CHAPTER 3

VITAMIN A CONTENT IN FORTIFIED WHITE MAIZE MEAL AS PURCHASED AND IN PORRIDGE AS CONSUMED IN SOUTH AFRICA

Food Research International, Article in press
(http://dx.doi.org/10.1016/j.foodres.2011.03.033)

A method to determine vitamin A in maize meal was optimised and validated. The method was accredited by the South African National Accreditation Services (SANAS). This method was subsequently used to determine the vitamin A content of maize meal samples, as well as the corresponding maize porridge samples. Retention of vitamin A in cooked porridge was calculated.

3.1 Abstract

In 2003, Department of Health of South Africa embarked on a mandatory fortification program of maize meal as part of a nutrition program to alleviate malnutrition. The aim of this study was to determine the vitamin A content in fortified white maize meal and the maize porridge prepared with it as purchased and consumed. The highest mean vitamin A concentration in the maize meal was 261 µgRE/100g and the lowest mean vitamin A concentration was <19 µgRE/100g. Pertaining to regulation the final minimum level of vitamin A in fortified maize meal shall not be less than 187.7 µgRE/100g (Department of Health, 2003). The average retention of vitamin A in maize porridge as the difference in vitamin A concentration between raw maize meal

and cooked porridge was calculated as 39.8%. Although fortification of maize meal can improve the vitamin A intake of the population, it must be regularly monitored and regulated to be beneficial. If not then fortification might as well be voluntary.

Key words: maize meal, porridge, vitamin A fortification, retention, staple foods

3.2 Introduction

Food fortification of staple foods with micronutrients is one of the food-based strategies employed to alleviate micronutrient deficiencies in a population. Vitamin A deficiency (VAD) is a major nutritional concern in poor societies, especially in lower income countries. Its presence as a public health problem is assessed by measuring the prevalence of deficiency in a population, represented by specific biochemical and clinical indicators of status (WHO, 2009a).

In South Africa, 1 in 3 preschool children has a serum retinol concentration < 0.7 µmol/L (SAVACG, 1996), and 55–68% of children aged 1–9 years consume < 50% of the recommended dietary intake of vitamin A (700 µg retinol equivalents) (NFCS, 2000). The main underlying cause of VAD as a public health problem is a diet that is chronically insufficient in bioavailable vitamin A that can lead to lower body stores and fail to meet physiologic needs (e.g. support tissue growth, normal metabolism, resistance to infection) (WHO, 2009a).

In 2003, the Department of Health of South Africa embarked on a fortification program of wheat flour and white maize meal as part of a multipronged approach to alleviate malnutrition. These foods were identified during the National Food Consumption

Survey (NFCS, 2000) as most often consumed (staple) food products, thereby reaching lower income consumers most vulnerable to micronutrient malnutrition. According to regulations protected, stabilized Vitamin A palmitate containing 75 000 µRE activity per gram premix must be added to the maize meal (special, super, sifted and unsifted) to give a final, minimum level of the micronutrient in the fortified maize meal of 187.7 µRE activity per 100 g (Department of Health, 2003).

The success of a fortification program depends, amongst other factors, on the content of the fortificants in the fortified products. A number of factors, 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. The choice of a vitamin A fortificant is largely governed by the characteristics of the food vehicle. Because preformed vitamin A (retinol) is an unstable compound, in commercial preparations it is esterified, usually with palmitic or acetic acid, to the more stable corresponding esters. Retinyl acetate and retinyl palmitate are the main commercial forms of vitamin A that are available for use as food fortificants in cereals (WHO, 2006). Maize meal can technically be fortified with vitamin A because vitamin A is stable in dry products without producing organoleptic changes. Vitamin A is quite stable when heated to moderate temperatures in the absence of oxygen and light. However, as is the case for some other vitamins, high humidity, high temperatures and the presence of oxygen and light can adversely affect the vitamin A content during the preparation of maize meal products such as traditional maize porridge (or “pap”). This reaction is also accelerated in the presence of trace metals (Mehansho et al., 2003; WHO, 2009b).

It would thus be feasible to add vitamin A to any kind of maize meal with the primary constraint being cost. Inclusion of an expensive micronutrient such as vitamin A can double or triple the cost of a cereal fortification program due to the cost of the

micronutrient, extra equipment needed for mixing, quality control through quantitative vitamin analysis and additional personnel (WHO, 2009b).

An early quality control step to make sure that the food fortification program will have an impact on vitamin A deficiency is to verify the vitamin A content in the fortified maize meal as well as in the cooked products. If these comply with regulations, a reduction in vitamin A deficiency can be assumed in the long term.

Therefore the aim of this study was to determine the vitamin A content of fortified white maize meal from different manufacturers (brands) as purchased from the shelves of different retailers, as well as in the traditional maize porridge as consumed. Due to financial constraints and the fact that the vitamin A fortificant is stable under dry storage conditions (WHO, 2009b), a shelf-life study was not done.

3.3 Materials and Methods

Note: Light should be avoided during preparation and storage of samples and standards to prevent degradation of vitamin A.

3.3.1 Samples

Sixty-two samples of fortified white maize meal from readily available brands (nine different brands) were collected from supermarkets in the Tshwane-metropolis between July 2005 and November 2008. See Addendum B for a list of brands. Samples were stored in their original packaging at room temperature in the laboratory until analysis. Analyses commenced within a week after every sampling. Brands with a higher market

share according to the Markinor/Sunday times Top Brands Results (2008) have higher representation within the data set. A wide variety of maize porridge preparation methods is known, but due to resource constrains only preparation of the traditional soft porridge was selected for laboratory simulation. The maize porridge was prepared according to a standardised method from seven different brands of maize meal purchased from the supermarkets. Each maize meal and its corresponding porridge sample were analysed in duplicate for moisture and vitamin A content using accredited methods according to ISO/IEC 17025:2005. The methods were accredited by the South African National Accreditation System (SANAS).

Figure 3.1: Different maize meal brands sampled during the study.

3.3.2 Preparation of porridge samples

Traditional soft maize porridge was prepared according to the following recipe: One litre (1L) of tap water was heated to boiling point in an aluminium saucepan. A 180 g sample of dry maize meal was added and stirred thoroughly. The heat was turned down and the porridge was left to simmer with the lid on for 30 minutes, whilst stirring

occasionally. The end-temperature of the samples was between 75 ℃ – 80 ℃. The samples were prepared with the assistance of people familiar with the preparation method, texture and consistency of this type of traditional porridge. Porridge samples were left to cool in covered glass containers and were stored under refrigeration (± 4℃) until the next day when they were analysed.

3.3.3 Gravimetric determination of dry matter

Dry matter was measured in the samples by determining the loss in weight of the sample after it had been dried in an oven at 105±1℃ for 16 hours. Weight loss is used to calculate dry matter content (AOAC, 2005a).

3.3.4 Determination of total Vitamin A as all-trans retinol

3.3.4.1 Chemicals and Standards

Diethyl ether, ethanol (99.9%), potassium hydroxide (KOH) and sodium chloride (NaCl) were obtained from Merck Chemicals. Butylated hydroxyl toluene (BHT) and retinol standard were purchased from Sigma-Aldrich. HPLC-grade methanol was obtained from Labscan and pure, crystallised ascorbic acid from Associated Chemical Enterprises. A stock standard solution of retinol was prepared in ethanol. Working standard solutions were prepared in ethanol and the concentration of each standard was determined with a spectrophotometer (Lambda 25, PerkinElmer), using an extinction coefficient of 1850 (λ_{max} = 325 nm).

3.3.4.2 Sample preparation

Approximately 5 g maize meal or 8 g porridge was weighed into a round bottom flask using a Precisa XT220A analytical balance (readability ± 0.1mg).

3.3.4.3 Saponification

The weighed sample was mixed with 25ml of a 0.5% ascorbic acid-ethanol-methanol solution until sample material was moistened. Glass beads were added and purged with nitrogen gas. The sample was saponified at boiling point for 30 min under reflux with 50% KOH (w/w). The flask was swirled from intermittently to prevent the material from adhering to the sides. After saponification the sample was cooled on ice for 5 minutes.

3.3.4.4 Extraction and phase transfer

The contents of the round bottom flask were filtered through Whatman no 4 filter paper into a separating funnel. The flask was rinsed with a minimum amount of water (no more than 15 – 30ml) and filtered into the separating funnel. Subsequently the round bottom flask was washed with diethyl ether containing 0.01% BHT and added to the separating funnel. The mixture was allowed to expand several times before the actual extraction (in such conditions emulsions can be largely avoided). The ether layer was decanted into another separating funnel. Extraction was repeated two more times combining all the ether fractions in the same separating funnel. The ether fraction was washed with distilled water until neutral. Should any emulsions form during the wash and extraction procedures, NaCl can be added. The ether fraction was then transferred to a 250ml volumetric flask and made up to volume with diethyl ether. An aliquot from

the ether extract was evaporated to dryness with a rotary evaporator under partial vacuum in a water bath at a temperature < 40°C. The residue was dissolved in ethanol and injected into the HPLC.

3.3.4.5 HPLC

The HPLC system (Shimadzu) consisted of a Quaternary gradient pump (model LC-20AD), a solvent degasser (model DGU-20A5), an auto-injector (model SIL-20A, 230V), a Photodiode Array Detector (DAD) with a thermostatted standard cell (model SPD-M20A) and control and integration software (LCsolution Ver. 1.1). A Nucleodur 250X4 mm reverse phase C18 column (5µm particle size) with guard column was used. Separations were achieved using a mobile phase of 97% methanol in deionised water and a flow rate of 1.0 mL/min. Separations were performed at 325 nm for the identification and quantification of retinol.

3.3.4.6 Calculation

Quantification was performed by using an external calibration procedure. The peak height of five different concentrations of a retinol standard and a blank (ethanol) were used for calibration. The calibration standards were checked for purity and concentration by spectrophotometric procedure.

3.3.4.7 Method validation

Retinol was determined by using peak height and regression analysis. From the calibration curve, linearity, range, limit of quantification (LOQ) and limit of detection (LOD) were determined. The LOQ and LOD were calculated from the calibration lines

that defined linearity, using the Long and Winefordner criterion (Long and Winefordner, 1983) as expressed in the following equations.

$$LOQ = \frac{10 \times S}{a}$$

$$LOD = \frac{3 \times S}{a}$$

where *a* is the slope of the calibration line and *S* is the standard error of the intercepted point. The LOQ, LOD and precision of the method are shown in Table 3.1.

Repeatability of the method was determined by analysing the same sample eight times on the same day. From this data the mean, standard deviation (SD) and coefficient of variation (CV%) were determined. Reproducibility of the method was determined by analysing a control sample (infant cereal with added vitamins) over a period of time (≥ 7 times). The mean, standard deviation and coefficient of variation were calculated. A control chart was implemented to monitor validity of the analysis. The action limits were set as the mean plus or minus three times the standard deviation of the reproducibility data. Warning limits were set as twice the standard deviation. The control sample (infant cereal) was analysed with every batch of ten samples or less. The results of the control sample were recorded on the control chart and evaluated. If the result fell outside the action limits, the analysis was repeated. Standard reference material (SRM2383 – baby food composite) and inter-laboratory comparisons (using fortified maize meal as a control sample) were used to prove accuracy.

3.3.5 Calculation of the retention of vitamin A in porridge

Retention of vitamin A was calculated based on the following equation (Bengtsson et al., 2008):

$$\%\ \ \mathrm{Re}tention = \frac{retinol\ \ content\ \ per\ \ g\ \ porridge\ \ (dry\ \ basis)}{retinol\ \ content\ \ per\ \ g\ \ meal\ \ (dry\ \ basis)} \times 100$$

The vitamin A result of each maize porridge sample was compared with its corresponding maize meal sample.

3.3.6 Statistical Analysis

Data was analyzed by Analysis of Variance (ANOVA), Pearson's correlation test and Principal Component Analysis (PCA), which were applied to determine and explain variation in the data. The data was analysed with SAS statistical software version 9.2 (SAS, 1999).

3.4 Results and Discussion

3.4.1 Method performance

Blake (2007) evaluated several official AOAC, CEN and ISO methods for the determination of fat soluble vitamins. These methods involve alkaline saponification of the test material to eliminate the fat without removing the fat-soluble vitamins, liberate natural retinol in the cells and to hydrolyse added vitamin A in fortified food products to retinol. After saponification, the vitamins are separated by liquid-liquid extractions with organic solvents. The organic phases are pooled and evaporated to dryness. This is then redissolved in the mobile phase and usually analysed by liquid chromatography.

Table 3.1: Limit of Detection (LOD), Limit of Quantification (LOQ) and Precision

	LOQ	LOD	Repeatability			Reproducibility		
	(µg/100g)	(µg/100g)	Mean	SD	CV%	Mean	SD	CV%
All-*trans* Vitamin A	20	7	0.536	0.057	10.593	0.588	0.082	14.017

The performance of the method was determined as summarised in Table 3.1. Precision was assessed using the criteria developed by AOAC International (2005b). The calculated Horwitz Ratio (HorRat) of 1.28 for repeatability and 1.13 for reproducibility is consistent with the guideline range of 0.5 – 2.0. Linearity was confirmed by least-squares regression analysis of the calibration standards. The UV-signals (peak height) were linear in the range 0 - 566 µg/100ml with an accepted linearity of $R^2 \geq 0.98$. Accuracy was determined by two different inter-laboratory studies as well as with a standard reference material. The values were compared and acceptable z-scores (<2) obtained. The method was validated for linearity, repeatability, reproducibility, LOD, LOQ and accuracy.

3.4.2 Vitamin A content of maize meal as purchased in supermarkets

Vitamin A concentrations were evaluated for outliers using the Q-test. Outliers were excluded from the data set. The mean vitamin A content per brand can be seen in Figure 3.2. Brand A had the highest mean vitamin A concentration (261 µgRE/100g), and is also the only brand analysed with a higher mean vitamin A concentration than the regulatory requirement of 187.7 µgRE/100g (Department of Health, 2003). Brand D had the lowest mean vitamin A concentration (<19 µgRE/100g).

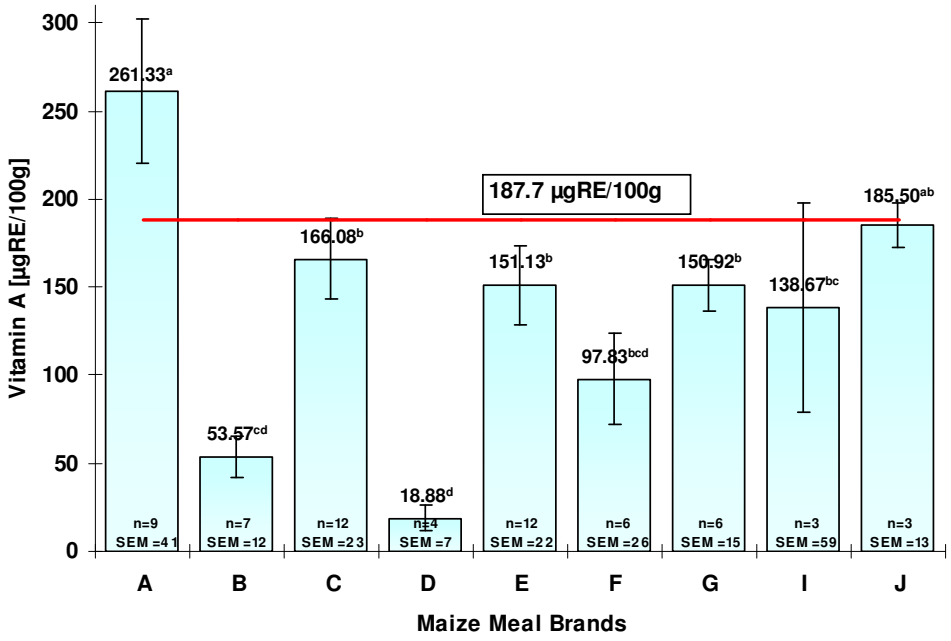

Figure 3.2: Mean vitamin A concentration (µgRE/100g) of different brands of maize meal as purchased in supermarkets in the Tshwane-metropolis

According to fortification principles, the maize meal is fortified with protected and stabilised Vitamin A palmitate to improve stability of the added vitamin. The protected particles tend to be heterogeneously distributed throughout the maize meal, and this may influence the precision of the analyses (Blake, 2007). This may also cause segregation of the maize meal leading to a variation of vitamin A content within one brand of maize meal. This could explain the large variation in results within a specific maize meal brand.

Although there is a regulatory requirement for vitamin A, a large variation in vitamin A content between different brands was observed. This variation may be an indication of poor quality control at the millers. Poor or variable quality of fortification premixes, unreliable and poorly fabricated equipment, and inadequate manufacturing and marketing facilities lead to poor product quality (Johnson, Mannar and Ranum; 2004).

Another reason for the low vitamin A concentration in the maize meal might be the incorrect storage conditions of the maize meal on the shelves of retailers. It was observed during the sampling of the maize meal that some of the maize meal was exposed to sunlight. As was previously mentioned, vitamin A is light sensitive (DSM/USAID, n.d.b). If the maize meal remains on the shelves for several days, it may have an effect on the vitamin A content.

Fortification mixes supplied by unregistered suppliers and the stability of the vitamin A are challenges identified by the Department of Health of South Africa (de Hoop; 2010). Major obstacles to the implementation of an adequate food control system (FCS) occur when material sourcing, production, packaging, storage, transport conditions and delivery systems are sub-optimal. The lack of efficient and skilled manpower to carry out an effective FCS both at production and government levels, coupled with limited training opportunities are other major obstacles (Clarke, 1995). Moreover, legislation and regulation in South Africa may not be well developed. Enforcement mechanisms are probably not yet adequately developed and established to ensure that government standards are met.

3.4.3 Vitamin A concentration of maize porridge

Vitamin A and dry matter content were determined for each of the maize meal samples and the corresponding porridge samples. An average retention of 39.8% was observed. Results are shown in Table 3.2.

Table 3.2: Vitamin A content (µgRE/100g dry matter) of maize meal and maize porridge samples of seven different brands

Brands	Maize Meal (Raw)	Maize Porridge (Cooked)	% Retention of Vitamin A
	*Vitamin A (µgRE/ 100g DM)		
A	174.7	85.5	48.9
B	93.7	45.5	48.6
C	238.8	83.9	35.1
D	10.5	5.90	55.8
E	237.8	45.3	19.0
G	245.4	90.5	36.9
J	201.6	69.1	34.3
		Average retention	**39.8±12.3**

*Vitamin values are reported on a dry weight basis.

The average cooking losses of vitamin A in super maize meal according to the CSIR-report on the stability of fortified food vehicles for the National Food Fortification Program was reported as 53% (Kuyper, 2000). This relates to an average retention of 47%. When the more recent nutrient composition values of super maize meal, as reported by Wolmarans, Danster and Chetty (2005) were used, retention of 39.5% was calculated for soft porridge, which compared favourably with results of this study. The result is best explained by the fact that vitamin A is stable under inert atmosphere. However, it rapidly loses its activity when heated in the presence of oxygen (Lešková et al., 2006).

A Pearson's correlation test (Table 3.3) and principal component analysis (PCA) were done to determine whether there was an association between the retinol concentration and dry matter (DM) in the maize meal (raw) and the retinol concentration and dry matter in the maize porridge (cooked). The correlation between retinol and dry matter in the raw maize meal is not significant ($r = -0.525$; $p > 0.05$). This is expected as retinol is not related or dependant on the dry matter content. The correlation between the dry matter in the raw maize meal and in the cooked porridge is significant ($r = -0.542$; $p \leq 0.046$). Although the dry matter contents in the raw maize meal and in cooked maize meal (porridge) are dependant on each other, it must be taken into account that the matrix of the maize meal changes during cooking because water is absorbed and heating causes starch gelatinisation. As is expected, the correlation between the retinol in the maize meal and in the maize porridge is high ($r = 0.833$; $p \leq 0.000$), but not identical. This is supported by the retention values calculated and is an important consideration in determining fortification levels.

Table 3.3: Pearson Correlation matrix between retinol content and dry matter (DM) of the raw maize meal and retinol content and dry matter (DM) of the cooked maize porridge

Variables	Raw-DM	Raw-Retinol	Cooked-DM	Cooked-Retinol
Raw-DM		-0.525 (p>0.054)	-0.542* (p≤0.046)	-0.576* (p≤0.031)
Raw-Retinol			0.087 (p>0.767)	0.833*** (p≤0.000)
Cooked-DM				-0.038 (p>0.897)

Significant levels: * p≤0.05; ** p≤0.01; *** p≤0.001)

The PCA explained 77.71% of the variation in the data. See Figure 3.3 for the biplot of retinol and dry matter (DM) in maize meal (raw) and maize porridge (cooked). On PCA1 (x-axis) 50.94% of the data was explained. The variables retinol-raw (31.38%), DM-raw (-25.89%) and retinol-cooked (23.98%) contributed the most to the variation. On PCA2 (y-axis) 26.77% of the data was explained by the variables DM-cooked (46.68%) and retinol-cooked (25.57%). If the retinol value in the raw and cooked samples were high then the dry matter was low.

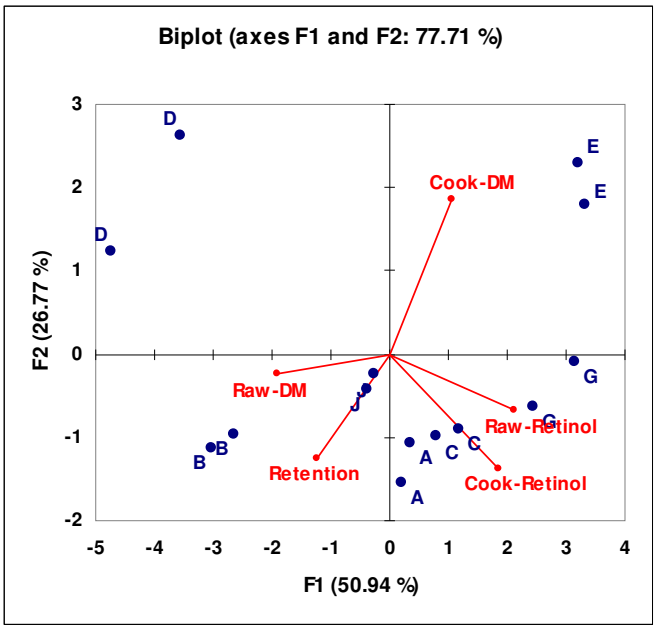

Figure 3.3: PCA Biplot of retinol and dry matter (DM) in maize meal (raw) and maize porridge (cooked)

To understand the contribution of the fortified maize meal to the vitamin A intake of children, the results of the different brands were translated (see Table 3.4) into Recommended Dietary Allowance (RDA), Daily Recommended Intake (DRI) and Recommended Safe Intake (RSI) values (FAO; 2001) . The RDA and DRI for children

1-3 years and 4-9 years is 300 and 400 µg retinol/day respectively. The RSI values used by the FAO to correct VAD in a population are 400, 450 and 500 µg retinol/day for children 1-3 years, 4-6 years and 7-9 years respectively. According to the National Food Consumption Survey (NFCS, 2000) the average portion size of maize porridge reported for children 1-3 years was 410 g/person/day and for children 7-9 years was 500 g/person/day. This relates to an average portion size of 455 g/person/day for children 1-9 years (12-108 months). This portion size was used in the calculation of the average intake of vitamin A from soft maize porridge based on the concentration levels as determined in this study.

The highest contribution to the RDA and RSI was made by maize meal Brand G and the lowest contribution by Brand D. On average, 17% of Recommended Dietary Allowance (RDA) for children 1-3 years and 13% of RDA for children 4-9 years old were met by the fortification of the maize meal (Table 3.4). It must be kept in mind that this would be a zero percentage if the maize meal was not fortified, but that it should be a 31% of RDA according to legislation. This contribution is even lower when compared to at the Recommended Safe Intake levels using by the FAO. When using the same retention values as calculated in this study, the contributions to the RDA for children from maize meal that is fortified according to the minimum levels as stipulated in the regulations (ie. 187.7 µg vitamin A/100g), will only be 16% and 12% respectively. This is approximately half of the 31% government intended to at least achieve through the mandatory fortification of maize meal (Department of Health, 2003).

Table 3.4: Vitamin A content (µgRE/100 g) of maize meal and maize porridge samples of seven different brands and the contribution towards the Recommended Daily Allowances (RDAs) and Recommended Safe Intake (RSI) of vitamin A for 1-9 year old children

Brands	Maize Meal	Maize Porridge	Vitamin A/portion size*	% RDA#	% RDA#	% RSI$	% RSI$	% RSI$
	Vitamin A (µgRE/100g)		(µgRE/100g)	(1-3 years)	(4-9 years)	(1-3 years)	(4-6 years)	(7-9 years)
A	155	16	71	24	18	18	17	14
B	83	8	34	11	8	8	7	7
C	212	15	67	22	17	17	15	13
D	9	1	5	2	1	1	1	1
E	210	9	42	14	10	10	9	8
G	217	18	80	27	20	20	18	16
J	180	13	56	19	14	14	12	11
			Average contribution	**17±9**	**13±6**	**13±6**	**11±6**	**10±5**

* Portion size: 445 g/person/day for maize porridge (NFSC, 2000)

#The RDA and DRI of vitamin A for children 1-3 years and 4-9 years is 300 and 400 µg vitamin A/day respectively.

$The RSI values of vitamin A are 400, 450 and 500°µg vitamin A/day for children 1-3 years, 4-6 years and 7-9 years respectively.

In essence, food fortification can contribute to the improvement of the overall vitamin A status of children aged 1– 9 years. This was also reported by Steyn, Nel and Labadarios (2008) in their analysis of dietary micronutrient intake pre- and post-fortification using existing dietary data. However, it is suggested that the level of vitamin A fortification be raised to at least achieve the intended 31% RDA contribution or even higher. A review by Allen and Haskell (2002) indicated that the risk of excessive vitamin A consumption from fortified foods in women and young children is likely to be negligible.

3.5 Conclusion

The quantitative difference in the vitamin A content of fortified white maize meal as purchased and consumed is shown. Vitamin A concentrations varied from the highest concentration of 226 µgRE/100g to the lowest concentration of <19 µgRE/100g. Reasons for the large variation in vitamin A concentration could be explained by substandard premixes, inadequate mixing of the fortification premix into the maize meal, segregation of the fortificant and the maize meal, storage losses or poor quality control by the milling companies. The average retention of vitamin A in maize porridge was calculated as 39.8%. The low retention observed might be an indication of poor stability of the vitamin A fortificant under cooking conditions.

The lower than regulated concentration levels and the low retention of the vitamin A found in this study, probably contribute towards the RDA for vitamin A for 1-9 year old children not being met. This may explain the results found during the National Food Consumption Survey Fortification Baseline Study (NFSC-FB-I) done in 2005. One of the main findings in this study was that the prevalence of poor vitamin A status in

children appeared to have increased when compared with previous national data (NFSC-FB-I; 2008). This emphasises the need for an efficient food control system (FCS) in South Africa in order that food fortification processes meet nutritional objectives. Evaluation of some of the more mature fortification programs, mainly in Latin America, suggests that the quality of vitamins, minerals and micronutrient premixes may be a barrier to achieving the required health and nutrition results (DSM; 2009). A consideration that should be high on the priority list of the overall micronutrient strategy is the adequate and efficient monitoring, evaluation, regulation and quality assurance of all premixes and maize meal.

Correcting VAD in populations at risk of deficiency is an investment in improving human development. Based on the results and evaluation of this study, it appears that fortification of maize meal can contribute to the micronutrient intake of children under nine years of age and improve the overall micronutrient density of their diets. It is necessary to take into account whether the efficacious nutrient supply can be met. The efficacious nutrient supply depends on the amount of vitamin A-containing foods consumed, vitamin A content of each food consumed and bioefficacy of vitamin A in the food consumed (Van Lieshout and West, 2004). It is therefore important to also verify the vitamin A concentration in bread as this is the other food vehicle used for fortification and to evaluate the bioavailability of the added vitamin A.

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3.6 Acknowledgements

Sincere gratitude is expressed to Mrs Liesl Morey at the ARC-Biometry Unit for the statistical analyses and to the National Research Foundation for financial support under the focus area group of Prof Johann Kirsten, School of Agricultural and Food Science, University of Pretoria.

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