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Determination of Vitamin A Content by High Performance Liquid Chromatography Method in Vegetable Oils Sold in Dakar Supermarkets

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Abstract

Vitamin A deficiency has been a public health problem in many low- and middle-income countries, causing a weakened immune system, putting a child at greater risk of early disease and mortality. It is a source of preventable childhood blindness. Following Decree No. 2009-872 of 10 September 2009, making the application of standards on refined edible oils enriched with vitamin A mandatory. A study was conducted with the aim of evaluating the vitamin A content by the HPLC method in vegetable oils marketed in the urban markets of Dakar. This study is spread over three localities in the city of Dakar according to their demographic concentration. The results found on average for vitamin A are quite revealing with 10.28 ± 2.17 mg/kg for the commune of Grand-Yoff, that of Pikine gives 11.34 ± 0.85 mg/L and finally the commune of Keur Massar 11.79 ± 0.71 mg/kg. The results give a fairly significant difference with P = 0.023.

Keywords

Vitamin A, Vegetable Oils, HPLC Method, Dakar

1. Introduction

Micronutrient deficiency, sometimes referred to as "hidden hunger," is often less visible than other forms of malnutrition but is nonetheless a major cause of serious health problems. In Senegal, micronutrient deficiencies are recognized as a significant public health problem despite limited data on their magnitude and geographic distribution in the country. Senegal's 2017-2021 Multisectoral Strategic Nutrition Plan (PSMN) (Government of Senegal, 2017) argues that micronutrient deficiencies constitute a major challenge in Senegal's nutrition landscape [1].

In 2017, global hunger increased for the first time in a decade, reaching 815 million hungry people2, an increase of 38 millions compared to 2016. While 155 million children under 5 years of age are stunted, caused by poor nutrition, levels of micronutrient deficiencies are alarming: 2 billion people suffer from micronutrient deficiencies, also known as "hidden hunger3". National and international health organizations are increasingly emphasizing micronutrient fortification programs as a promising way to address deficiencies in low- and middle-income countries [2].

Vitamin and mineral deficiencies, or "hidden hunger," are a real public health problem worldwide, particularly in sub-Saharan Africa, where a third of the population continues to suffer from them.

In Senegal, the proportion of children under six years of age affected by subclinical vitamin A deficiency is 61%, while anemia, which is most often the result of dietary iron deficiency, affects 82.6% of children aged 6 to 59 months and 59.1% of women aged 15 to 49 (2005 Senegal Demographic and Health Survey) [3].

The health and socioeconomic consequences of these nutritional deficiencies are considerable: increased mortality and morbidity, impaired physical and mental development, and reduced work and learning capacities. Food fortification is undeniably recognized as one of the most cost-effective interventions worldwide.

According to the FAO and WHO guiding principles, food fortification is defined as "the action of deliberately increasing the content of an essential micronutrient (vitamins and minerals, including trace elements) in a food in such a way as to improve the nutritional quality of the diet and provide a public health benefit with minimal health risk [2].

In West Africa, vitamin A fortification of oils is already underway in Ghana, Nigeria, and Côte d'Ivoire. In light of these important challenges, the Government of Senegal initiated the fortification process, which resulted in the adoption of Senegalese standards for vitamin A-fortified edible oils and soft wheat flour fortified with iron and folic acid. To effectively combat micronutrient deficiency, it is necessary to make the fortification of these products mandatory and enforce the relevant standards [3].

Preformed vitamin A, or retinol, is exclusively of animal origin. Carotene or carotenoids act as provitamins. Of the many plant carotenoids, the most important is beta-carotene, which is converted to vitamin A by enzymatic action in the intestinal wall [2]. Dietary vitamin A deficiency primarily affects the eyes and can lead to blindness. Xerophthalmia, or dry eyes (from the Greek xeros = dry), is the term that covers the various effects of deficiency. Vitamin A deficiency also affects other organs and contributes to increased childhood mortality, especially in cases of measles [3].

Inadequate intake of carotene or preformed vitamin A, poor intestinal absorption, or increased demand are the three causes of deficiency, the first being by far the most common.

The best sources of retinol are liver, fish liver oils, egg yolk, and dairy products. However, in developing countries, most poor people get up to 80 percent of their vitamin A intake from carotene in plant foods. This carotene is a yellow pigment, but it can be masked by chlorophyll in dark green leafy vegetables, for example. It is found in many green and yellow vegetables and fruits, in yellow corn, and in some yellow roots such as sweet potatoes. Another very rich source is palm oil, which is commonly consumed in West Africa but is not widely consumed [3].

Vitamin A deficiency is the leading cause of blindness in many endemic areas. Xerophthalmia is usually due to insufficient intake of vitamin A and beta-carotene over a prolonged period. Other factors that can aggravate matters include intestinal parasites, gastroenteritis, or malabsorption. Measles usually precipitates the progression to xerophthalmia through anorexia and stomatitis, which further reduce dietary intake and increase vitamin A requirements. In addition, the virus can directly affect the eye and aggravate damage due to vitamin A deficiency [3].

Vitamin A, preformed or transformed from beta-carotene, is stored in the liver and then transported to other organs by its carrier protein, RBP (Retinol Binding Protein). A protein deficiency can therefore increase a vitamin A deficiency by reducing the synthesis of RBP. The biological activity of vitamin A is now expressed in retinol equivalents (RE) rather than international units (IU). One RE is equivalent to 1 µg of retinol or 6 µg of beta-carotene. The WHO recommends an intake of 300 RE per day for children and 750 RE for adults [3]. According to the long-term preventive approach, the prevention of deficiency is based on increasing the production and consumption of foods rich in vitamin A or carotene by vulnerable populations, coupled with nutritional education. In the meantime, supplementation with massive doses of vitamin A every four to six months can be used, or fortification of certain foods can be considered [3].

Vitamin A is essential for good vision and cell differentiation. Deficiency leads to growth retardation, mucosal damage, reproductive disorders, eye damage, and ultimately blindness. Children with vitamin A deficiency are often deficient in several micronutrients and are likely to be anemic, growth retarded, and at increased risk of severe morbidity from common childhood infections such as diarrhea and measles. Pregnant women with vitamin A deficiency may be at increased risk of mortality. Approximately 30 percent of preschool-aged children are vitamin A deficient, and nearly 5.2 million preschool-aged children suffer from night blindness. A 2013 Lancet article attributed 105,700 infant deaths to vitamin A deficiency. More than 19 million pregnant women in developing countries are also vitamin A deficient, and 9.7 millions are clinically night blind [4].

It is in this context that Senegal has committed itself through this ministerial decree Decree No. 2009-872 of September 10, 2009 making mandatory the application of standards on refined edible oils enriched with vitamin A and soft wheat flour, enriched with iron and folic acid. To launch the promotion of foods enriched with vitamin A constitutes one of the key interventions of the strategic plan to combat child malnutrition and women of childbearing age and vitamin A supplementation to reduce the risk of anemia by improving hemoglobin and ferritin levels [5]. Moreover, good nutrition constitutes a major determinant of health and a key factor for the development of a country because it is an essential condition for the physical, mental and psycho-affective growth of the individual [6]. The aim of this study was to evaluate the vitamin A content by the laboratory analytical method in order to be in line with the will of the Senegalese state concerning its policy towards the compulsory enrichment of refined vegetable oils with vitamin A.

2. Material and Method

This was a cross-sectional quantitative study conducted from June to July 2022.

The study covered three areas, namely three districts in the city of Dakar. The choice was made due to the high demographic concentration of Dakar's population; the municipalities of Grand-Yoff, Pikine, and Keur Massar were selected based on population density.

The shops were randomly selected, three at least 1 km apart. A total of ten types or brands of vegetable oil were listed in each location, for a total of thirty (30) samples across the three municipalities. The samples were hermetically sealed with aluminum foil to prevent contact with UV rays and sent to the analysis laboratory.

2.1. Method of Analysis

The method used is that of the dosage of vitamin A in oil by High Performance Liquid Chromatography (HPLC brand Thermo Jasco LC 4500 coupled with a compact UV-visible detector UV-4570) according to standard [7].

2.2. Saponification Oil

- Weigh 2 g of oil into a flat-bottomed flask.
- Add 50 ml of 0.1% BHT ethanol.
- Add 5 ml of a 60 g/100ml KOH solution.
- Cap, add a little nitrogen, mix, and saponify for 16 hours under gentle stirring in the cold in the presence of a magnetic stir bar (start the timer).

2.3. Retinol Extraction

- Pour in the saponified oil and slowly add 50 ml of double-distilled water (to avoid emulsions) and 50 ml of hexane. Mix thoroughly and shake well.
- Allow to settle and collect the aqueous phase in an Erlenmeyer flask and the organic phase in a round flask.
- Repeat the extraction of the aqueous phase twice with 50 ml of hexane.
- Repeat with just 30 ml of hexane.
- Return the recovered organic phases to the separating funnel and rinse 3 to 4 times with 50 ml of double-distilled water.
- Collect the organic phase in a round flask by filtering with anhydrous Na_2SO_4.
- Rinse all glassware with at least 20 ml of hexane to collect all the contents on

the side of the funnel and the funnel.

- Evaporate to dryness using a rotary evaporator. (35˚C)
- Resolve the dry residue in 5 ml of 50/50 methanol/DCE
- Place in an amber vial and analyze using HPLC

2.4. Chromatographic Conditions

- C18 reversed-phase HPLC column, 15 cm, 5 µm, T˚ = 35˚C.
- Mobile phase: 89/11 (V/V) $MeOH/H_2O$ reversed phase; with 0.01% ammonium acetate.
- Flow rate: 1 ml/min.
- Injection volume: 20 µl.
- Absorbance at 325 nm.

All samples were analyzed in duplicate, using a 45˚C water bath, in which the samples were immersed, with alternating evaporation times between each weighing sample.

However, it should be noted that saponification can also be carried out at a temperature of 80˚C for just 15 minutes.

Statistical Data Analysis: The laboratory analysis results were entered into an Excel 10 spreadsheet and processed using Sigma Plot 13 software.

Expression of the result follows the following formula: C vit A (mg/kg)

$$C = \frac{Ce \times Vd}{Pe}$$

Où C vitA: represents the vitamin content A, en mg/kg,

Ce: represents the concentration read by the chromatograph,

Vd: represents the dissolution volume,

Pe: represents the test sample,

$$C\ (\text{mg/kg}) = \frac{Y - 9056}{114534} \tag{1}$$

$$X = (y - 9056)/114534$$

Table 1 below summarizes the results of this vegetable oil study conducted in the three strata of Dakar.

The data were calculated using Excel 2010 and SigmaStat 11.0 software.

3. Results

The results are expressed in mg/kg of oil, as summarized in Table 1 below;

Table 1. Data on results obtained.

Sample	Strate	Nature of sample	T˚C bain marie	Time evaporation	Results mg/kg	Average mg/kg
E1	GY	HVS	45˚C	1er/20 mn 2é/10 mn	11.32 13.42	12.37 ± 1.48

Continued

E2	GY	HVS	45˚C	1er/15 mn 2e/8 mn	12.92 12.15	12.53 ± 0.54
E3	GY	HVS	45˚C	1er/15 mn 2e/8 mn	12.52 12.36	12.44 ± 0.11
E4	GY	HVS	45˚C	1er/20 mn 2e/8 mn	6.89 6.15	6.52 ± 0.52
E5	GY	HVT	45˚C	1er/8 mn 2e/5 mn	13.04 12.57	12.80 ± 0.33
E6	GY	HVS	45˚C	1er/8 mn 2e/8 mn	6.60 6.01	6.30 ± 0.41
E7	GY	HVT	45˚C	1er/8 mn 2e/8 mn	12.20 12.11	12.15 ± 0.04
E8	GY	HVT	45˚C	1er/8 mn 2e/8 mn	11.78 10.96	11.36 ± 0.4
E9	GY	HVS	45˚C	1er/8 mn 2e/8 mn	10.89 11.01	10.95 ± 0.06
E10	GY	HVT	45˚C	1er/8 mn 2e/8 mn	10.80 11.02	10.91 ± 0.11
E11	GY	HVS	45˚C	1er/8 mn 2e/8 mn	7.80 7.70	7.75 ± 0.07
E12	GY	HVS	45˚C	1er/8 mn 2e/8 mn	8.89 8.90	8.90 ± 0.007
E13	GY	HVS	45˚C	1er/8 mn 2e/8 mn	9.60 10.80	10.20 ± 0.84
E14	GY	HVT	45˚C	1er/8 mn 2e/8 mn	10.10 10.30	10.20 ± 0.14
E15	GY	HVT	45˚C	1er/8 mn 2e/8 mn	8.90 8.80	8.85 ± 0.07
E16	PIK	HVS	45˚C	1er/8 mn 2e/8 mn	13.22 12.92	13.07 ± 0.21
E17	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	12.15 12.61	12.38 ± 0.32
E18	PIK	HVT	45˚C	1er/15 mn 2e/8 mn	12.00 10.14	11.07 ± 1.31
E19	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	10.81 10.80	10.80 ± 0.007
E20	PIK	HVS	45˚C	1er/8 mn 2e/8 mn	11.25 11.14	11.19 ± 0.07

Continued

E21	PIK	HVS	45˚C	1er/15 mn 2e/8 mn	10.26 09.77	10.015 ± 0.34
E22	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	12.05 11.80	11.92 ± 0.12
E23	PIK	HVS	45˚C	1er/8 mn 2e/8 mn	11.75 11.98	11.86 ± 0.11
E24	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	12.05 12.96	12.50 ± 0.45
E25	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	10.66 10.80	10.73 ± 0.07
E26	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	11.52 11.60	11.56 ± 0.056
E27	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	10.50 10.70	10.60 ± 0.14
E28	PIK	HVS	45˚C	1er/8 mn 2e/8 mn	10.87 10.98	10.92 ± 0.07
E29	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	11.15 11.05	11.10 ± 0.07
E30	PIK	HVT	45˚C	1er/8 mn 2e/8 mn	10.37 10.53	10.46 ± 0.12
E31	KM	HVS	45˚C	1er/8 mn 2e/8 mn	11.10 10.54	10.82 ± 0.39
E32	KM	HVT	45˚C	1er/15 mn 2e/8 mn	12.50 11.97	12.23 ± 0.37
E33	KM	HVT	45˚C	1er/8 mn 2e/8 mn	10.89 10.67	10.78 ± 0.15
E34	KM	HVS	45˚C	1er/15 mn 2e/8 mn	10.56 11.07	10.81 ± 0.36
E35	KM	HVS	45˚C	1er/15 mn 2e/8 mn	12.80 11.99	12.39 ± 0.57
E36	KM	HVT	45˚C	1er/8 mn 2e/8 mn	12.22 12.11	12.16 ± 0.07
E37	KM	HVS	45˚C	1er/8 mn 2e/8 mn	11.24 11.55	11.39 ± 0.15
E38	KM	HVT	45˚C	1er/8 mn 2e/8 mn	12.76 12.70	12.73 ± 0.03
E39	KM	HVS	45˚C	1er/8 mn 2e/8 mn	12.89 13.24	13.06 ± 0.24

Continued

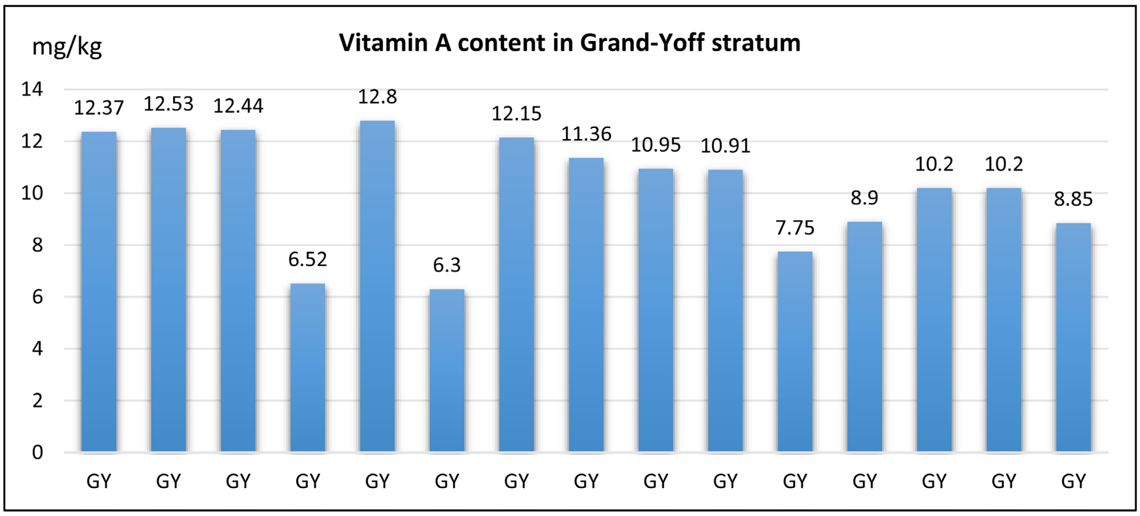
E40	KM	HVT	45˚C	1er/8 mn 2e/8 mn	11.23 11.85	11.54 ± 0.31
E41	KM	HVT	45˚C	1er/8 mn 2e/8 mn	11.90 11.80	11.85 ± 0.07
E42	KM	HVT	45˚C	1er/8 mn 2e/8 mn	12.45 12.15	12.30 ± 0.21
E43	KM	HVS	45˚C	1er/8 mn 2e/8 mn	12.30 11.90	12.10 ± 0.28
E44	KM	HVS	45˚C	1er/8 mn 2e/8 mn	11.28 10.92	11.10 ± 0.25
E45	KM	HVS	45˚C	1er/8 mn 2e/8 mn	11.97 11.23	11.61 ± 0.50

GY = Grand-Yoff, KM = Keur Massar, PIK = Pikine, HVS = Huile végétale Soja, HVT = Huile végétale Tournesol.

3.1. Strata of Grand-Yoff

Analysis of variance yields a positive test with (P = 0.843) according to the Shapiro-Wilk test.

The differences in mean values between the treated elements are quite significant; this possibility of difference may be due to random sampling variability. Graph 1 below shows the more or less random nature of the observed values.

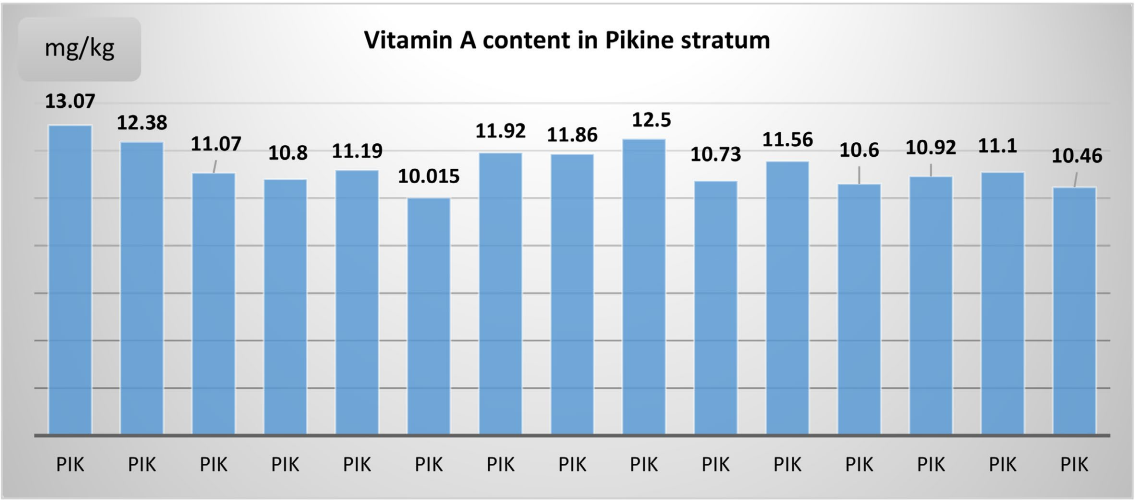


Graph 1. Vitamin A content of samples measured in the Grand-Yoff stratum.

Of the 15 samples constituting the stratum, only two samples E4 and E6 seem to have similar values or similar to the other samples of the same stratum (6.52 and 6.30 mg/kg) in vitamin A content, they have almost low values compared to the other samples which have better vitamin A content, 12.37 mg/kg for the first followed by 12.53 mg/kg then 12.44 and finally 12.80 mg/kg for sample No. 5. However, we note more or less similar values with samples 11, 12 and 15 with the respective values of 7.75 mg/Kg, followed by 8.90 mg/kg and 8.85 for the last sample of the Grand Yoff stratum which gives on average a value of 10.28 ± 2.17 mg/L. There is a fairly significant difference among the samples of the Grand-Yoff stratum with (P = <0.001).

3.2. Strata of Pikine

While Graph 2 below clearly illustrates a more or less constant variation in the results data observed in the Pikine stratum.



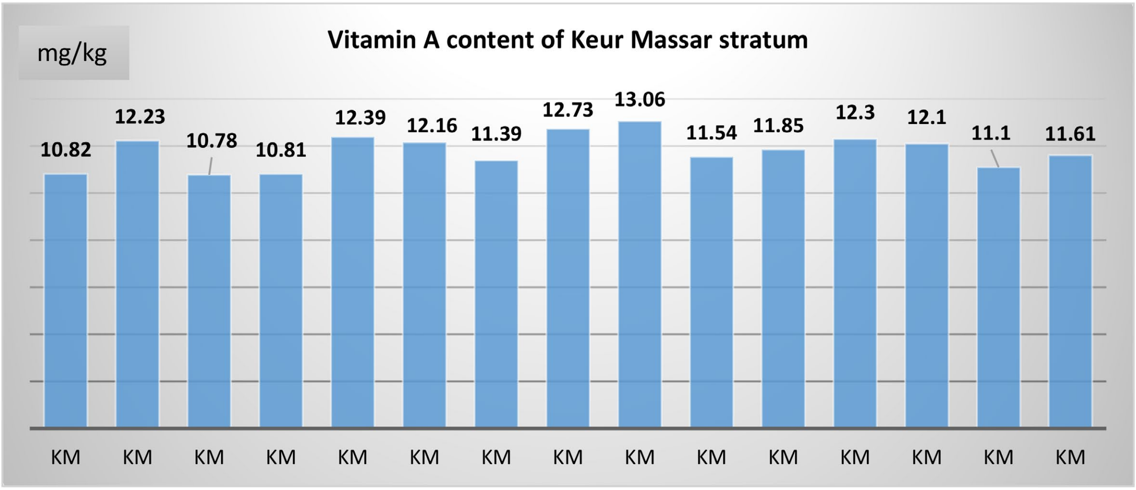
Graph 2. Vitamin A content of samples measured in the Pikine stratum.

According to the analysis of variance with (P = 0.686). The Pikine stratum offers a range of values greater than or equal to 11 mg/kg, which means the good conditions of conservation and storage of oil products marketed in Pikine supermarkets. With the average value of 11.34 ± 0.85 mg/L, much higher than that of the Grand-Yoff stratum which has the value of 10.28 ± 2.17 mg/kg. There is a fairly significant difference among the samples of the Grand-Yoff stratum with (P = < 0.001).

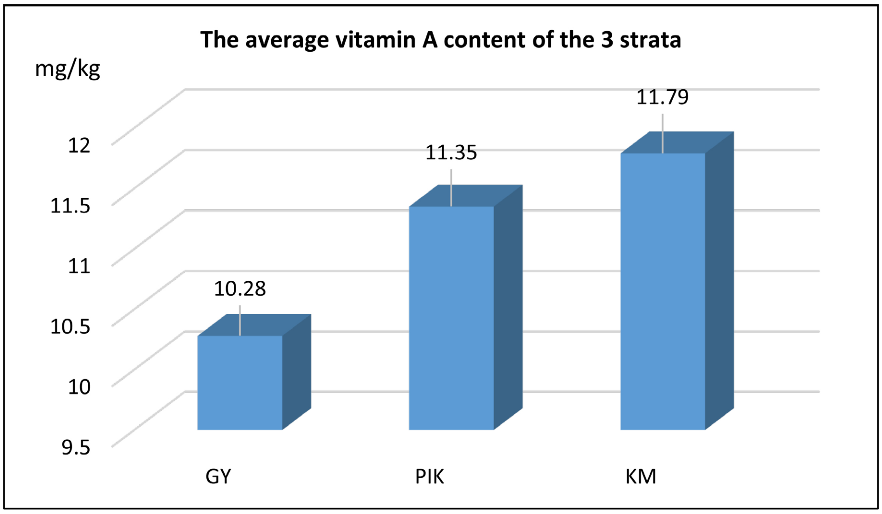
3.3. Strata of Keur Massar

The Keur Massar stratum shows a more obvious disparity according to the 15 samples taken, we can clearly see a division on a scale of ½; in other words at 50% as shown in the following Graph 3.

The Keur Massar stratum yields an average value of 11.79 ± 0.71 mg/L, which demonstrates the good dynamics of the quality of the oils sold in this area, with a good policy of supplementing oils sold in the Dakar market. The normality test yields (P = 0.560) according to (Shapiro-Wilk) following the analysis of variance. There is a statistically significant difference in the Keur Massar stratum with (P = <0.001). Graph 4 below shows the average content of the values of the three strata.



Graph 3. Vitamin A content of samples measured in the Keur Massar stratum.



Graph 4. The average vitamin A content in the three strata.

Even if the average contents of the two strata, that of Pikine and Keur Massar, are visibly similar 11.35 ± 0.85 mg/kg and 11.79 ± 0.71 mg/kg, these values are far higher than that of the Grand-Yoff stratum which presents a lower value of 10.28 ± 2.17 mg/kg, this exactly reflects that the commune of Grand-Yoff has problems of conservation and also of oxidation which could be linked to the exposure to heat or also to the humidity of the stock rooms, to which would be added in particular the displays in copper or iron probably. There is a significant difference between the values of the three strata studied with ($P = 0.002$).

4. Discussion

The results of this study provide a fairly interesting scope according to the different strata analyzed. It emerges that the Grand-Yoff stratum gives a significant statistical difference according to ANOVA, which implies that there is a statistically significant difference ($P = < 0.001$), however the difference may result solely from the variability of random sampling.

The analysis of variance between the different samples gives a normal distribution, this normality is equivalent according to the Shapiro Wilk test P = 0.090.

A statistically significant difference (P = 0.023) was noted between the different strata studied according to the Kruskal-Wallis test following the ANOVA analysis of variance (P = 0.023).

This difference can only come from the state of conservation on the one hand but on the other hand from the presence of exposure to UV rays which could be the source of a degrading effect on the quality of the oils and also of oxidation with the peroxide index or the presence of ketone compounds in particular which can alter the products stored in local supermarkets. The differences in the median values between the treatment groups are greater than what could be expected by chance. Note that the deficiency in vitamin A and micronutrients is not an end in itself, this could be corrected by the essential contribution through the use of food fortification which is an effective and sustainable means [8].

These study findings align with the same policy focus exercised since 1994 by Nutrition International, which leads the global effort to provide vitamin A supplements to developing countries worldwide with the goal of reducing under-five mortality. The government of Senegal aims through these projects and programs to advance food fortification in Senegal, such as the Fortification Strengthening Program (PRF), which ensures the implementation of fortification standards, particularly with regard to the fortification of oil and flour, through compliance monitoring, capacity building, development of quality assurance systems, distribution of fortified products, as well as social marketing and communication [9]. Vitamin A deficiency is a public health problem in many low- and middle-income countries. Vitamin A deficiency weakens the immune system, which puts a child at a higher risk of illness and early death. It is also the leading cause of preventable childhood blindness. In pregnant mothers it causes night blindness during pregnancy and newborns suffer from vitamin A deficiency [10].

Access to and increased consumption of vitamin A-rich foods by vulnerable populations is essential to reduce deficiency, particularly through fortified foods. Despite efforts to improve diets, vitamin A supplementation has been shown to be effective in reducing the impact of deficiency, particularly in children aged six months to five years [11].

Given the differences observed in the three strata, it can be stated that peroxides are intermediates in the oxidation reaction leading to the deterioration of lipids in the oxidation of oils in some districts of Dakar. Therefore, a high peroxide index is an indication of the sensitivity of an oil to rancidity [12]. It should also be noted that the oxidation rate is considerably increased by the catalytic effect of copper or copper alloys, even if it is only present in trace amounts (ppm). This is why copper and copper alloys must be strictly excluded from installations. Other metals such as iron also have catalytic effects but less pronounced than those of copper [13].

5. Conclusions

The oils marketed in Senegal come on the one hand from national production which is industrial with Sonacos and on the other hand from imports to cover the needs of consumers in the Senegalese market which is too large, in addition there is an artisanal production which is less despite awareness efforts to curb this practice which does not offer all the guarantees in terms of hygiene and food safety. Faced with the diseases of xerophthalmia, vitamin A deficiency and anemia, which primarily affect young children and women of childbearing age, Senegal has enrolled in the vitamin A oil fortification program, through Decree No. 2009-872 of September 10, 2009, making mandatory the application of standards on refined edible oils enriched with vitamin A and soft wheat flour, enriched with iron and folic acid.

In West Africa, the fortification of oils with vitamin A is already effective in Ghana, Nigeria and Ivory Coast. In view of these important issues, the Government of Senegal has initiated the fortification process which resulted in the adoption of Senegalese standards on edible oils fortified with vitamin A and soft wheat flour enriched with iron and folic acid [2]. The aim of this study was to evaluate the vitamin A content which is in perfect alignment with the policy defined by the State of Senegal on the mandatory fortification of refined vegetable oils with vitamin A on the one hand, and on the other hand to alert and define a new trajectory to allow the surveillance and regular monitoring of the health control plan on foodstuffs marketed on the Senegalese market with the support of competitiveness to satisfy the strong aspiration of the population for better well-being [14]. This will involve, among other things, diversifying the productive base to strengthen the resilience of the economy through agro-industry and industrial development by establishing the best long-term resilience which would consist of diversifying sources of production and improving growth [15].

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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[13] Annexe III. Avant-projet de norme pour les graisses et les huiles comestibles non visees par des normes individuelles. Norme NS 3-72 sur l'huile comestible de palme raffinée enrichie en vitamine A.

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