Stability of Vitamin a in Selected Nigerian Bread Made From Commercial Fortified Wheat Flour

UCHENDU Florence Ngozi
Department of Health Sciences
School of Science and Technology
National Open University of Nigeria
14/16 Ahmadu Way, Victoria Island
Lagos, Nigeria.

ATINMO Tola
Department of Human Nutrition
Faculty of Public Health
College of Medicine
University of Ibadan
Ibadan, Nigeria.

OYEWOLE Oyediran
Department of Epidemiology
Health promotion and Education
Faculty of Public Health
College of Medicine
University of Ibadan
Ibadan, Nigeria.

Abstract

Purpose: *Vitamin A deficiency (VAD) is a public health problem in Nigeria. Stability study was carried out to determine vitamin A contents of selected brands of commercial wheat flour and bread.*

Methods: *Retinyl Palmitate content of three samples of commercial wheat flour and bread randomly selected were analyzed at 0, 5 (bread) and 0, 60 days (flour) respectively at room temperature using High Performance Liquid Chromatography.*

Results: *Vitamin A contents of wheat flour and bread were 7.1 to 10.1 i.u. /g (wheat flour) and 0-3.4 i.u./g (bread). Significant difference existed between the mean vitamin A content of wheat flour and label declaration (30 i.u/g) (P< .05). Vitamin A stability was 0-91% (wheat flour) after 60 days and 0-68% (bread) after 5days. Mean stability was better in bread than in flour.*

Conclusion: *Wheat flour and bread contain vitamin A lower than the standard (30 i.u/kg).*

Key words: Vitamin A deficiency, Wheat flour fortification, Nigerian bread, Wheat flour, Vitamin A fortification

1.0 Introduction

Vitamin A deficiency (VAD) is a global public health problem in 118 countries, especially in Africa and South-East Asia (Rostami et al. 2007). An estimated 190 million (approximately 33%) pre-school-aged children and 19 million pregnant and lactating women are affected (Klemm, et al. 2010). The prevalence and number of cases of preschool children that are vitamin A deficient in Africa is 32% (33, 406 million) (West, et al. 2008). Recent data show that 30% of preschool children are vitamin A deficient (HarvestPlus, 2009). The main demographic groups most susceptible to vitamin A deficiency (VAD) are pre-school children (0-59 months), pregnant women and lactating mothers and sometimes school-aged children and adolescents (Chakravarty, 2000; Dary and Omar, 2002).

It is also becoming clear that vitamin A deficiency can extend through school age and adolescent years into adulthood (Ramakrishnan and Darnton-Hill, 2002). Vitamin A deficiency is a leading cause of high morbidity and mortality among preschool children, pregnant women and lactating mothers. A common cause of vitamin A deficiency might be a shift in the local diet to imported and ready-to-eat foods (Englberger et al. 2005). The increasing use of highly refined foods, and foods prepared from highly purified ingredients, may contribute to dietary vitamin A inadequacies in certain population (Manan, 1994).

Strategies to achieve this goal are vitamin A supplementation, food fortification of commonly eaten foods, nutrition education, dietary diversification, promotion and support for breastfeeding and biofortification. The fortification of commonly consumed processed foods is an alternative that has a number of advantages over supplementation (Sullivan and Bagriansky, 2008). It is a long-term strategy, preventive, cost effective and resolves the many issues of equity and access because it is population based (sphemory.edu, 2007). Food fortification is the addition of a nutrient to a food, whether or not is normally contained in the food for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups (WHO/FAO, 2006). It is a strategy to improve overall baseline micronutrient status (Timmer, 2010). Food vehicles especially staples in various countries have been identified and fortification is going on both on voluntary and mandatory bases. Examples of foods that have been fortified with vitamin A are wheat and maize flour, sugar, oil, condiments, complementary food, salt etc.

Nigeria is the only country in Sub-Saharan Africa to mandate the fortification of three staple foods with vitamin A namely, wheat and maize flour, vegetable oil and sugar (Micronutrient Initiative, 2007). About 80 % of flour, 87% of vegetable oil, and 90% of sugar met the minimum quality standards (Gana, 2011**).** Wheat and maize flour fortification is a preventive food-based approach to improve micronutrient status of populations over time that can be integrated with other interventions in the efforts to reduce vitamin and mineral deficiencies when identified as public health problems (WHO, 2009). While fortification of flour with vitamin A has been initiated in a few countries, questions remain about the cost of adding vitamin A to flour, as well as the stability of the vitamin A in flour and flour products (FFI, 2008). The aim of the study therefore is to determine the stability of vitamin A in commercial flours and bread.

1.1. Background of the study

While fortification of flour with vitamin A has been initiated in a few countries, questions remain about the cost of adding vitamin A to flour, as well as the stability of the vitamin A in flour and flour products (FFI, 2008). Technological issues to food fortification need to be fully resolved especially with regards to appropriate levels of nutrient, stability of fortificant, nutrient interactions, physical properties and acceptability by consumers (WHO/FAO, 2006).

1.2. Justification of the study

This study is useful to Nutritionists, Food technologists, Stakeholders in vitamin A wheat flour fortification and general public. Combating vitamin A deficiency through vitamin A wheat flour fortification will reduce childhood morbidity and mortality aimed at achieving the 4th Millennium Development Goal by 2015. This study will help to assess the effectiveness of bread produced from vitamin A fortified commercial wheat flour in meeting this goal. The result obtained may guide government policies on vitamin A fortification of wheat flour. The study will also add to existing knowledge.

2.0. Materials and Method

2.1. Food and raw material sampling

Commercial flour and bread samples were collected from bakeries. Three medium sized bakeries volunteered to participate in the study. Composite samples of flour brands used in baking the bread samples were randomly collected from only two bakeries before the bread was baked. At least 30% (standard laboratory procedure) of each sample was randomly selected immediately after baking as done by Oyunga-Ogubi et al. (2009). A total of 3 wheat flour and 3 bread brands were sampled for analysis.

2.2. Labeling of samples

Samples were labeled A, B, C, D, AB, BB, CB, DB, etc for easy identification.

2.3. Storage Studies

After initial analysis, vitamin A contents of bread and flour samples were determined at 0, 5 (bread) and 0, 60 (flour) days of storage respectively at room temperature.

2.3.1 Baking Stability of vitamin A in bread

Retinyl palmitate content of bread and flour used in baking the bread were determined using HPLC as described in Eitenmiller and Landen Jr. (1998) and Norden and Kendall (2000). Vitamin A losses were computed in percentage to estimate baking losses as done by Mahmood et al. (2008).

3.0. Vitamin a Extraction

The quantities of vitamin A were measured by direct method similar to that of Thompson 1998. The quantities of vitamin A in flour were measured using High-performance liquid chromatography, UV detector (Shimadzu Corporation, Kyoto,Japan) after 60 days. Vitamin A was de-proteinized from 5.0g sample using 10ml of Isopropanol (HPLC grade). About 0.10% Butylated Hydroxyl-Toluene (BTH) was weighed and added to each of the samples and vortexed for 2 minutes. 5ml of water was pipetted into the mixtures to improve the extraction efficiency. The samples were vortexed and allow standing for 2 minutes. Vitamin A extraction was done using Hexane. The vitamin A extracts were filtered into the 1.5ml ember vial bottles using 0.45µm disposable filter and syringe and injected into the chromatograph. Reading was taken at 325nm with a flow rate of 1.5ml/min and injection volume of 20µl. The Mobile Phase used was Methanol 98%, Water 2%. An Alltech column LC-18, 5micron (Dimensions 4.6mm x 150mm) was used. The All- Retinyl Palmitate, Sigma grade, type IV was used as internal standard. Standard was treated like sample. The minimum required quantity for this assay was 30 i.u/g. Vitamin content in the sample was calculated using the following formula as done by Eitenmiller and Landen Jr. (1998).

$$Cs = \frac{(As)(F_R)(V_E)}{(Ws)}$$

Where:
Cs = Concentration of sample in i.u/kg
As = Area of sample
F_R = Response /Correction factor of standard

$$F_R = \frac{\text{Area of the actual concentration of analyte (vitamin A)}}{\text{Area of vitamin A after calibration}}$$

V_E = Volume of Hexane used for extraction.
Ws = Sample weight

4.0. Result

4.1. Vitamin A contents of wheat flour and bread

Table 1 shows the vitamin A contents of wheat flour and bread as analyzed. The result of laboratory analysis showed that the flour samples contained vitamin A lower than the standard, 30, 000i.u/kg. Significant difference existed between the mean vitamin A content of wheat flour and label declaration (30 i.u/g) ($P< .05$). Mean vitamin A content was 8632.27±1525.9 and that is 28.7%.

4.2. Vitamin A contents of bread

All the bread samples contained vitamin A except one (Table 2). It was observed that the bread sample made with flour that had the highest vitamin A content had zero vitamin A.

4.3. Vitamin A stability in wheat flour and bread

4.3.1 Vitamin A stability in wheat flour

Table 3 shows the percentage stability of vitamin A in wheat flour. The stability of vitamin A in the samples had linear relationship with time ($P<.05$). The flour with the highest vitamin A content had zero content after 60 days. Only one sample (A) had very good stability (91.4%).

4.3.2. Vitamin A stability in bread

Table 4 indicates that the mean stability of vitamin A in bread is 63.2 ± 5.58. Fresh sliced bread made with flour with the highest vitamin A content had zero vitamin A stability. Mean stability lost was 39.2% ±5.3.

5.0. Discussion

The low level of vitamin A in the flour samples analyzed (7, 064-10, 000 i.u/kg) might be due to lack of total compliance and poor quality control of vitamin A premix and flour during production, transportation and storage. One flour sample had very good stability above that obtained by BASF, 2007 (80%); Solon et al. 2008 (81%) and Klemm et al. 2010 (70%) showing that good vitamin A stability in flour is achievable if the right measures are put in place. The initial vitamin A level in flour was significant with time ($P<.05$) corroborating the fact that vitamin A degrades with time (Manna 1994; Iherekonye and Ngoddy, 1995; Wirakartakusumah and Hariyadi, 1998). Vitamin A is affected by light, high temperature, oxygen, metallic ions, food composition and enzymes as a result of its chemical structure with many double bonds that are susceptible to degradation (Wirakartakusumali and Hariyadi, 1998). An abnormal observation was made where the vitamin A in one of the flour samples seemed not to obey this trend of degrading with time. At 18 days old, it had the highest vitamin A content, 10, 000 i.u/kg whereas those at 10-23 days old had between 8,700-7,000 i.u/kg. On the average, the flour samples had a mean percentage vitamin A content of 28.35% (8507.75 ±1270.5 i.u/kg). There is no standard yet for vitamin A content of flour products in Nigeria. The vitamin A contents of bread (1.0 – 3.4 i.u/g) obtained in this study was lower than that obtained by Murphy et al. (1995) (9.8-11.9i.u/g) but similar to that of Anyika and Uwaegbute, 2005 (1.3 i.u/g). However, these results depend on the level of fortification of the different samples. The vitamin A in the sliced bread made with two brands of flour could have been higher if the two flour samples used in the baking contained vitamin A.

The vitamin A present was contributed by only one flour brand (B flour) since the second brand had zero vitamin A content. The abnormal behaviour of the B flour sample impacted negatively on the second sliced bread sample. The flour had the highest vitamin A content but the bread it was used to bake had zero vitamin A content. This flour also had zero vitamin A content at 79 days old while others even older than it (84 days) still retained some level of vitamin A contents. The flour had an indication of virtual or trace vitamin A potency. The flour showed high presence of vitamin A but the vitamin A is not there or the vitamin A may have undergone the process of isomerization in the presence of light, oxygen, acid, iodine and copper.

According to Manan, 1994, isomerization of retinoid compounds is often by exposure to light with or without the addition of any catalyst. Another reason that could explain the phenomenon is that since vitamin A is very unstable, it might not have been encapsulated. Encapsulation enhances the stability of vitamin A but also increases the vitamin A premix cost and the overall over-head cost of production. The heat/environmental stability of vitamin A depend on the manufacturing procedure, palmitate/acetate type and the level of encapsulation and at times the source (moral standing of manufacturers). The vitamin in form of acetate is less stable than retinyl palmitate and can be snuffed out of it by mere exposure to sunlight (Ozara, 2010; Bagriansky, 1999). Another reason may be due to the flour treatment such as bleaching, enzymes, improvers and additives such as reactive iron compounds included for the purpose of enrichment could also have some reaction with vitamin A during high baking temperatures in the oven which is above 200 ^{o}C (Fellers and Bean, 1977). Vitamin A is oxidized fast at 150 ^{o}C and above (Boyacioglu, 2010) whereas baking temperature is within the range of 180-200^{o}C.

5.1. Vitamin A stability in wheat flour and bread

Mean stability is better in bread than in flour. This might be due to the additional vitamin A contents of butter/margarine and sugar used in baking the bread. The mean vitamin A stability lost in bread (39.2%) is slightly lower than that obtained by Solon et al. (1999) (40%). It is also within the normal range of losses recorded for dry fortified foods. Stability losses in bread have been estimated at 30-50% which is within the normal range of stability of vitamin A in dry fortified products (Dary and Omar, 2002). The result also fall within the range obtained by Nicholas Piramal India Limited, (2007), 20-40%. However, this disagrees with the result of Piza & Nilson, (1998), Solon et al. (2000) and Klemm et al. (2010). Piza & Nilson (1998) and Solon et al. (2000) stated that the typical loss in wheat flour fortified with vitamin A during the production of bread is 30% while Klemm et al. (2010) stated 20-30%. The difference between the mean vitamin A stability in bread (63.2%) and that obtained by Solon et al. 2008 (64%) who determined the recovery and stability of vitamin A in baked "pandesal" was not much (0.8%) even though their result was during the first month. The result suggests that fortified wheat flour could provide an effective dietary means of improving vitamin A status. However, the result differs from that obtained by Parrish et al. 1980 who studied the recovery of vitamin A in processed foods made from fortified flours and recovery of vitamin A in some of them after shelf storage.

Products prepared were white pan bread, corn bread, corn mush, cakes, pancakes, spaghetti and corn curls. Retention generally was 90% or more (range 79 – 105%) in fresh products made from flours stored less than 6 months, with similar retention (range 72 – 108%) when the products were stored at room temperature for 5 or 6 days, except for corn curls where retention in the fresh product was less than 50%. Good stability is achievable if the use of the right vitamin A premix and total quality management is enforced.

Conclusion

Fortified wheat flour is capable of enriching bread with vitamin A if compliance and adequate quality control is ensured.

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Table 1. Initial vitamin A content of wheat flour

Sample Flour:	Age (days)	Initial vitamin A content (i.u/kg)
A	10	8,134.3
B[1]	12	8,720.0
C	18	10,112.4
D	23	7,064.4

B[1] Saponification method used

Table 2. Vitamin A content (i.u/kg) of fresh bread

Sample Fresh bread	Vitamin A content (i.u/kg)
AB	998.7
BB[1]	3,438.1
CB	0.0
DB	1,524.0

.

Table 3. Storage stability (%) of retinyl palmitate in wheat flour

Sample Flour brand:	0 day	60 days	Stability (%)	Loss (%)
A	8,134.3	1844.6	22.7	77.3
B[1]	8,720.0	7973.0	91.4	8.6
C	10,112.4	0.0	0.0	100.0
D	7,064.4	1255.1	17.8	82.2

Overall mean stability: 22.58 ± 10.26.
B[1] Saponification method used

Table 4. Storage stability of retinyl palmitate in bread

Sample Bread:	Fresh (0 day) (i.u/kg)	5 days (i.u/kg)	Stability (%)	Loss (%)
AB[1]	998.7	678.8	68.0	32.0
BB[2]	3438.1	2221.8	64.6	35.4
CB[3]	0.0	0.0	0.0	100.0
DB[4]	1524.0	869.7	57.1	42.9

[1,2]Unsliced bread
[3,4]Sliced bread

Fig. 1. Mechanical production of bread. Source: questionmarkmag.com

Fig. 2. Vitamin A fortification label claim on flour bag.
Source: http://www.micronutrient.org/english/view.asp?x=596