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Effect of a multi-step preparation of amaranth and palm nut sauces on their carotenoid content and retinol activity equivalent values

Waliou Amoussa-Hounkpatin,[1,2] Claire Mouquet-Rivier,[2] Adéchola Pierre Polycarpe Kayodé,[1] Joseph Djidjoho Hounhouigan[1] & Sylvie Avallone[3]*

1 DNSA, FSA, University of Abomey-Calavi (UAC), 01 BP 526, Cotonou, Benin
2 IRD, UMR 204, IRD/Montpellier1/Montpellier2/SupAgro (NUTRIPASS), F-34394, Montpellier Cedex 5, France
3 Montpellier SupAgro, UMR 204, IRD/Montpellier1/Montpellier2/SupAgro (NUTRIPASS), F-34394, Montpellier Cedex 5, France

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Summary The preparation of a traditional sauce based on amaranth leaves cooked palm nuts or red palm oil (RPO) in Benin was described. The recipes included an optional step of leaf blanching at 100 °C, heating the RPO or boiling the palm nuts and finally cooking all the ingredients together. The influence of this multi-step preparation on the carotenoid content of the final dish was measured. During blanching of amaranth leaves, violaxanthin was the only carotenoid to be significantly affected by the thermal treatment. Retinol activity equivalent (RAE) remained high after blanching even when alkaline traditional potash was added. Heating the RPO was the most critical step because it considerably and very rapidly (in <3 min) decreased α-carotene, β-carotene and RAE values (more than 70%). Sauces calling for palm nuts, RPO and amaranth leaves were equally advantageous in terms of final RAE value. These ingredients and sauces can thus be used in programmes to reduce vitamin A deficiency.

Keywords Amaranth leaves, Benin, boiling, carotenoids, oil heating, red palm oil.

Introduction

In developing countries, vitamin A is recognised as a determining factor in adult and child mortality from infectious diseases; its deficiency remains the commonest cause of preventable childhood blindness (Sommer *et al*., 1996). Eliminating vitamin A deficiency (VAD) would save 16% of the global burden of disease in children (*Mason et al.,* 2003). Adequate intake of local provitamin A-rich foods is a viable food-based strategy to alleviate VAD. In southern countries, the major dietary sources of vitamin A are provitamin A carotenoids from plants, especially β-carotene and, to a lesser extent, α-carotene and other vitamin A active carotenoids (Pepping *et al*., 1988). Food containing preformed vitamin A such as dairy products, eggs and liver is rarely eaten by vulnerable people because of socio-economic constraints. In Benin, VAD is recognised as a public health problem because of low intakes of vitamin A, provitamin A and fat (Mason *et al*., 2003; INSAE & Macro International Inc., 2007).

Green leafy vegetables, red palm oil (RPO) and palm nuts are grown and consumed throughout West Africa. Depending on soil and agro-climatic conditions, these foods can be rich in β-carotene (Shukla & Singh, 2000; Veda *et al*., 2010). In Africa and Asia, amaranth leaves (*Amaranthus cruentus*) are an inexpensive source of protein, carotenoids, vitamin C, dietary fibre and essential minerals (Prakash & Pal, 1991; Shukla *et al*., 2003, 2006; Adebooye *et al*., 2008). Palm oil is the most widely consumed plant oil in the world. In its crude form, RPO is the richest source of provitamin A carotenoids (50–70 mg/100 g; Edem, 2002). It contains high amounts of bioavailable α-carotene and β-carotene because of the liquid and fatty medium (Choo *et al*., 1993; You *et al*., 2002). Regular consumption of small amounts of RPO has been shown to have a positive impact on the vitamin A status of children (Zagré *et al*., 2003; Zeba *et al*., 2006). RPO is also a source of several antioxidants (vitamin E, carotenoids) involved in the prevention of cancer and cardiovascular diseases (Gann *et al*., 1999; Ng *et al*., 2000; Edem, 2002; Kritchevsky *et al*., 2002).

*Correspondent: Fax: +33 467 41 63 30;
e-mail: sylvie.avallone@ird.fr

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However, vegetables and palm nuts need to be cooked in an appropriate way to avoid loss of carotenoid (Hedrén *et al*., 2002). Indeed, the way food is prepared is known to have an impact on the dietary patterns of populations. According to the cooking time and/or temperature, thermal treatments (*i.e*. blanching, boiling, cooking, frying) have very distinct effects on the carotenoid pattern of processed food (Mayer-Miebach & Spiesz, 2003). While the heat treatment involved in cooking is a necessary step in making the food palatable and in improving the digestibility of food components, undesirable changes associated with cooking are a reduction in micronutrient contents caused by chemical reactions (Veda *et al*., 2010). At lower temperatures (60–100 °C), most carotenoids are well retained and isomerisation is negligible during blanching, pasteurisation, cooking, low temperature drying and frying (Maiani *et al*., 2009). At the same time, the breaking down of food matrices (*e.g*. cell walls and membranes) facilitates the liberation and accessibility of bound carotenoids and increases their bioavailability (Maiani *et al*., 2009). Furthermore, inactivation of oxidative enzymes prevents further (and greater) losses during slow processing (Rodriguez-Amaya, 1997). The reduction in the particle size of food (*e.g*. grinding or milling) or the incorporation of an oil phase in food formulations also improve carotenoid bioaccessibility (Hedrén *et al*., 2002; Huo *et al*., 2007). At higher temperatures (*i.e*. frying), carotenoid losses are greater. Carotenoids with their unsaturated structure are very fragile and can be degraded according to two main reactions: isomerisation (a change of configuration from its natural *trans* form to various *cis* isomer species) and oxidation (the major cause of loss) through a radicalar mechanism with free radical attacks.

The nutritional value of African crops has been described in various articles and reports (WHO/IRD, 2001; Addis *et al*., 2009; Greffeuille *et al*., 2010), but few data are available on the characterisation of dishes formulated with processed local ingredients such as red palm oil, leafy vegetables (amaranth leaves) or palm nuts even though such dishes are very frequently consumed by vulnerable people. In this article, we describe multi-step preparations of traditional sauces frequently consumed by 6- to 35-month-old children in Benin based on amaranth leaves and palm nuts. The most critical steps in terms of carotenoid losses were identified during household preparation.

Materials and methods

Materials

Approximately 500 g of amaranth leaves (*Amaranthus cruentus* L.) was purchased in a garden on the outskirts of Natitingou (Benin). They were grown in the same field (27 m^2) during the dry season (from February to March 2010) and conducted rapidly to Montpellier in cold atmosphere. Leaves with the same colour, size and aspect were chosen, and approximately 2.5 g of amaranth leaf was used to determine the effect of the hydrothermal cooking on dry matter (DM) contents.

Traditional potash, a soluble ingredient that corresponds to the dried residue of an alkaline extract of wood ash, is used in various African countries during leafy vegetable blanching. This ingredient was purchased at the local market in Natitingou and conducted to Montpellier. Mature palm nuts (*Elaeis guineensis* Jacq.) were purchased in the same market and conducted rapidly to Montpellier in cold atmosphere; approximately 2.5 g of palm nut was used to determine the effect of cooking on DM content and 400 mg for carotenoid analysis. For cooking experiments, volvic® water (a natural water with stable and standardised mineral composition) was used. It is commercialised in a polyethylene bottle of 1.5 L. All the experiments and analysis were performed in Montpellier's laboratory.

Monitoring the preparation of traditional sauces and sampling

The preparation of three potentially vitamin A-rich sauces eaten by young children (Amoussa-Hounkpatin *et al*., 2012) and consumed by the whole family was monitored in ten households in Natitingou (Benin). These were as follows:

- Four sauces based on blanched amaranth leaves and RPO.
- Three sauces based on blanched amaranth leaves and palm nut juice.
- Three sauces based on fresh amaranth leaves and palm nut juice.

A rapid appraisal had previously helped identify the ingredients used to prepare the sauces. The households that prepared sauces were randomly selected. The step-by-step preparation of sauces was observed during home visits, and the recipes were written down in detail. The types and quantities of ingredients and the chronology of each unit operation used to prepare the sauces were recorded, along with the cooking temperature and total preparation time. At the end of the preparation, a homogenous representative sample of the sauces was taken and divided into three portions. The ingredients and the samples of final product were placed in separate tightly sealed plastic containers and kept on dry ice during transport to the laboratory. One portion was immediately used to measure the DM content of the sauce. The second portion was freeze-dried and crushed before lipid analysis. The third portion was frozen (−20 °C) to determine carotenoid and retinol contents.

Physicochemical analysis

The DM contents of fresh samples were determined by oven drying at 105 °C to reach the constant weight.

Recipes and cooking procedures in the laboratory

To identify the most critical step in terms of retinol activity equivalent (RAE) decrease, the preparation of the sauces was reproduced in Montpellier's laboratory. In Benin, the name of the sauce corresponds to the main ingredient used in the recipe. The palm sauces were based on amaranth leaves and (a) palm nuts or (b) red palm oil (Fig. S1). Three thermal treatments (amaranth blanching, palm nuts boiling, RPO heating) were carried out just like in the households (Table 1, Figs S1 and S2) with four replications for each treatments. The samples were placed in sealed Pyrex tubes and heated on a hot plate stirrer (MR Hei-Tec, Heidolph, Germany) connected to an electronic temperature controller (EKT 3001, Reax 2, Heidolph, Germany), providing a temperature accuracy of ±1 °C. The hot plate was covered with a reaction block, which ensured high thermal homogeneity of the twelve vials (Drysyn, Heidolph, Germany).

Amaranth leaves were cooked at 98 °C for 27 min. The stems were discarded and the leaf/water ratio reach 0.135 as observed in household. Traditional potash was sometimes added to soften leaf tissues (1.8 g of solid extract for 100 g of fresh leaves). The final concentration of the traditional potash was then 2.2 g L^{-1} (pH of 9.9, pH meter; WTW, Weilheim, Germany). After cooking, the leaves were drained and cooled during 5 min. Palm nuts (8.5 g) were boiled in 17 g of water for 21 min at 98 °C and then crushed with a mortar and pestle for 15 min to extract their red juice. When the juice had reached the desired texture and colour, the solid residues were discarded; it was then boiled with other ingredients including fresh blanched amaranth, tomatoes, hot pepper, black pepper, onion, garlic, shrimps, smoked fish, a broth cube, baobab seeds or groundnut paste. RPO heating was done from 0 to 4 min at 180 and 200°C and ingredients were then added.

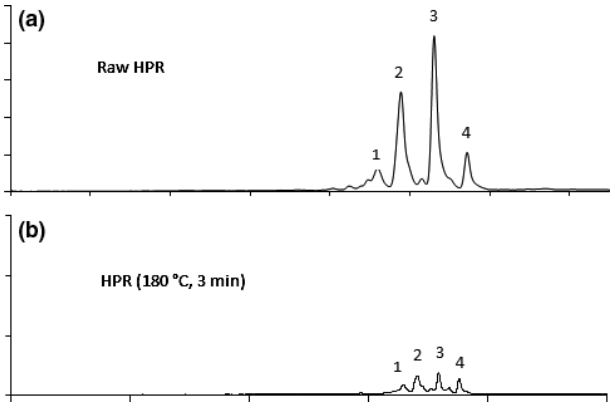

Figure 1 Chromatograms of (a) red palm oil and (b) red palm oil heated at 180 °C during 3 min. (1) 13-*cis*-β-carotene, (2) α-carotene, (3) β-carotene, (4) 9-*cis*-β-carotene.

Carotenoid identification and quantification

HPLC standards (α-carotene, β-carotene, lutein, retinol, retinyl acetate, retinyl palmitate) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). Violaxanthin, β-cryptoxanthin and lycopene were obtained from Carotenature GmbH (Lupsingen, Switzerland). Carotenoid extraction was adapted from Taungbodhitham *et al.* (1998). Amaranth leaves (approximately 150 mg) and palm nuts (approximately 200 mg) were homogenised with a Ultra Turrax (IKA, France) for 1 min in a glass tube with 10 mL of ethanol hexane 4:3 (v/v). Analysis of amaranth leaves, cooking water, palm nut pulp and RPO was performed with four repetitions. The cooking water (approximately 10 mL) was treated like the solid samples (*i.e.* mixed with 10 mL of ethanol hexane 4:3 and homogenised with a Ultra Turrax for 1 min). Extracts were filtered through a glass funnel, transferred to a glass tube, washed with 10 mL of 10% NaCl, 10 mL of distilled water and left for 10 min for partitioning. The organic extract was dried with N_2 and, immediately before injection, redissolved in 2 mL of acetone and filtered through a 0.2 μm PTFE minisart SRP4 (Sartorius, Germany). Crude and thermal-treated RPO were diluted with acetone, and the mixtures were then centrifuged (−9 °C, 900 g, 15 min) to precipitate the saturated fat of palm oil and protect the HPLC column. We have previously checked that the carotenoids still remained in the upper phase after a short and cold centrifugation. The samples were filtered through a 0.2 μm PTFE minisart SRP4 (Sartorius, Germany).

Carotenoid analysis was performed with an Agilent 1100 series chromatograph (Dionex, Sunnyvale, CA, USA). The column was a polymeric YMC_{30} (4.6 mm i.d × 250 mm, 5 μm particle size; YMC, Inc, Wilmington, NC, USA). The mobile phase compromised two mixtures (methanol and milli-Q water, 60:40, v/v, and methanol, methyl tert-butyl-ether and milli-Q water, 28.5:67.5:4, v/v/v) at a flow rate of 1 mL min^{-1}. The gradient used went from 100/0 to 0/100 over a period of 65 min. When RPO or dishes based on palm nuts were analysed, the column was washed every day with ethyl acetate during 2 h at a low flow at 0.2 mL min^{-1} to prevent fat deposition on the HPLC device.

Table 1 Physical parameters of the steps used to prepare amaranth and palm sauces

	Temperature (°C)	Duration (min)
Palm nut and amaranth sauce		
Amaranth blanching ($N = 7$)	98 ± 5 [86; 101]	18 ± 8 [7; 27]
Palm nut boiling ($N = 5$)	98 ± 1 [97; 99]	21 ± 4 [17; 27]
Cooking ($N = 4$)	96 ± 1 [94; 98]	56 ± 16 [39; 75]
Red palm oil and amaranth sauce		
Amaranth blanching ($N = 7$)	98 ± 5 [86; 101]	18 ± 8 [7; 27]
Red palm oil heating ($N = 3$)	186 ± 13 [176; 200]	4 ± 4 [1; 8]
Cooking ($N = 3$)	96 ± 2 [94; 98]	34 ± 17 [25; 43]

N, number of repetitions. Values are means ± standard deviations [min; max].

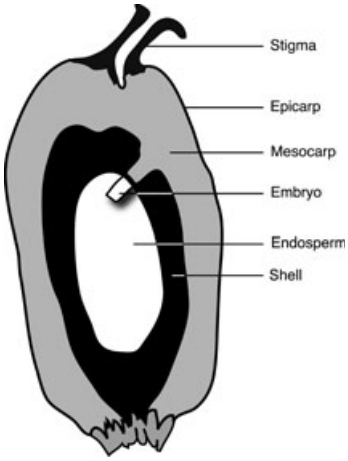

Figure 2 Cross section of a palm nut.

A UV-visible photodiode array detector (Dionex UVD 340U) was used, and chromatographs were quantified at the wavelength of maximum absorption of the carotenoids in the mobile phase ($\lambda = 450$ nm). The carotenoids were identified by comparing their retention times and spectra with standards when available; co-chromatography was sometimes used. The carotenoid isomers were identified according to their retention times and absorption spectra given by the literature (Fig. 1) (Aman *et al.*, 2005). Wavelength absorption maxima, ratio of absorption intensity at the near-UV maximum (327–339 nm) to the absorption intensity at the main absorption maximum and the peak ratio III/II were calculated. The amounts of isomers of β-carotene were expressed in *trans*-β-carotene-equivalent. Our extraction procedure and HPLC analysis were validated using Standard Reference Material 2385 (slurried spinach, from the National Institute of Standards and Technology, Gaithersburg, MD, USA).

A conversion factor of twelve for β-carotene and of twenty-four for the other provitamin A (α-carotene, β-cryptoxanthin) and for one retinol was used to determine the RAE expressed in mg RAE/100 g of each product (Murphy, 2002).

Statistical analysis

Data were assessed by analysis of variance using the Statistical software Statgraphics plus 5.1 (Virginia, USA). The Fisher's test and Duncan's multiple range tests were used to separate means. Significance was accepted at $P < 0.05$.

Results and discussion

Characterisation of the ingredients (amaranth leaves, palm nuts and RPO) of the sauces

The amaranth leaves had an initial DM content of 14.5%, and β-carotene, lutein and violaxanthin were their main carotenoids (Figs 3 and 4). The palm nuts were composed of 17.5% of kernel (endosperm), 40.7% of hull (shell), 31.3% of pulp (mesocarp) and 5.7% of skin (epicarp; Fig. 3). The DM content of the pulp is 79.2%, and three provitamins A were detected (Fig. 6): α-carotene (22.8 ± 10.2 mg/100 g wet basis, WB), β-carotene (29.4 ± 9.2 mg/100 g WB) and 9-cis-β-carotene (4.2 ± 0.4 mg/100 g WB). The RAE value of the palm nut pulp was very high (4.5 ± 1.5 mg/100 g WB).

In the same way, RPO contained a high amount of α-carotene (41.8 ± 4.3mg/100g), β-carotene (41.8 ± 4.2 mg/100 g), 9-*cis*-β-carotene (10.0 ± 1.1 mg/100 g) and RAE value (7.4 ± 0.6 mg/100 g). The total carotenoid content (93.6 mg/100 g) of RPO showed that this product is one of the richest oils in the palm tree biodiversity. Average carotenoid contents (50–70 mg/100 g) are reported throughout the world (Sambanthamurthi *et al.*, 2000; Edem, 2002), and the contents can reach 160 mg/100 g for some Beninese populations (Rajanaidu *et al.*, 2006).

Influence of blanching on the DM, carotenoid and RAE values of amaranth leaves

After cooking in water or in traditional potash, the weight of amaranth leaves greatly increased (by 180%) because of hydration during cooking. As a result, the DM content of the leaves decreased significantly to, respectively, 4.3% and 4.6% with both cooking procedures. The DM content of the initial water was 0.13 g/L. It deeply increased because of the diffusion of nutrients from the leaves into the water ($P < 0.001$). In the presence of traditional potash, the DM of the cooking medium was significantly higher (6.4 g/L) because

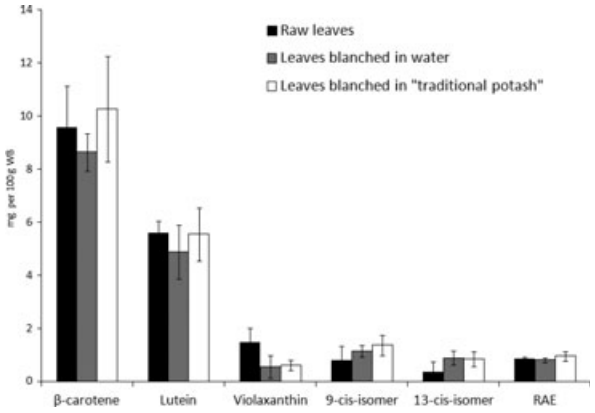

Figure 3 Carotenoid contents and retinol activity equivalent (RAE) in fresh and blanched amaranth leaves ($n = 4$).

of the addition of this solid residue (with high amount of DM *approximately* 99%). The final DM content of the cooking medium without traditional potash reached 4.2 g L^{-1}. DM diffusion from the amaranth leaves into the cooking medium reached 19% with water and 16% with traditional potash added to the water. Cooking in traditional potash slightly prevents the diffusion of DM from the leaves into the cooking medium because the medium is more concentrated and diffusion is thus reduced.

The blanching step did not significantly affect the carotenoid profiles of the amaranth leaves (Fig. 2). As a consequence, the RAE value of the amaranth leaves was the same before and after blanching. However, 9-*cis*-β-carotene and 13-*cis*-β-carotene contents were slightly higher in cooked amaranth leaves than in fresh leaves ($P < 0.05$) because of isomerisation reaction during thermal treatments. The use of traditional potash during blanching of the leafy vegetable did not increase the degradation of the carotenoids even though this product is alkaline. After soaking in the alkaline solution, significant losses of hydrosoluble vitamins such as thiamin, riboflavin and niacin occurred during cooking (Prodanov *et al.*, 2004). Carotenoids thus appear to be more stable in alkaline conditions than hydrosoluble vitamins. Previous studies demonstrated that blanching did not affect the carotenoid level of leafy vegetables (Addis *et al.*, 2009). Carotenoid isomerisation is rather limited during processing at low temperatures (60–100 °C; *i.e.* blanching, pasteurisation, cooking, low temperature drying), and the effects of thermal processing on the losses and bioavailability mainly depend on the severity of the treatments (Maiani *et al.*, 2009).

Influence of cooking and of heating the oil on DM, carotenoid and RAE values of palm products

When palm nuts were crushed, only the soft red-coloured pulp was solubilised in the hot water. According to our data, approximately 20% of the

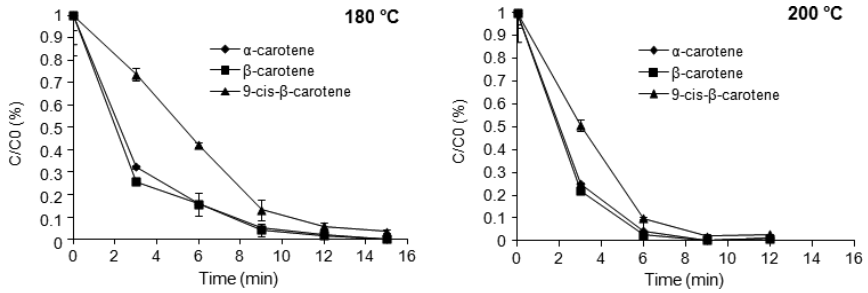

Figure 4 Carotenoid degradation during red palm oil heating at 180 and 200 °C ($n = 4$).

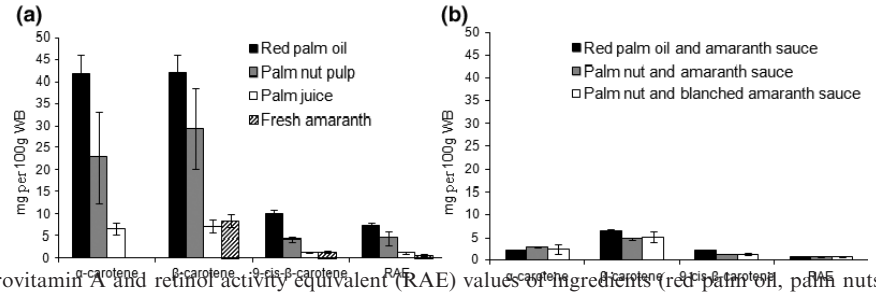

Figure 5 Provitamin A and retinol activity equivalent (RAE) values of ingredients (red palm oil, palm nuts, fresh amaranth leaves) and of the intermediate product (palm juice) (a) and final sauces (b).

carotenoids present in the palm pulp was extracted after crushing. When RPO was heated at 180 and 200 °C (Fig. 4), substantial and similar losses (70% and 80%) of β and α-carotene were observed after 3 min. In these conditions, carotenoid isomerisation and 9-*cis*-β-carotene degradation occurred because of high temperature during oil heating. The longer RPO is heated, the more carotenoids are lost. Degradation was faster at 200 °C than at 180 °C. As a consequence, heating RPO resulted in rapid and total loss of RAE. In the household, RPO was heated from 1 to 8 min. According to the kinetics observed in the laboratory, in these conditions, carotenoid losses would range from 25% at 1 min to 95% at 8 min. There is thus a major advantage to be had by quick frying of RPO when preparing this sauce.

The RAE values of the resulting sauces ranged from 0.69 mg/100 g (palm nut and blanched amaranth sauce), 0.70 mg/100 g (palm nut and amaranth sauce) to 0.82 mg/100 g (RPO and amaranth sauce) as eaten (Fig. 3). Whatever the formulation or thermal treatment, the use of palm nuts or RPO as ingredient of the sauce results in a vitamin A-rich dish. Indeed, plant foods are considered to be rich in vitamin A when their content is more than 0.3 to 1 mg RAE for 100 g of dish eaten (WHO/IRD, 2001). When these traditional sauces are consumed by young Beninese children, they contribute significantly to fulfilling the children's vitamin A needs.

Discussion

Palm nut and RPO are two provitamin A-rich products that are available and financially accessible to all individuals in West Africa and other countries where palm trees are grown. They could be the ideal tool to combat vitamin A deficiency wherever this is a public health problem. The promotion of the sauces based on these ingredients and cooked in appropriate ways should thus be encouraged. Analysis of *in vitro* accessibility of carotenes from leafy vegetables cooked in Tanzania showed that adding RPO instead of sunflower oil doubled the quantity of accessible β-carotene (Hedrén *et al*., 2002). If vegetable relishes with added oil are eaten on a daily basis, it should be possible to cover the recommended vitamin A intake.

Red palm oil has a preferential place in alleviating VAD among the provitamin A plant-based foods because of the absence of food matrices and its lipid medium, both of which are favourable for the absorption of carotenoids (Delisle *et al*., 2003). The lipid content of the RPO and palm nut sauces was, respectively, 61.2 ± 0.2 and 54.9 ± 0.1 g/100 g DM (data not shown). The DM content was 27.5 ± 0.1 g/100 WB for RPO sauce and 15.7 ± 0.1 g/100 g WB for palm nut sauce. During our consumption survey, the median quantities of these sauces ingested by young children were 33 g for RPO sauce and 37 g for palm nut sauce (data not shown); these quantities correspond to 5.8 and 3.1 g of lipid intakes per meal, respectively. These lipid intakes per meal are theoretically sufficient to ensure the bioavailability of provitamin A carotenoids in human and could help improving the vitamin A status of the children. Indeed, a recent study showed that a minimum of 2.4 g fat per meal is required to ensure the absorption of provitamin A carotenoids in meals containing leafy vegetable and hence to improve the vitamin A status of the consumer (Ribaya-Mercado *et al*., 2007; Tang, 2010).

Conclusion

Leafy vegetable blanching and the production of juice from palm nuts had a negligible effect on the RAE values of the initial products. The most critical step was the RPO heating (at 180–200 °C) because RAE decreased in few minutes. The thermal treatment applied to the palm nut juice is less detrimental to RAE value than the very hot treatment of RPO. Nevertheless, the resulting dishes have interesting provitamin A profile and can contribute to alleviating vitamin A deficiency and its corollaries in Benin.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart of preparation of amaranth and palm sauces with (a) palm nuts or (b) red palm oil.

Figure S2. Effect of blanching on the dry matter (DM) contents of amaranth leaves and of the blanching medium ($n = 4$). Letters (a, b) indicate a significant difference between samples.

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