

Full Length Research Paper

Vitamin A stability in Nigerian wheat flour and fortification compliance level

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Stability and compliance level of fortified Nigerian retailed flour has not been determined. The aim of study therefore was to evaluate vitamin A stability in retailed flour and assess compliance status. Seventeen wheat flour samples were randomly selected from 12 bakeries across six Local Government Areas in Lagos, Nigeria. Pre- and post-storage retinol analyses of retailed flour stored for 30 days were carried out using high performance liquid chromatography. Stability results for flour were grouped under 1, 2, and 3 months conditions. Fortification compliance was calculated based on three assumptions, using Nigerian Industrial Standards (NIS) (≥ 30.0 IU/g). WHO guidelines (Feasible Fortification Level/Range (FFL)) of approximately 25 % loss (22.5 -30.0 IU/g) and 50 % acceptable compliance range (ACR) for vitamin A (15.0-30.0 IU/g). Sample stability and compliance were calculated in percentages. Data were analysed using T-test and ANOVA at $p < .05$. Mean vitamin A (retinol) contents of flour were 18,221.3 IU/kg (1 month), 9,181.9 IU/kg (2 months) and 6,432.7 IU/kg (3 months). Pre- and post-storage vitamin A stabilities in flour at 1, 2, and 3 months were 60.7, 30.6, and 21.4%. Only 11.8% of samples met NIS. Pre-storage vitamin A content compliance in flour was 23.5% and non-compliance rate was 76.5%. Post-storage compliance rate decreased to 5.9% while non-compliance rate increased to 94.1%. Significant difference existed between vitamin A content of flour and NIS. Low stability and compliance were observed in flour samples. Revised quality of premix, effective monitoring and enforcement should be ensured.

Key words: Vitamin A, fortification, wheat flour, stability, compliance.

INTRODUCTION

Micronutrient fortification of flour plays a significant role in the prevention and eradication of micronutrient deficiencies in vulnerable populations (Sun et al., 2008; Huo et al., 2011, 2012). Many countries especially in Sub-Saharan Africa (SSA) and Asian Countries are mandatorily fortifying wheat flour, maize flour, wheat meal, corn meal,

and rice with different micronutrients such as vitamin A, iron, B-complex, zinc, and folate. Currently, 19 low- and low middle –income countries are fortifying or proposing to fortify wheat flour with vitamin A (Klemm et al., 2010). There has been mandatory vitamin A fortification of wheat and maize flour in USA (1974), Venezuela (1996),

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Philippines (1996-2000), Egypt (1999), Indonesia (2000), Guatemala (2002), South Africa (2003), Zambia (2003), Nigeria (2004), Morocco (2005) and Ghana (2009). In South Africa, by the end of 2003, all maize meal and white and brown bread flour (and bread baked with this flour) have been fortified with vitamin A, iron, thiamine, riboflavin, niacin, pyridoxine, folic acid and zinc (DOH, 2008). In Zimbabwe, maize has been fortified with vitamin A (Vitamin Global Initiative, 1997). Nigeria started vitamin A fortification of flour in 2002 at a very high level of 9 mg/kg (30,000 IU/Kg).

Stability testing provides evidence on how the quality of the food substance or product is influenced over time under various environmental conditions such as temperature, relative humidity, moisture, pH, light, air and metallic ions (DHHS, FDA/CDER/CBER, 2003). Studies conducted in USA and Philippines showed that the stability of vitamin A in wheat flour and baked products is good (Mansoor, 2007; Dary and Omar, 2002). Vitamin A losses due to shipping and storage and during food preparation has been estimated in wheat flour at 30-50 % which is within the normal range of stability for vitamin A in dry fortified products (Dary and Omar, 2002). Further losses of vitamin A during food processing form an additional concern, because the fortification level should be based on the vitamin level in the food at the time of consumption (Nalubola et al., 1998).

Micronutrient fortification of staples serve as a short and long-term intervention strategy and if complied with by all stakeholders, it would have helped to effectively accomplish the first millennium development goal (MDGs) of halving, between 1990 and 2015, the proportion of people who suffer from hunger (physical or hidden) especially in Sub-Saharan Africa and Asian countries. However, there is a critical gap between fortification legislation and compliance which is affecting the impact of food fortification (Garrett and Luthringer, 2015).

Knowledge of the level of non-compliance in any study is essential for assessing impact. It is also a reminder that when any measure is adopted for routine application there will always be those who for various reasons fail to participate (McLaren and Kraemer, 2012). For effective fortification impact to be achieved, it is essential to ensure that the food vehicle consistently supplies adequate amounts of nutrients at the point of consumption to the at-risk groups (Yusufali et al., 2012). Measures to provide quality control are necessary to guarantee food fortification in pre-established concentrations. Great variation was found in the concentration of vitamins as pre-established on the labels (Liberato and Pinheiro-Sant'Ana, 2006). A serious problem is measuring errors in the vitamin doses used for fortification during food processing (Liberato and Pinheiro-Sant'Ana, 2006).

In Honduras, despite mandatory sugar fortification, vitamin A was not detected in 34% and 21% of the sugar

consumed in rural and urban regions respectively (Nestel, 1993). A compliance range of 12 to 33% has been reported in wheat flour (Ogunmoyela et al., 2013). The micronutrient levels in fortified products should be monitored on a regular basis by calibrated/standardized laboratory equipment with modern analytical technology such as spectrophotometry and HPLC in order to dictate mixing errors or potency variations that would result in over- or under-fortification (Blum, 1997). A study in South Africa, found low compliance levels in bread flour and maize meal (Yusufali et al., 2012). The conclusion was that the low compliance was as a result of insufficient addition of premix at the mills as opposed to losses due to vitamin A stability. Low compliance reduces micronutrient availability and intake by vulnerable consumers of fortified products and potentially prevents the eradication of micronutrient deficiencies expected from the flour fortification programme (Yusufali et al., 2012). The average compliance pass rate for Global Alliance for Improved Nutrition (GAIN)-supported staple food fortification programmes in 25 countries was approximately reported as 40% (Garrett and Luthringer, 2015). Nigeria has fortified flour with vitamin A since 2004 but the vitamin A stability and compliance level after storage have not been assessed. Assessment of vitamin A stability and industrial compliance are very important in the success of vitamin A deficiency (VAD) eradication in Nigeria. The aim of this study therefore was to determine vitamin A stability in retailed wheat flour and assess fortification compliance status.

MATERIALS AND METHODS

Collection and selection of flour samples

Seventeen wheat flour samples were randomly selected from 12 bakeries across six Local Government Areas (Agege, Ikorodu, Mushin, Ojo, Oshodi/Isolo and Lagos Island) in Lagos State, Nigeria. The following information was recorded for each sample: (i) date of production written on flour bags/labels (inserted inside some of the flour bags) in order to determine how long the sample has stayed or the post-production time; (ii) date of sampling/analysis; (iii) brand name; (iv) batch number if given and (v) Laboratory sample code for identification of each sample brand was AA, BB, CC and DD.

Sample storage

Wheat flour samples were kept in plastic corked containers and stored for 30 days at room temperature similar to that done by Solon et al. (1998, 1999, 2008) and Cort et al. (1976).

Vitamin A (retinol) content

The high performance liquid chromatography (HPLC) method by AOAC (2000) reported elsewhere (Uchendu and Atinmo, 2012; Uchendu et al., 2012) was used in the vitamin A analysis of samples. Duplicate samples were analysed within 24 h of collection and mean values taken.

Table 1. Pre and post-storage vitamin A contents of wheat flour Vitamin A content (IU/Kg).

S/N	Flour brand	Vitamin A content (IU/Kg)		
		1 month	2 months	3 months
1	AA	44,574.2	20,211.2	
2	AA	n/a	11,936.0	7,283.0
3	BB	n/a	5,266.5	866.4
4	BB	n/a	16,110.6	11,148.7
5	DD	31,670.0	26,113.3	
6	AA	13,494.2	7,680.6	
7	AA	22,250.3	5,615.0	
8	AA	8,134.3	1,844.6	
9	BB	8,720.0	7,973.0	
10	BB	12,771.7	2,882.7	
11	AA	24,350.0	6,262.7	
12	AA	14,960.0	9,560.1	
13	CC	27,048.5	18,576.7	
14	DD	12,256.9	3,316.8	
15	CC& DD	17,690.8	2,305.6	
16	DD	10,112.40	0.00	
17	BB & DD	7,064.40	1,255.10	
	Mean	18,221.26	9,181.90	6,432.70
		±10,698.11	±7,441.5	±5,193.62
	P-value	0.001	.000	.016

*Recommended value =30,000 IU/Kg. **Samples written n/a were 2 months old at point of collection. P-value significant at p<.05.

Calculation of percentage vitamin A stability in samples

All wheat flour samples were assumed to be fortified with the vitamin A recommended value of 30,000 IU/kg (9.0 µg RE/kg). Vitamin A stability was calculated as percentage of the recommended value as follows:

$$\% \text{ vitamin A stability} = \frac{\text{Mean pre or post-storage vitamin A contents of flour}}{\text{Recommended value (30,000 IU/Kg)}} \times 100$$

Calculation of vitamin A stability losses in samples

Vitamin A losses were computed by subtracting vitamin A stability values from 100%.

Calculation of compliance

Fortification compliance was calculated using the method of Ogunmoyela et al. (2013). Three assumptions were made as follows:

- i) All the samples were assumed to have been fortified with current Nigerian International standard (NIS) or recommended value for flour (30 IU/g).
- ii) World Health Organisation (WHO) guideline of acceptable range of 25% (Feasible Fortification Level/Range (FFL)) 25% loss (22.5-30 IU/g) due to losses during distribution and storage was applied (WHO/FAO, 2006).
- iii) An acceptable range (ACR) of 50% (15-30 IU/g) was used to determine if the level of fortification was adequate taken into

consideration additional factors such as premix quality and stability, in-process addition challenges.

Number of samples that had the required ranges based on the assumptions was calculated as follows:

$$\% \text{ Compliance level} = \frac{\text{Number of samples that met the recommendations}}{\text{Total number of samples analyzed}} \times 100$$

Samples were grouped according to compliance and non-compliance status under NIS (≥ 30 IU/g), FFL (29,999-30,000), ACR (15-30 IU/g), and not detected.

Statistical analysis

Stability results were grouped under 1, 2, and 3 months conditions. Descriptive statistics such as frequency counts, total, mean, percentages and standard deviation (\pm SD) were used to describe data. The obtained data were subjected to student T-test and analysis of variance (ANOVA) using Statistical Package for Social Scientists (SPSS), software (version 15 for windows SPSS Inc., Chicago) to compare and identify significance ($p < .05$) between means of treatments.

RESULTS AND DISCUSSION

Vitamin A stability in wheat flour

Table 1 shows that the pre- and post-storage vitamin A

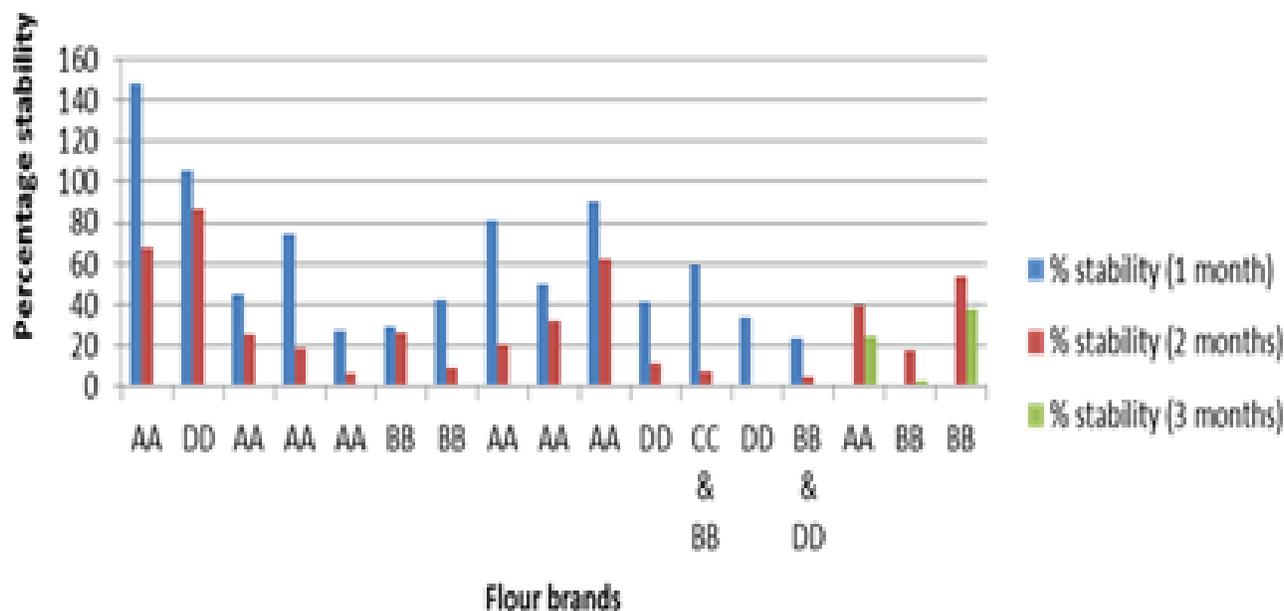


Figure 1. Pre and post storage vitamin A stability in flour.

contents of wheat flour samples was lower than NIS minimum requirement for wheat flour in Nigeria; 30,000 IU/Kg (NIS: 121:2000). All the samples showed presence of vitamin A but only two samples (11.8 %) (AA and DD) were found to be adequately fortified, above the standard. Vitamin A concentration in wheat flour was 19-83% below the recommended value in 88% (15/17) of the samples tested and exceeded the recommended value in 12% (2/17) with overages of 5.6 to 48.6%. The mean vitamin A content at 1 month was however similar to that obtained by Cort et al. (1995) but higher than that obtained by Ogunmoyela et al. (2013). There was a significant difference between the obtained vitamin A content of wheat flour and the Nigerian recommended level ($p < 0.05$). Also, vitamin A content of samples was significantly different at different storage periods ($p < 0.05$).

Figure 1 shows the mean percentage vitamin A stability in wheat flour as 60.7% (1 month), 30.6% (2 months) and 21.4% (3 months). This was lower than that obtained in Philippines (Solon et al., 1998; Solon et al., 2008). Flour was able to retain 60% of the vitamin A added under one month storage probably because vitamin A is added at point of bagging with no heat application. The stability of vitamin A is affected by physical and chemical factors such as temperature, (water activity (a_w) and moisture content), pH, oxygen, light, time, metallic ions, food composition and enzymes (Wirakartakusumah and Hariyadi, 1998; Manan, 1994). It has been reported that once premix is added at intended ratio concentrations to wheat flour, the stability of vitamin A continues to vary according to temperature, humidity, duration of storage, and other conditions of storage (Klemm et al., 2010). The instability might also be due to its chemical structure,

having many double bonds susceptible to degradation (Wirakartakusumah and Hariyadi, 1998). The inclusion of 5% cassava flour into Nigerian wheat flour does not affect the vitamin A stability in flour because it is still 100% flour (95% wheat flour + 5% cassava flour) before vitamin A premix is added so there is no dilution effect. Another reason responsible for the low vitamin A values in the flour samples might be that the recommended vitamin A value 30,000 IU/kg was not added at the fortification point in the majority of the samples. But if the recommended value was added and the samples retained only 18,221.3 IU/kg at 30 days mean post production time, poor quality of premix ingredients could be responsible. All the flour millers used the same Standard Organisation of Nigeria (SON) standards but from different suppliers. There is evidence that some supplier's mixes are of better quality and therefore lasts longer than others (Johnson et al., 2004). The quality of the premix is related to the quality of encapsulation. In South Africa, there is a proven indication of vitamin A source problems where there are two or three different sources using acetate instead of palmitate or using less stabilised forms which are cheaper (DOH/UNICEF, South Africa, 2009). Adjusting for 30 to 50% lost consideration during transportation, distribution, storage and processing of wheat flour and its products according to Klemm et al. (2010), it implies that the minimum acceptable range for Nigerian vitamin A content for flour is 15,000 - 21,000 IU/kg (4,504.5 - 6,306.3 μgRE). Less than half (35.3%) of the flour samples met this range at 1 month storage, 23.5% at 2 months storage and none at 3 months storage. This trend cut across the different flour brands in the market.

The sample that had the highest vitamin A content

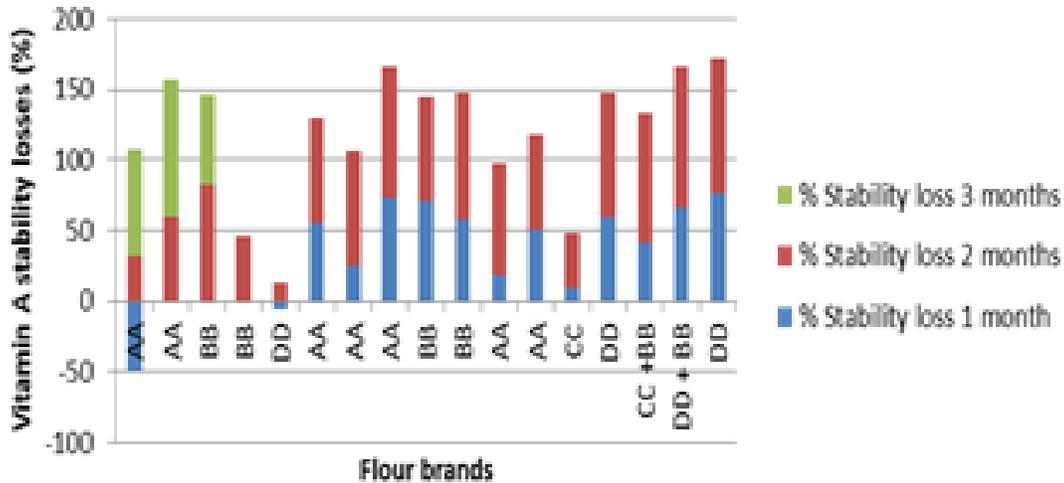


Figure 2. Post-storage vitamin A stability losses in flour samples.

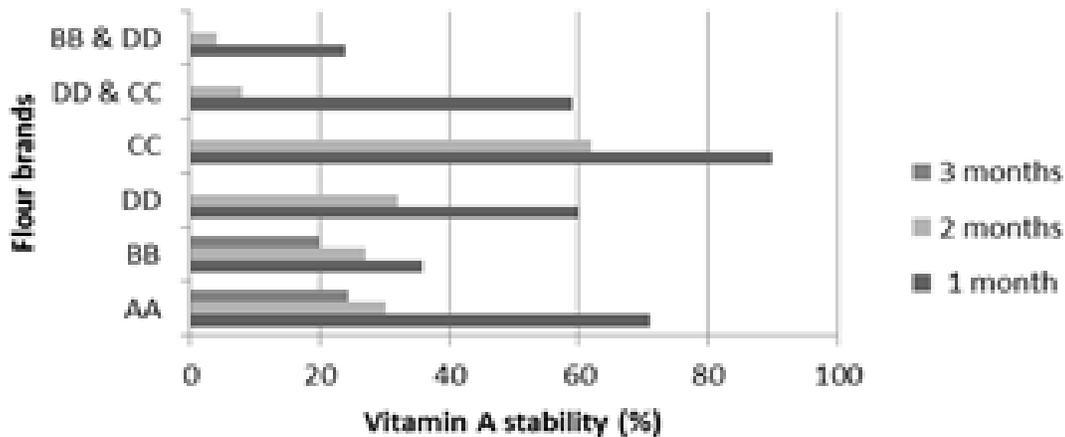


Figure 3. Mean vitamin A stability according to flour brands.

(44,000 IU/kg) more than the recommended value might suggest poor quality control measures in the fortification dosing process or addition of excess overage (Omar, 2005; Liberato and Pinheiro-Sant'Ana, 2006).

Figure 2 shows the post-storage vitamin A stability losses in wheat flour samples. Mean stability loss was 39.3 ± 35.7 (1 month), 69.4 ± 29.3 (2 months) and $78.6 \pm 17.2\%$ (3 months). Vitamin A stability loss in wheat flour after one month storage was below forty percent (39%) and is within the normal range of losses (30 to 50%) recorded for dry fortified foods products (Dary and Mora, 2002). However, after 1 month, vitamin A stability declined. This decrease was significant with time ($p < 0.05$) and agrees with the report that vitamin A degrades with time (Wirakartakusumah, 1998; Manan, 1994). At three months storage, wheat flour lost more than 70 % of its vitamin A content. This high level of degradation during storage calls for urgent attention

because the general aim of vitamin A fortification of flour is to make vitamin A available to vulnerable groups through consumption of fortified flour products in order to eradicate vitamin A deficiency in Nigeria.

Figure 3 groups vitamin A stability in flour samples according to flour brands and indicated that the vitamin A stability by brands was sample AA, 71% (1 month), 30% (2 months), and 24% (3 months); sample BB 36% (1 month), 27% (2 months), and 24% (3 months); sample CC 90% (1 month) and 62% (2 months); sample DD 60% (1 month) and 32% (2 months); mixed samples (CC and DD) 59% (1 month) and 8% (2 months) and BB and DD samples had 24% (1 month) and 4% (2 months) respectively. For most of the flour brands, vitamin A stability was good at one month. It was best in CC (91%) followed by AA brand (71%) then DD (60%) and the least BB flour (36%). After 1 month of production, AA flour brand was leading in vitamin A stability followed by CC

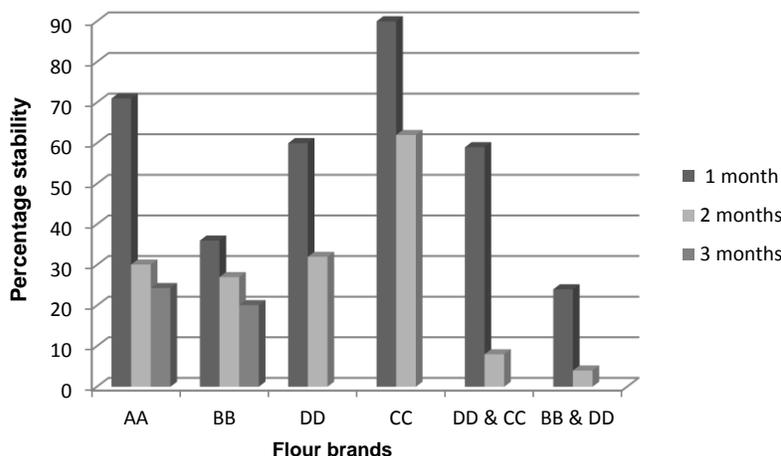


Figure 4. Decrease in vitamin A stability in flour brands.

Table 2. ANOVA Table showing vitamin A stability in different flour brands stored at 1 to 3 months.

S/N	Flour brands	% Stability			Mean**	SD	F	p-value
		1 month	2 months	3 months				
1	AA (7)*	71	30	24	50.5	29.0	10.092	.047
2	BB (4)	32	22	20	27.0	7.1		
3	DD (3)	60	32	-	46.0	19.8		
4	CC (1)	90	62	-	76.0	19.8		
5	CC+DD(mixed) (1)	59	8	-	33.5	36.1		
6	BB+DD (mixed) (1)	24	4	-	14.0	14.1		

*Numbers in parenthesis are number of sampling occurrences. P-value significant at $p < 0.05$.

and DD. However, beyond 1 month, there was a sharp decline in vitamin A stability in all the samples except in BB flour brand. While others declined in geometric progression, BB decreased in arithmetic progression (Figure 4). At three months storage, CC and DD flour brands had zero vitamin A contents. BB flour brand might have a superior quality premix than the others. This comparison excluded samples that were only collected once in all the bakeries. It is also observed in this table that AA flour brand enjoyed more patronage by bakers than other brands followed by BB flour brand. Out of the 17 flour samples used in the bakeries, 41.1% samples were AA flour, 23.5% BB, 5.9% CC, 17.7% DD and 11.8% blended flour brands. Table 2 shows that vitamin A stability significantly differed in all the flour brands in Nigeria. This might be as a result of the quality of vitamin A used by each miller.

Type of matrix used for the vitamin A premix encapsulation might be one of the major factors that affected the vitamin A stability of Nigerian wheat flour. Nigeria uses 250 CWS premix which is encapsulated with modified food starch. A study has found that starch matrix has the lowest stability among other matrices. Vitamin A stability of various matrices used as vitamin A coatings

were reported as mannitol (90%), lactose (89%), mannitol + sucrose (88%), mannitol + dextrose (83%), dextrose (81%), sucrose (80%), calcium sulphate (75%), kaolin (75%), aluminium hydroxide (73%), mannitol + starch (70%), mannitol + aluminium hydroxide (60%), and starch (59%) after one month storage (Kee-Neng et al., 1962). This study shows that starch as a coating matrix for vitamin A has the lowest stability (59%) among other matrices. The stability of flour obtained in this study at one month post-production time (60.7%) is similar to the stability of the modified starch matrix used (59%). This might explain the low stability obtained in this study and the reason Nigerian fortified products could not withstand the effect of vitamin A degrading factors.

Encapsulation of vitamin A is meant to protect it from all external degrading factors and it is successful in developed countries. However, the stability of the ingredients used in the encapsulation becomes the determining factor on the level of stability to be achieved. If the ingredients used in formulation are not stable, the premix might not be stable. Encapsulation can only provide additional moisture barrier if the formulation ingredients are stable. The form of vitamin A and premix to be used in fortification should be the highest grade,

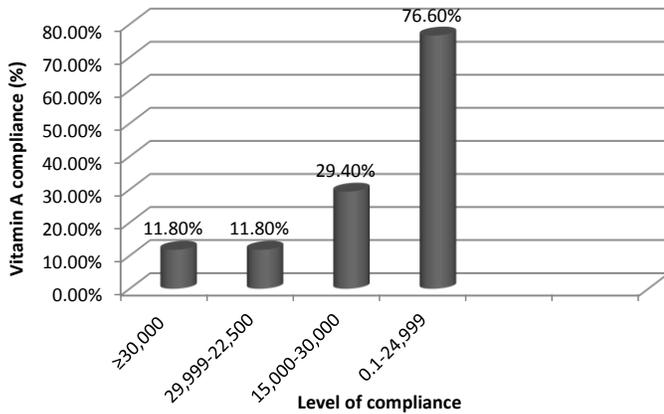


Figure 5. Pre-storage vitamin A content compliance in flour.

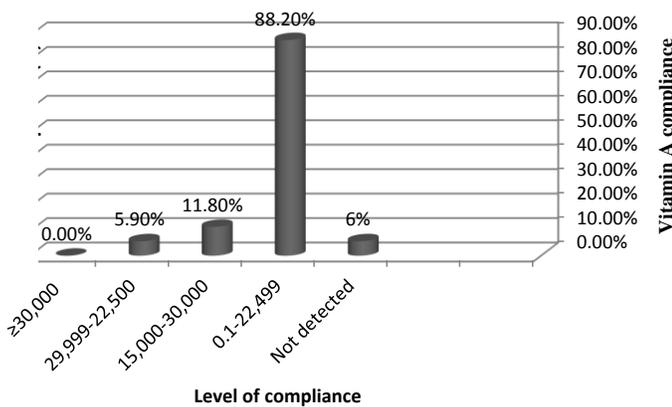


Figure 6. Post-storage vitamin A content compliance in flour.

appropriate for the intended food vehicle, stable under ambient conditions and for the duration of expected use, and introduced into the food supply in accordance with existing industry standards (Klemm et al., 2010). There is a research need to determine the level of encapsulation and ingredients in the Nigerian premix.

Nigeria is in the tropical region which is prone to high atmospheric temperature or hot weather during dry season and high humidity during rainy season. Poor handling procedures at retail level could have affected the vitamin A stability in flour especially if the vitamin A was not encapsulated and flour is not packaged adequately. Flour is exposed or displayed outside shops for customers to see and buy. Even when they are packed inside, the shops or warehouses are very hot. At retail levels, flour is tired in black nylons in small kilogram measurements for sale. Some retailers open the industrial bag (50 kg) and retail directly from it and retail selling in cups might take them some months before the bag is finished. Flour retailers should be trained on the proper ways of handling flour. Fortified flour should be

packaged in 500 g, 1 kg, and 2.5 kg to prevent retailing from 50 kg bags which should be for industrial use. In the past (1970s), there used to be 2.5 kg flour bags meant for home use.

Improper packaging of wheat flour from millers might be another challenge for vitamin A stability in Nigeria. Polypropylene bags are used and this might not properly protect flour from moisture and air during transportation and storage. Only one flour brand (AA) has its polypropylene bag laminated. This might be one of the reasons why this brand had higher vitamin A stability (71%) than other brands after 30 days storage. This problem was also reported in Pakistan where different packaging materials such as jute, cotton and polypropylene are being used for packaging of flour (Butt et al., 2003). These packages do not protect the wheat flour properly from contamination by insect pests, microbes, sand, dust and environmental moisture.

Vitamin A content compliance level in wheat flour

Figure 5 shows the pre-storage vitamin A content compliance in wheat flour to be only 11.8 % based on WHO guidelines (Feasible Fortification Level/Range (FFL) of approximately 25% loss (22,500-30,000 IU/Kg). Total compliance (≥30,000 IU/Kg) was 23.6% and non-compliance level was 76.47%. Out of 17 flour samples, only 29.4% were compliant at the 50% acceptable compliance range (ACR) for vitamin A (15,000-30,000 IU/Kg). Figure 6 shows that compliance after stability studies dropped from 23.6 to 5.9% while non-compliance increased from 76.5 to 94.1%. At ACR, compliance level dropped from 29.4 to 11.8%. Vitamin A content of flour obtained from analysis was lower than Nigerian recommended level. This is an indication of low compliance. This result is in agreement with that obtained by BASF (2009), Yusufali et al. (2012), Ogunmoyela et al. (2013), Garrett and Luthringer (2015). From these results, it is clear that the low compliance of ‘fortified’ samples might limit the anticipated impact of the current Nigerian fortification program. The fact that a product claims to be fortified does not really mean that it contains the specified vitamin A level. Despite the fact that fortification is mandatory for wheat/maize flour in Nigeria, the cost of high vitamin A dosage (30 IU/Kg) with other micronutrients in the fortification of wheat flour without any incentives from Government might pose a challenge to millers and these become limiting factors to total compliance. Vitamin A compounds needed for fortification of dry matrixes such as flour and sugar are at least four times more expensive than the oily forms, and their stability is inferior. Hence, dry foods tend to be fortified with less vitamin A, which requires higher consumption (Dary and Mora, 2002). Vitamin A is expensive and the issue of cost-benefit analysis should be addressed by reducing vitamin A recommended level to the range of 5.0 to 7.0 IU/g.

Conclusions

Low stability and compliance were observed. Vitamin A stability was only good in 1 month wheat flour. Quality and quantity of vitamin A premix ingredient added at fortification point, high premix cost and inadequate packaging might be the major factors responsible for low stability and compliance. High quality premix, reduced NIS standard, effective regulatory monitoring and enforcement should be ensured to achieve the aims of vitamin A fortification of flour in Nigeria.

Conflict of Interests

The authors have not declared any conflict of interests.

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