academicJournals

Vol. 7(11) pp. 415-420, November 2013
DOI: 10.5897/AJFS2013.1086
ISSN 1996-0794 ©2013 Academic Journals
http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Simultaneous high performance liquid chromatography analysis of water soluble vitamins in some foods prepared by a Nigerian eatery

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Accepted 25 September, 2013

The present study carried out simultaneous analysis of water soluble vitamins (thiamine, pyridoxine, niacin, folic acid and ascorbic acid) in some foods prepared by a Nigerian eatery using a reverse phase high performance liquid chromatography. The results (mg /100 g) indicate that pyridoxine ranged from 0.16 in amala to 1.14 in fried rice, niacin from 0.18 in doughnut to 0.70 in meat pie, thiamine from 0.30 in doughnut to 2.40 in cowpea. Ascorbic acid was lowest in doughnut and highest in *eba* composite meal while folic acid ranged from 30 µg /100 g in doughnut to 195 µg /100 g in meat pie. Thiamine was significantly higher in village kitchen meals and below detection limit in meal of rice alone. The gram per serving of the village kitchen would give 108-200% and 32-48 % of the recommended dietary allowance (RDA) for thiamine and folic acid, respectively, whereas the level of ascorbic acid was too low to meet the RDA. The study shows that fast foods have low content of water soluble vitamins and its consumption could compromise intake of some vitamins.

Key words: Thiamine, pyridoxine, niacin, folic acid, ascorbic acid, eatery.

INTRODUCTION

Micronutrient deficiency diseases (MDD) are widespread and affect large numbers of people in developing countries. It is estimated that one in every three people in the world is at risk for one or more micronutrient deficiencies, while approximately 2 billion people world-wide suffer from some kind of micronutrient deficiency, causing a wide array of disorders and increasing the risk of death, disease and disability (WHO, 2005). Vitamin (an important micronutrient), is essential for most of the body's functions and is required by the body in small amounts for metabolism, to promote health and proper growth especially among children (Olaniyi, 2000). Vitamin deficiency diseases do not only occur in poverty stricken communities; in affluent societies, deficiency diseases also occur often as the result of poor choice of food or unhealthy eating habits. With fast food eateries springing up in nooks and crannies of Nigeria, consumption of fast food is gradually becoming a habit among Nigerian youths and elite. The eateries probably because of pre-ference for taste, apart from conventional fried or baked fast foods, incorporated village kitchen meals (village kitchen meals are local meals of the people where the eatery is located).

Fast food operations involved non-conventional coo-king methods like deep frying, microwave and pressure cooking in order to save time. From general point of view, thermal processing employed in food preparation has been found to lead to loss of water soluble vitamins.

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According to Berechet and Segal (2007) water soluble vitamins may be lost during cooking in two ways: first, by degradation which can occur by destruction or by other chemical changes such as oxidation; and second by leaching into the cooking medium.

The increasing interest in good eating habit has provided a greater awareness of the vital role that micronutrients play in growth and health. Diets which do not contain adequate amounts of these vitamins often result in deficiency diseases; for instance with thiamine deficiency, a number of important body functions can be disturbed. They include brain function, nerve function (especially of the legs), and heart function; these three impairments are called Wernicke-Korsakoffs psychosis', dry beriberi and wet beriberi (Ozawa et al., 2001). Pyridoxine deficiency may lead to depression, convulsions, abnormal nerve functions (especially in the limbs), dermatitis, cracking of skin at the corner of the mouth and the lips, a smooth tongue, and anaemia (Anyakora et al., 2008).

Pellagra is a condition resulting from niacin deficiency, in which there are symptoms of dermatitis in skin exposed to the sun, diarrhoea and dementia (Berger, 1985). The consequences of deficiency of folic acid include anaemia and defective lining of the gut, adversely affecting absorption of many nutrients. Since the number of blood platelets (which play a part in blood clotting) can be low with folic acid deficiency, a tendency to prolonged bleeding can also occur (Bailey et al., 2010), while vitamin C have been reported to reduce the damage by free radicals and check degenerative disease its deficiency, leads to scurvy (Rickman et al., 2007).

The National Agency of Food and Drug Administration and Control (NAFDAC), in conjunction with the Micronutrient Initiative (MI), of Nigeria, have contributed immensely to the creation of this awareness of the impact of eating foods deficient in micronutrients especially vitamins (Anyakora et al., 2008). With the increase in establishment of fast food eateries and popularity of consumption of fast foods, it is therefore of utmost importance to know if conventional fast foods and village kitchen meals prepared from eateries will contribute significantly to the dietary vitamin intake. The objective of this study was to determine the content of some water soluble vitamins (niacin, pyridoxine, folic acid, thiamine and ascorbic acid) in selected foods prepared by a Nigerian eatery.

MATERIALS AND METHODS

Samples

Samples were collected as consumed from five outlets of a popular fast eatery located in Lagos, Nigeria. The meals were weighed as served and pooled together, then those served with soup were mixed together with the soup before being blend using warring blender ((National model MX-795N, Matsushita, Malaysia). The samples were kept in a freezer and the extraction of vitamins was initiated within 12 hof sample collection.

Analysis of vitamins

Analysis of water soluble vitamins was carried out simultaneously using High Performance Liquid Chromatography with UV detector (Agilent Technologies Model 1200, Germany) following the method of Khor and Tee (1996) with slight modifications.

Reagent and chemicals

Vitamin standards (pyridoxine, niacinamide, folic acid, ascorbic acid and thiamine) and HPLC grade methanol were purchased from Sigma (Sigma-Aldrich, Germany). Sodium monohydrogen phosphate was purchased from BDH.

Chromatographic conditions

The HPLC used was a reversed phase type. The chromatographic separation column consisted of a stainless steel (4.6 × 150 mm) Eclipse XDD, and 5 µm mbondapack C_{18} column. The HPLC had an integrated UV detector which was set at 254 nm wavelength to monitor the column effluent. The mobile phase comprised of 90% of 0.01 M sodium mono-hydrogen phosphate and 10% HPLC grade methanol. The mobile phase was sonicated for 30 min before use. The elution was isocratic and the flow rate was 0.60 mL / min.

Extraction and analysis of vitamins from samples

The samples (2.0 g) were weighed into 200 mL Erlenmeyer flask, 30 mL of 0.1 M Hydrochloric acid was added and the mixture was heated at 40°C for 5 min, the mixture was filtered through ashless filter paper and centrifuged for 10 min at 1800 rpm. The supernatant was sonicated and 20 µL were injected into the column and separated. Peaks were identified by comparison of the retention time to that of the known standards and the value of vitamins was obtained from computer printout (Chemstation software).

Preparation of standards

The stock standard solutions of thiamine, pyridoxine and niacin were prepared by dissolving 50 mg of the standard in 0.1 M hydrochloric acid in 25 mL standard flask and made up to mark with the acid. For preparation of folic acid and ascorbic acid, 0.1 M sodium hydroxide and deionized water was used. The working standards were prepared from the stock standard solutions by taking 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL of the stock into 10 mL standard flask and made up to mark with appropriate solvent. The solutions were sonicated for 30 min and 20 µL of various concentrations of vitamin standards were injected into the HPLC column separately and the retention times were noted. The solutions of the standards were mixed in equal proportion and 20 µL was injected into the column. The retention time of each vitamin in the complex mixture was noted and used to identify the vitamins in the sample. The detection limit for the vitamins was 0.05 mg/ml.

Statistical analysis

Statistical analysis was carried out by the use of Microsoft Excel Statistical Packages (Microsoft Corporations, USA) and Graph-Pad Instat-3 Packages (Graph Pad software Inc, USA). All analyses were carried out in triplicate and the results presented as mean and

Table 1. Characteristics of the standard curve.

Vitamin	Retention Time (min)	Regression Equation (y = mx+b)	Correlation coefficient (r)
Ascorbic acid	2.3	y = 92511.36027x + 1048.70238	0.999
Pyridoxine	3.2	y = 18536.68580x + 16.31764	0.999
Thiamine	3.9	y = 14213.42610x + (-284.46318)	0.995
Folic Acid	4.8	y = 25719.56704x + (-327.04686)	0.998
Niacin	5.9	y = 44725.448x + (-586.167)	0.996

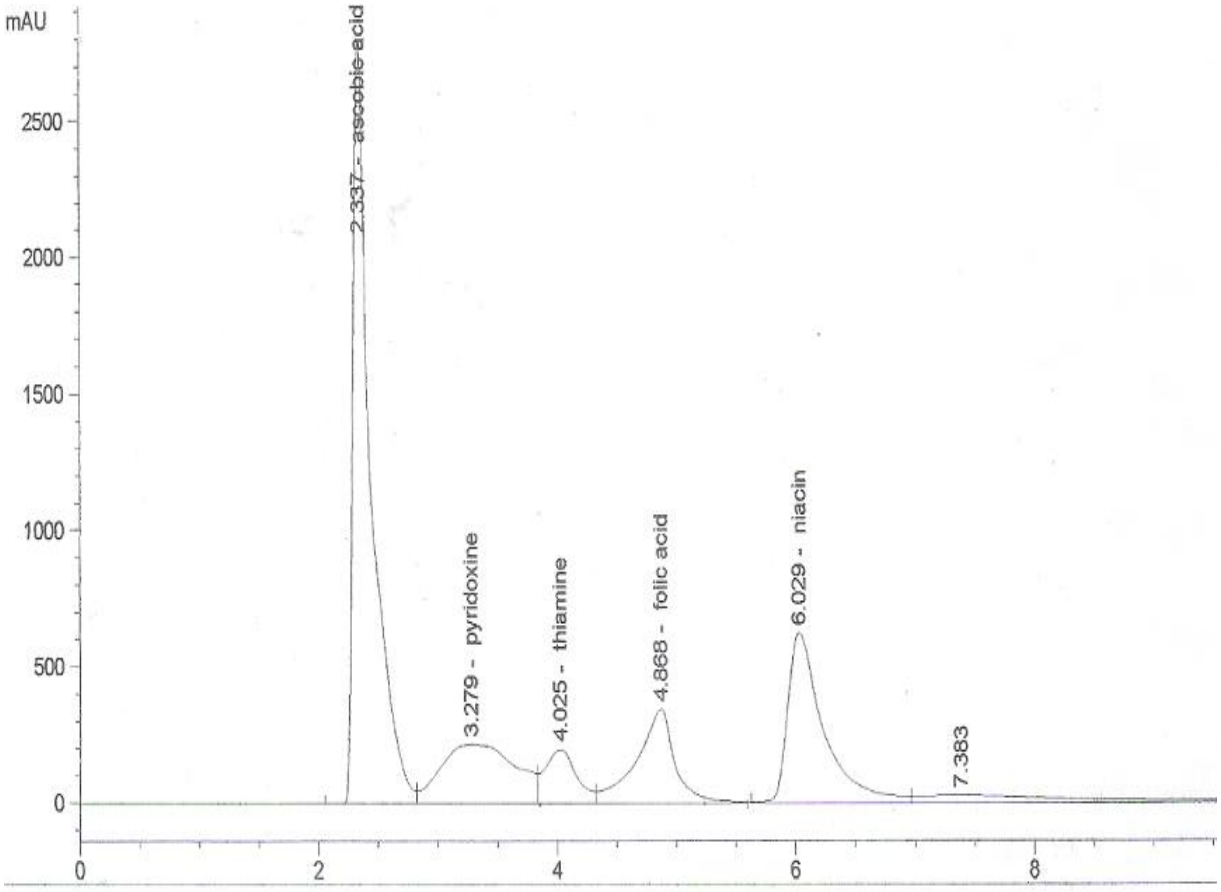

Figure 1. Chromatogram of the vitamin standards.

standard deviation. Analysis of variance was used to assess and compare results.

RESULTS AND DISCUSSION

The chromatographic characteristics and column is presented in Table 1 and Figure 1, while the mode of preparation of the samples is presented in Table 2. The results of analysis indicated that the highest moisture content (Table 3) was reported for cowpea meal (40 %), and it was observed that moisture was significantly higher in village kitchen meals than fast foods. Preparation of village kitchen meals involved the use of large volume of water which by implication could mean more dilution or less nutrients per volume of food compared to fast food fried and baked products. The village kitchen meals were served with soup and meat and/ or fish which apart from large portion size per serving could significantly affect the vitamin status of the meals compared with fast foods fries. While young adolescent and adult would require munching at least two serving size doughnut, beef roll, meat pie to satisfy appetite, a serving size of village kitchen meals and fast food rice kitchen meals and fast

Table 2. Sample description and mode of preparation.

Sample	Description and Method of Preparation
Conventional fast foods	
Jollof rice:	Rice cooked (or baked) with ground tomatoes, peppers, sometimes with meat and other spices.
Fried rice:	Rice cooked in vegetable oils, liver chop, meat, carrot, green beans and spices are added to taste
Meat pie	Snack made from wheat flour dough containing pastry of seasoned meat, potatoes and other vegetables
Doughnut:	Snack made from wheat flour dough mixed into a circular ball fried in deep frying oven and then robbed with sugar or honey
Hotdog	Snack made from wheat flour dough containing cooked sausage made from meat slurry, rolled up in a pastry and baked in the oven
Beef roll	Snack made from wheat flour dough containing cooked sausage is rolled up in a pastry and baked in the oven
Village kitchen meals	
Eba	Prepared from gari (peeled, grated cassava tuber, fermented, partially dehydrated and baked or fried) mixed with hot water to thick paste served with vegetable soup and fish
Amala	Prepared by mixing dried yam flour powder in boiled water into a thick paste, served with vegetable soup and fish
Pounded yam	Prepared by pounding boiled yam in a mortal with pestle to smooth paste served with vegetable soup and fish
Cowpea (Mashed Beans)	Prepared by cooking cowpea (*Vigna unguiculata L. Walp*), pepper, palm oil and other ingredients are added to the broth and mashed to paste.

food rice (dry weight presented) (Table 3) could satisfy appetite.

The result of pyridoxine content indicated that village kitchen meals recorded significantly lower value than fast foods. The least value was reported for *amala* (0.16 mg) and highest for fried rice (1.14 mg). In fast foods, pyridoxine value followed this decreasing order: fried rice > meat pie > jollof rice > beef roll. The high pyridoxine recorded in meals with lower moisture content is consistent with observation that pyridoxine in food is susceptible to light induced degradation and exposure to water which can cause leaching and consequent losses (Perveen et al., 2009), thus low level of pyridoxine observed in village kitchen meals (*amala, eba* and pounded yam) could be also adduced to high volume of water employed in preparation that could cause leaching of the vitamin into the cooking broth. The range of 0.34 to 0.60 mg /100 g was reported for soy product and 0.416 mg /100 g for buckwheat flour (Labiedzinka and Szefer, 2006).

The level of thiamine unlike pyridoxine was significantly higher in village kitchen meals than fast foods. Thiamine in village kitchen meals appear in this decreasing order; cowpea > amala > pounded yam, while baked hot dog and meat pie recorded higher values than doughnut which is processed by deep fat frying. Several studies have shown significant decreases in thiamine content during thermal processing although the extent of degradation depends on the commodity. Baking, pasteurization, or boiling of foods fortified with thiamine has been reported to decrease its content by up to 50% (Prodanov et al., 2004). The high content of thiamine recorded for village kitchen meals is consistent with the report that deficiency disease associated with thiamine is rare in developing countries except for those who suffer eating disturbance or alcoholism (Gordon, 2003). The impact of heat in village kitchen meals would be highly reduced due to volume of water and this could explain in part the reason for higher thiamine compared to fast food fries and baked foods.

Analysis of rice alone (fried/ Jollof) without meat or fish indicated that thiamine was below detection limit. This observation is in agreement with the report that rice (especially polished rice) is a poor source of thiamine, which was the main reason why “beriberi” a noticeable deficiency disease of thiamine has been reported to be endemic in areas where rice was the staple diet (Ozawa et al., 2001). The result presented for thiamine in rice in this study reflects the thiamine in composite meals and not rice alone, therefore, in Nigeria, where polished rice is becoming staple diet in homes, incorporation of complementary source of thiamine becomes inevitable if occurrence of thiamin deficiency diseases is to be averted.

The highest level of niacin (0.70 mg) was recorded in meat pie and least for beef roll and *amala* (0.18 mg). There was no significant difference between village kitchen meals and fast food rice but doughnut and beef roll recorded significantly lower values. Most data suggest that niacin is stable to processing, retention rates of 93% or higher were found after canning and subsequent storage of green peas, green beans, peaches and sweet potatoes on both wet and dry weight bases (Rickman et al., 2007).

Table 3. Hydro- soluble vitamin content of some foods prepared by a Nigerian eatery (mg / 100 g) and % realization of RDA (per serving).

Sample/ Vitamin	Moisture %	weight / per serving (dry)	Pyridoxine	% RDA	Thiamine	% RDA	Niacin	% RDA	Ascorbic acid	% RDA	Folate**	% RDA
Jollof rice	24 ± 0.1^{b}	239* ± 10^{b}	0.75 ± 0.02^{c}	44	0.29 ± 0.01^{d}	54	0.27 ± 0.02^{b}	19	0.94 ± 0.04^{b}	0.65	80 ± 0.9^{d}	41
Fried rice	19 ± 0.2^{c}	362* ± 13^{a}	1.14 ± 0.0^{a}	66	0.18 ± 0.02^{e}	53	0.53 ± 0.1^{b}	37	0.53 ± 0.01^{d}	0.75	53 ± 1.0^{e}	13
Meat pie	14 ± 0.1^{c}	116 ± 5.0^{c}	1.04 ± 0.01^{b}	61	0.30 ± 0.11^{d}	25	2.7 ± 0.3^{a}	50	0.5 ± 0.03^{d}	0.9	195 ± 5.0^{a}	48
Beef roll	16 ± 0.2^{c}	75 ± 0.8^{g}	0.67 ± 0.01^{d}	39	0.08 ± 0.0^{e}	6	0.18 ± 0.0^{c}	12	0.23 ± 0.01^{e}	0.2	30 ± 3.0^{f}	8
Doughnut	15 ± 3.1^{c}	60 ± 2.0^{h}	0.34 ± 0.03^{f}	20	0.11 ± 0.0^{e}	9	0.2 ± 0.0^{c}	14	0.2 ± 0.02^{e}	0.2	30 ± 3.0^{f}	8
Hot dog	15 ± 1.9^{c}	60 ± 4.2^{h}	0.5 ± 0.01^{e}	29	0.33± 0.01^{d}	27	0.38± 0.01^{b}	27	1.0 ± 0.05^{b}	1.1	130 ± 3.0^{a}	32
Eba	35 ± 2.8^{a}	148* ± 13^{d}	0.30 ± 0.02^{f}	17	3.3 ± 0.05^{a}	25	0.3 ± 0.0^{b}	21	1.1 ± 0.06^{a}	1.1	37 ± 2.0^{f}	9
Amala	38 ± 4.0^{a}	124* ± 8.0^{e}	0.16 ± 0.02^{g}	09	1.4 ± 0.03^{c}	116	0.18± 0.04^{c}	12	0.27 ± 0.04^{e}	0.3	58 ± 4.0^{e}	14
Pounded Yam	36 ± 5.0^{a}	158* ± 10^{d}	0.25 ± 0.0^{g}	14	1.3 ± 0.1^{c}	108	0.24 ± 0.0^{b}	17	0.2 ± 0.0^{e}	0.2	46 ± 1.0^{e}	11
Cowpea meal	40 ± 2.8^{a}	180* ± 15^{c}	0.29 ± 0.0^{f}	17	2.4 ± 0.02^{b}	200	0.44± 0.05^{b}	31	0.76 ± 0.0^{c}	0.8	169 ± 3.0^{b}	43

Values are mean ± standard deviation of triplicate analysis. Values with the same superscripts within the column are not significantly different ($p < 0.001$). *Foods served with meat or fish; **folic acid reported as µg /100g.

The content of folate (Table 3) ranged between 30 to 195 µg/100g; the least value was reported for doughnut and the highest was recorded for meat pie. The trend in the level of folate is in the order: meat pie > cowpea meal > hot dog. Legumes have been reported to contain high level of folate; this is evident in that cowpea meal recorded high folate whereas the relatively high folate content recorded in meat pie and hot dog could be as a result of fortification of the flour with folate. Leichter et al. (1978) reported that folate is relatively stable to heat and humidity hence the loss of folate in foods has been adduced to leaching and not to degradation of the molecule. One of the major results of a folate deficiency is that in the early phase of red blood cell formation, the cell cannot divide because they cannot form new DNA. The cells remain in immature form known as megaloblasts. The bone marrow of a folate deficient person produces mostly immature megaloblastic cells; few mature red blood cells arrive in the blood stream, thus the blood capacity to carry oxygen decreases causing a form of anaemia known as megaloblastic anaemia (Lieberman and Bruning, 1990).

The content of ascorbic acid (Table 3) ranged from 0.2 to 1.1 mg / 100 g with the highest value reported for eba. The content of ascorbic acid was generally low in all samples compared to fruits and vegetables; for instance Fox and Cameron (1989), reported 15 - 50 mg / 100 g for raw fruits. It is a common knowledge that ascorbic acid is heat labile and is by far the least stable nutrient during processing; it is highly sensitive to oxidation and leaching into water-soluble media hence is easily destroyed during food processing. Ascorbic acid is one of the ligands that promote absorption of iron but its action on copper is dose dependent (Hazell and Johnson, 1986). The action of ascorbic acid on food iron is usually related to its reducing power and metal chelating activities thereby forming soluble complexes.

For consumption of conventional fast food fries, on the average, a consumer can munch two servings of doughnut, hotdog or meat pie in order to satisfy hunger. Assuming the grams per serving (applying food weight on dry weight basis) of any of these food products was consumed per day, the percentage realization of the recommended dietary allowances for vitamins would indicate that jollof rice and meat pie would provide between 44 and 61% of pyridoxine whereas village kitchen meals would provide below 20%; for niacin all meals would provide a range of 12-40% though niacin is a semi-essential vitamin in man since niacin could be spared by tryptophan, from which it could be synthesized at the rate of 60 mg of the amino acid for every one mg of niacin (Gordon, 2003). This way, the body could synthesize up to 50% of the needed niacin. The use of tryptophan in this manner could however jeopardize the protein nutriture of the diets, making tryptophan a major limiting amino acid. On the other hand, the percentage realization of the RDA revealed that village kitchen meals would give 108-200%, whereas doughnut and beef roll could provide less than 10% of the RDA for thiamine, also cowpea, meat pie and hot dog could give 32-48 % while doughnut and beef roll would provide less than

10% of the RDA for folate. The level of ascorbic acid observed in this study is too low and could not meet the RDA of 90 mg hence the meals must be complemented with fruits and vegetables.

The study reveals that village kitchen meals contain higher level of some water soluble vitamins than conventional fast foods; it is envisaged that consumption of fast foods could lead to poor intake of the vitamins.

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