.5.3.5 pH*

The stability of vitamin A is also affected by acidity. Below a pH of 5.0, vitamin A is unstable (Wirakartakusumah and Hariyadi, 1998). Increasing the acidity of food through fermentation is often used as preservation method. An example of a fermented/beverage that is popular in many parts of southern Africa, including South Africa, is *mahewu*, *amahewu*, also known as *magou* or *mageu.* Mageu is prepared by the fermentation of maize with lactic acid bacteria (Byaruhanga, Bester, and Watson, 1999). Lactic acid bacteria fermentation can cause a pH < 4.0 (Mensah, 1997).

### *2.5.3.6 Losses of added micronutrients*

Some of the added micronutrients are lost during the milling process due to a combination of exposure to heat, oxygen and light. Some of the very light or small particle size materials with a large surface area may be physically removed with the dust during pneumatic suction, while larger particles may be removed by sieving. This can present as low values of vulnerable micronutrients (vitamin A, riboflavin) (Johnson, Mannar and Ranum, 2004). When mixing dry fortificants with dry foods, careful selection of the physical characteristics of the fortificant compound is

important to ensure adequate mixing and to minimise segregation on storage (Clarke, 1995).

#### *2.5.3.7 Premix and preblend considerations*

This is especially important in small-scale fortification. The concentrated premixes made for large scale fortification can be used in small scale fortification once they are properly diluted to a preblend. The cereal being fortified is used as the diluent. The dilution factor will be determined by the weight of cereals typically processed for each customer at the small mill. This can range from 2 to 20 kg at different mills. A fortification preblend has a much shorter shelf life than the parent premix – typically a few weeks rather than years – so it must be made in limited quantities in close proximity to the site where it will be used (Johnson, Mannar and Ranum, 2004).

#### *2.5.3.8 Effect of further processing*

In some cases the staple food that is brought to the small mill may need further processing before it is cooked at home. For example, de-hulled maize is brought to the mill for milling, but after the milling the maize meal is still moist. In this case the maize meal is spread out on mats in the sun to dry. Figure 2.6 shows this practise in the rural area near Giyani, Limpopo, South Africa. Sun-drying will effectively destroy most of the added vitamin A, riboflavin and folic acid (Johnson, Mannar and Ranum, 2004).

**Figure 2.6:** Sun-drying of hammer-milled maize meal in a rural village near Giyani

### *2.5.3.9 Packaging*

Products that are improperly packaged and subsequently transported over long distances under hot and humid conditions experience micronutrient losses.

Packaging selection is greatly influenced by shelf-life considerations and cost. Vitamin A must be protected from oxygen and light. Amber glass containers are the best choice for these fortified products because they are not permeable to oxygen and protect against light. However, glass is heavy, fragile, and expensive, so plastics are often used instead. Oxygen readily passes through plastic and will come into contact with the product. Light-proof containers, for example, dark glass or dark plastic, cans, and aseptic boxes will minimize the exposure to light. Because of high costs and the lack of availability of packaging material in developing countries, packaging assumes great importance and should be a major factor that is taken into account at the beginning of a fortification program (Johnson, Mannar and Ranum, 2004). For example, loss of vitamin A in sealed cans of oil is minimal, while losses from fortified cereals or fortified sugar can be in the order of 40% depending on ambient conditions and storage time (Allen et al., 2004).

Guidelines of the Micronutrient Initiative (MI) for premix packaging indicate that it should be packaged in air and watertight containers well protected from exposure to light. Typical packaging is a polyethylene bag inside a heavy, cardboard box, fibre cartons or metal containers (Johnson and Philar, 2005). The package should be such that the bag can be easily resealed and the box closed after a portion of the product has been removed. Premixes should be kept in their original containers in a cool dry place prior to use. Once opened exposure to light and air should be minimised to prevent product degradation (Johnson, Mannar and Ranum, 2004).

In the CSIR final report on the stability tests and sensory evaluation of fortified food vehicles for the South African National Food Fortification Program it was found that during storage the stability of vitamin A in raw super maize meal was better in polyethylene bags than in paper bags (Kuyper, 2000). However, maize meal is mostly sold as large volumes in polyethylene bags, but in small volumes in paper bags. Figure 2.7 shows examples of various packagings.


**Figure 2.7:** Examples of maize meal packaging (25kg and 12,5kg in polyethylene bags and 1kg in a paper bag) as presented to consumers.

#### *2.5.3.10 Effect of storage*

The stability of micronutrients in fortified maize flour stored at room temperature is good. One study showed that yellow maize flour retained all its vitamin $B_6$, over 95% of vitamins A, $B_1$, and $B_2$, and about 85% of folic acid activity after six months storage at room temperature (Ranum, 1999). Flour enriched with a vitamin-mineral premix by Cort et al. (1976) also demonstrated excellent stability on storage at room temperature. Under conditions of accelerated storage at elevated temperature (45°C), however, there was substantial loss of vitamin A beyond 4 weeks of storage. Parrish et al. (1980) also reported good stability of enriched wheat flour stored at room temperature, but losses of about 50% in flour stored at 40°C for 6 months. Warm and humid storage conditions adversely affect the stability of some micronutrients, such as vitamin A (DSM/USAID, n.d.a). This must be considered in humid environments where warehouses are not climatically controlled and temperatures can rise to 45°C as often happens in certain rural parts of the country.

#### *2.5.3.11 Food preparation*

A second type of nutrient loss occurs during food preparation. These food preparation losses affect how much of each micronutrient will actually be consumed (Johnson, Mannar and Ranum, 2004). Repeated heating, as may be experienced with vegetable oils used for frying, is known to significantly degrade vitamin A (Clarke, 1995). The stability of vitamins and minerals in cooked foods made with fortified maize flour is good. Only vitamin A showed a loss of between 10 and 15% after cooking maize flour for five minutes. According to analyses done in South Africa, the losses of vitamin A during the traditional cooking of maize meal is “somewhat higher” than for maize flour, probably due to the different time-temperature conditions (DSM/USAID, n.d.a). In the final report prepared by the CSIR

on the stability tests and sensory evaluation of fortified food vehicles for the South African National Food Fortification Program it was found that the mean cooking losses of vitamin A after 20 minutes of steaming in super maize meal were 53%, in special maize meal mean cooking losses were 41% and in sifted maize meal 45% (Kuyper, 2000).

**Figure 2.8:** Examples of maize porridge cooked from white maize meal

### 2.5.4 Summary

To achieve the required level of nutrients in fortified products reaching the consumer, manufacturers have to estimate processing and storage losses and add the necessary excess during production. To provide the best product to the consumer, the concept of overage should be introduced. Overage is the use of data on nutrient stability to calculate the amount of added nutrient so that the anticipated level of the nutrient at the end of the product's shelf life is in accordance with the level indicated on the package (Wirakartakusumah and Hariyadi, 1998). The introduction of new processes, equipment and packaging materials can affect processing and storage losses and hence fortification procedures (Clarke, 1995).

It would be feasible to add vitamin A to any kind of flour or maize meal. The primary constraint is the cost. Inclusion of vitamin A can double or triple the cost of a cereal fortification program. Vegetable oil may be a better carrier because the form of vitamin A that can be used in oil is cheaper and the stability is somewhat better. However, in many countries, wheat flour or maize meal may be the only processed foods consumed widely.

## 2.6 Sampling

Food sampling concerns the selection of the individual units of food, food products or bulk foodstuffs from the food supply or source, whether it be from the land, market place, manufacturing/food outlet or from the homes of the members of the study population (field sample). One of the main objectives of food sampling is to provide representative mean values for individual components (nutrients) in foods (Greenfield and Southgate, 2003).

The sampling procedure depends on the aim of the study, e.g. should the sample be representative for the whole country or only for a specific area or project or should the sample cover different seasons or be collected during one growing season. Sample units should preferably be randomly selected.

The following points highlight the important aspects of the sampling procedure.

- Where is the food consumed and by how many?
- How is the food consumed – raw or cooked?

- Are market statistics available? This provides information on the importance of the foodstuff in the food chain. Determine and collect the most used foods/recipes/cooking methods per region/sampling area.
- The population (total amount) of food items may be supplied to or distributed through an entire nation or region or be only typical of a particular sub-population group (e.g. ethnic group or tribe) (SAFOODS, 2010).

A sample is a single unit or a collection of units (e.g. packages, bunches, number of roots, fruits or items) representative of the total population of the food. Sample units must be taken from the available types and forms of the food for which the nutrient composition estimates are being determined. Most sampling schemes adopt a standard of at least 10 food sample units. However, available funds are often a limiting factor in the number of samples that can be analysed (SAFOODS, 2010).

Proper handling and transport of the samples is important to prevent nutrient losses. The samples must be properly identified and described. The sell by dates or batch numbers, the time and date of collection, and the location of collection should be reported. Samples must be packed in suitable containers; especially those that need refrigeration to avoid loss or damage to the food product, particularly of moisture loss or dark containers to avoid vitamin losses. Detailed guidelines on sampling procedures and the handling of the samples must be provided to the person responsible for the collection of the sample (Greenfield and Southgate, 2003).

In this project, convenience sampling was used to sample the major maize meal brands determined by market share and shelfe space. Maize meal samples were selected randomly from shelves in retail stores and outlets of varying size. Immediately after purchase, samples were transported directly to the laboratory in containers that protected them against direct light and heat.

## 2.7 Measuring the vitamin A content of South African fortified white maize

Development or selection of analytical procedures should be based on consideration of accuracy and precision of measurements, available facilities and equipment, simplicity of procedure and rapidity of determination. Only internationally recognised methodologies should be used (Clarke, 1995). A number of points impact on the suitability of various methods for the determination of vitamin A. These factors include:

- size of the test portion,
- efficiency of extraction procedures,
- chromatographic techniques, and
- adequacy of method validation (Blake, 2007).

### 2.7.1 Size of the test portion

For the purpose of testing vitamins A, E and β-carotene the European Committee for Standardisation (CEN, 2000) and the AOAC International (AOAC, 2006) state that test portions of a wide range of food products varying in weight between 5 and 10g should be used.

### 2.7.2 Extraction procedures

Extractions are usually made either by saponification/solvent extraction or by direct solvent extraction. Supercritical fluid extraction has been reported as an alternative, but has not yet been officially accepted (Blake, 2007). Saponification is commonly

used to liberate bound or esterified forms of the vitamin. Saponification is generally performed under reflux conditions with additions of antioxidants such as ascorbic acid or butylated hydroxytoluene (BHT) together with nitrogen flushing to reduce oxidation losses (Hulshof, 2005). After saponification, a liquid-liquid extraction (LLE) step, with non-polar organic solvents is performed. The organic phases are pooled, evaporated to dryness and redissolved in the liquid chromatography (LC) mobile phase. Solid phase extraction (SPE) as an alternative to LLE can also be used, but needs further evaluation (Blake, 2007).

### 2.7.3 Chromatography

Several techniques have been used for analysis including liquid chromatography (LC), gas chromatography (GC), spectrophotometry and capillary electrophoresis (CE). However the most widely used and preferred method is LC with UV detection (Greenfield and Southgate, 2003; Blake, 2007). The official methods recommend either reverse phase C18 (RP-C18) or straight phase C18 columns (AOAC, 2006; CEN, 2000). The calibration standards for vitamin A must be checked for purity by spectrophotometric procedure and a correction applied (Hulshof, 2005).

### 2.7.4 Method validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose. Many analysts focus on validating a method for precision, limit of detection (LOD), limit of quantification (LOQ), linearity and range (Green, 1996). The use of inter-laboratory studies and reference material is a prerequisite for checking correct application of analytical methods and to check accuracy (Blake, 2007).

In this study a method was optimised and validated to determine vitamin A in maize meal, maize porridge and liver samples. Alkaline saponification of the test material to eliminate fats, liberate natural retinol in the cells and hydrolyse added vitamin A to retinol was used. This was followed by ether extraction of unsaponifiable material. Quantification was done by HPLC and photo diode array (PDA) detection. The concentrations of the standards were calculated by using the Beer-Lambert Law.

## 2.8 Studying the relative bioavailability of vitamin A in fortified maize meal

Measuring the change in serum retinol concentrations following intervention can be used to determine the relative bioavailability of vitamin A, but numerous factors affect results from this approach. The vitamin A in the blood is tightly regulated and dependent on vitamin A status and the amount administered in the dose or meal (Van Lieshout et al., 2001). Other methods to evaluate bioavailability include postprandial chylomicron response (Parker et al. 1999), Caco-2 cells as an in vitro model of the human small intestinal mucosa to predict absorption (Garrett, Failla and Sarama, 1999; Liu, Glahn and Liu, 2004), stable isotope tracers (Vitamin A Tracer Task Force, 2004), and animal models (Baker, 2008). Results from the postprandial chylomicron response model are highly variable among subjects, limiting their use (Parker et al.; 1999). Caco-2 cells investigate bioaccessibility at the intestinal level, but do not reflect influences by the liver or other organs regulating enzyme activity and altering conversion factors. Isotope tracer studies in human hosts are the best method (Vitamin A Tracer Task Force, 2004), but their expense is limiting and factors such as diet and vitamin A status are difficult to control.

Although it would be ideal to use human subjects directly to answer this critical question regarding vitamin A availability, it was not possible because of the finite financial scope of the project. Animal research has contributed a great deal to what we know today about nutrition and metabolism. Appropriate animal models may contribute to a better understanding of vitamin A absorption (Baker, 2008). Animals also have the advantage of allowing invasive tissue sampling to assess nutrient status. Monitoring compliance with dietary protocols is easier with animals. Other considerations include availability of facilities and cost of the experiments to be performed.

### 2.8.1 Animal models in nutrition research

Animal models were instrumental in solving vitamin deficiency diseases. In his article on animals in nutrition research Baker (2008) discusses a list of examples, such as:

- beri-beri (chick thiamine deficiency),
- scurvy (guinea pig ascorbic acid deficiency),
- pellagra (dog, rat, pig, and chick deficiency of niacin and tryptophan),
- rickets (dog, rat, and chick deficiency of Ca, P, and/or vitamin D),
- night blindness (rat deficiency of vitamin A),
- dermatitis (rat deficiency of vitamin $B_6$),
- low fertility and muscle dystrophy (rat deficiency of vitamin E),
- haemorrhagic disease (chick deficiency of vitamin K), and
- anaemia (monkey, rat, and chick deficiency of folate and/or vitamin $B_{12}$).

Many of these diseases were initially thought to be of infectious and of bacteriologic origin. However, when an association with diet was noted, and when this was

followed by development of animal-model bioassays with defined purified diets, progress was quickly made in defining the disease condition and in reversing or preventing it with the proper vitamin containing food or (later) with the vitamin itself.

Chickens were selected as an appropriate animal model for this study because they are manageable, affordable and most importantly the metabolism of vitamin A in chickens is closely related to that of humans. Vitamin A is stored in the liver and chickens are also very susceptible to vitamin A deficiencies with symptoms very similar to human subjects (NRC, 1994). Chickens respond more rapidly to vitamin deficiencies than pigs (Baker, 2008). Therefore an effect caused by different diets will become evident over a shorter period of time. Significant results are most likely to be obtained in a study using chickens to determine the relative bioavailability of the vitamin A fortificant in fortified maize meal when comparing different maize meal diets with each other.

## 2.9 Concluding Remarks

Vitamin A deficiency is a public health concern in South Africa as is he case in many low income countries particularly in preschool-aged and school-aged children, as well as women of reproductive age. Fortification of foods with vitamin A is a potentially effective food-based intervention to prevent or control vitamin A deficiency in low-income countries where undernutrition and poverty coexist. According to the literature survey, maize meal is a suitable vehicle for vitamin A fortification. Fortification should be guided by estimates of intakes of vitamin A in the diet, levels of fortificant required to meet dietary requirements, stability of the fortificant under ambient conditions, stability under usual conditions of food preparation (e.g., high temperature and humidity) and product storage conditions.

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