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# Red palm oil-supplemented and biofortified cassava gari increase the carotenoid and retinyl palmitate concentrations of triacylglycerol-rich plasma in women

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#### ABSTRACT

Boiled biofortified cassava containing  $\beta$ -carotene can increase retinyl palmitate in triacylglycerol-rich plasma. Thus, it might alleviate vitamin A deficiency. Cassava requires extensive preparation to decrease its level of cyanogenic glucosides, which can be fatal. Garification is a popular method of preparing cassava that removes cyanogen glucosides. Our objective was to compare the effectiveness of biofortified gari to gari prepared with red palm oil. The study was a randomized crossover trial in 8 American women. Three gari preparations separated by 2-week washout periods were consumed. Treatments (containing 200-225.9 g gari) were as follows: biofortified gari (containing 1 mg  $\beta$ -carotene), red palm oil-fortified gari (1 mg  $\beta$ -carotene), and unfortified gari with a 0.3-mg retinyl palmitate reference dose. Blood was collected 6 times from -0.5 to 9.5 hours after ingestion. Triacylglycerol-rich plasma was separated by ultracentrifugation and analyzed by high-performance liquid chromatography (HPLC) with diode array detection. Area under the curve for  $\beta$ -carotene,  $\alpha$ -carotene, and retinyl palmitate increased after the fortified meals were fed (P < .05), although the retinyl palmitate increase induced by the red palm oil treatment was greater than that induced by the biofortified treatment (P < .05). Vitamin A conversion was 2.4  $\pm$  0.3 and 4.2  $\pm$  1.5  $\mu$ g pro-vitamin A carotenoid/1  $\mu$ g retinol (means  $\pm$ SEM) for red palm oil and biofortified gari, respectively. These results show that both treatments increased  $\beta$ -carotene,  $\alpha$ -carotene, and retinyl palmitate in triacylglycerol-rich plasma concentrations in healthy well-nourished adult women, supporting our hypothesis that both interventions could support efforts to alleviate vitamin A deficiency.

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Abbreviations: AC,  $\alpha$ -carotene; AUC, area under the curve; BC,  $\beta$ -carotene; BFG, biofortified cassava gari; high-performance liquid chromatography, HPLC; ROG, red palm oil-fortified gari; RP, retinyl palmitate; TAG, triacylglycerol; WG + RP, white cassava gari with retinyl palmitate tracer.

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# 1. Introduction

Vitamin A deficiency is a leading cause of morbidity and mortality, especially in young children and pregnant and lactating women [1]. Food-based interventions focused on alleviating vitamin A deficiency in susceptible populations are especially useful in rural areas because they may provide vitamin A-forming carotenoids as well as income to farmers [2].

Cassava is an inexpensive and easy to grow tuber that can survive droughts and is resistant to many pests [3]. Common varieties of cassava are poor sources of pro-vitamin A carotenoids [4,5], but recently, multinational nongovernmental organizations such as HarvestPlus have developed biofortified cassava with relatively high concentrations of  $\beta$ -carotene (BC) [2,6–8]. Several yellow-fleshed cassava cultivars with moderately high concentrations of BC have been released to rural populations in Nigeria, where cassava is a staple crop [9].

Previous studies showed that biofortified cassava enriched with BC successfully maintained vitamin A status in Mongolian gerbils [10] and increased BC and retinyl palmitate (RP) concentrations in the triacylglycerol (TAG)-rich plasma of healthy adult women [11]. Results from the previous human study [11] were used to simulate the amount of vitamin A which would be consumed if all native white cassavas were replaced with biofortified cassava. In a model based on these previous results, replacing native with biofortified cassava would provide more than 90% of the recommended dietary intake of vitamin A [12].

However, this study used boiled cassava, which is not the preferred cassava-based food in Africa. Cassava contains cyanogenic compounds that must be decreased to make it safe to consume [13,14]. Several food-processing methods have been developed in sub-Saharan Africa to remove cyanide: the most popular being sun drying and garification [15–18]. In garification, cassava is minced, fermented, dewatered, and then roasted to form coarse flour. More information is required on the effects of garification on the bioavailability and bioconversion of carotenoids from biofortified cassava.

The concentration of carotenoids in cassava-based foods can be increased either by substituting biofortified cassava for common unfortified cassava or by adding a food with a very high concentration of BC or vitamin A to the cassava. Red palm oil is an extremely rich source of BC and  $\alpha$ -carotene (AC), 2 common pro–vitamin A carotenoids. As such, it has been used to prevent vitamin A deficiency in individuals and small-scale human intervention trials for decades [19–28].

In this study, we compare the effect of biofortified gari (BFG) to an equal amount of red palm oil–fortified cassava gari (ROG), containing equivalent amounts of BC, on the BC, AC, and RP concentrations of TAG-rich plasma. Treatments were fed to 8 healthy well-nourished American women in a crossover double-blind study. Our objectives were to compare the effectiveness of BFG and ROG containing similar amounts of BC for increasing BC, AC, and RP concentrations in healthy adult women, and to calculate the retinyl equivalency of cassava prepared as BFG or ROG. Our hypothesis was that both interventions would be effective at increasing BC and RP in TAG-rich plasma.

# 2. Methods and materials

## 2.1. Subjects

Twelve healthy, nonsmoking, nonpregnant women aged 19 to 43 years participated in the study. Women were eligible for the study if they had a body mass index of 18 to 35 kg/m<sup>2</sup>, blood pressure less than 135/90 mm Hg, and TAG, cholesterol, total protein, electrolytes, kidney and liver function tests (such as blood urea nitrogen), hemoglobin, hematocrit, red blood cells, and white blood cells within clinically normal ranges. Exclusion criteria included the use of medications that affect retinoid, carotenoid, or cholesterol absorption from food such as fat, TAG, or cholesterol-lowering medications; medicines containing high dosages of retinoids, vitamin A, or carotenoid supplements; illegal drugs; or tobacco. In addition, subjects could not be allergic to cassava or palm oil (Table 1). This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the University of California, Davis Institutional Review Board, No. 48011, renewed yearly. Written informed consent was obtained from all subjects. The clinical trial registry name was BC absorption and bioconversion to vitamin A from biofortified cassava gari, with registration ID number NCT02210507, and the registry Web site was ClinicalTrials.gov.

Four women left the study prior to completion, 1 for scheduling conflicts and 3 because they disliked either the taste or the large portion of the gari served in the study. The demographic and physiological data of the participants who dropped out of the study were not different from those for the participants who completed the study. A recruiting diagram is shown in Fig. 1.

### 2.2. Chemicals

Methanol, isopropanol, and ammonium acetate were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Table 1 – Subject demographics and blood chemistries (n = 8)		
	Means	SEM
Age (y)	27.1	2.9
Body weight (kg)	65.7	3.4
BMI (cm²/kg)	24. 9	0.78
Glucose (mmol/L)	4.61	0.12
Glucose (mg/dL)	82.8	2.1
Total cholesterol (mmol/L)	4.81	0.22
Total cholesterol (mg/dL)	186	8.6
HDL cholesterol (mmol/L)	1.51	0.15
HDL cholesterol (mg/dL)	58.3	5.8
LDL cholesterol (mmol/L)	2.97	0.18
LDL cholesterol (mg/dL)	115	6.8
TAG (mmol/L)	0.72	0.096
TAG (mg/dL)	63.4	8.5
Hemoglobin (mmol/L)	2.09	0.039
Hemoglobin (mg/dL)	13.5	0.25
Hematocrit (%)	40.3	0.76
Cholesterol/HDL	3.3	0.23
Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.		

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