### Modelling Beta-carotene Retention in three Nigerian Palm Oil Soups

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### ABSTRACT

**Background:** Pre-heating of crude palm oil during the preparation of soups is a common practice in Nigeria, used to enhance flavour and taste. However, it leads to degradation and loss of pro-vitamin A carotenoids. **Objective:** The study modelled the effect of pre-heating and cooking on beta-carotene retention in commonly consumed palm oil soups in Ibadan North LGA in Nigeria.

**Methods:** Seventy-five people participated in a quantitative dietary recall, and 60 participants were selected based on their frequent consumption of palm oil soups. Three most-frequently consumed palm oil soups – egusi (melon soup), efo-riro (vegetable soup) and obe ata (palm oil stew) were selected from 15 palm oil dishes recalled by participants. Retention of beta-carotene in the composite soups was calculated using an exponential decay model (model I) at 190°C for the pre-heating phase of palm oil and a polynomial model (model II) for the cooking phase of the soups. Beta-carotene retention in palm oil was calculated experimentally by simulating the pre-heating process and measuring beta-carotene concentrations at 5 minutes, 15 minutes and 30 minutes. Beta-carotene content of soup ingredients was calculated using nutrient values from the West African Food Composition Database, while considering the yield factors for each soup.

**Results:** Seventy-eight percent, 82%, and 91% of carotenoid loss was estimated for egusi, efo-riro, and palm oil stew respectively, corresponding to 13,822  $\mu$ g/100g, 12,297  $\mu$ g/100g and 5,880  $\mu$ g/100g of beta-carotene retained in the soups respectively.

**Conclusion:** Pre-heating of palm oil contributed mostly to carotenoid loss. Caution should be taken when preheating of palm oil to minimize nutrient loss.

**Keywords:** Pro-vitamin A carotenoids, beta-carotene retention, mathematical modelling, Palm oil, Food Composition

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### INTRODUCTION

Vitamin A retention in cooked dishes is a key determinant of vitamin A intake (1-3). Numerous factors such as cooking temperature, cooking time/duration, cooking pressure and cooking method affect the amount of vitamin A lost or retained in cooked dishes (4). The accurate estimation of vitamin A intake is highly dependent on the method adopted for calculating its retention in consumed dishes. Hence, appropriate methods for calculating nutrient retention of ingredients in dishes are needed to avoid erroneous estimations. Most food consumption surveys/studies measure vitamin A intake using nutrient values of consumed foods obtained from food composition tables (5-6). However, few food composition tables account for changes in nutrient composition caused by differences in cooking methods, which in principle can affect nutrient intake estimation (1).

In Nigeria, palm oil is widely used in food preparation and there are over fifty palm oil-based dishes. These dishes include, soups such as palm oil stew (obe ata), vegetable soup (efo-riro), melon soup (egusi), okra soup, ogbono, bitter-leaf soup (onugbu), oha soup, afang soup, achara, edikaikong, bean soup (gbegiri), miyan kuka, ilasa, native soup etc; and other dishes such as fried plantain, yam, and potato, palm oil rice, akara, moimoi, okpa, abacha, achicha, fio-fio, breadfruit (*ukwa*), *akidi*, plantain pottage, and yam pottage, (7,9). These dishes are widely consumed by Nigerians (6, 10).

In many households, in south-west Nigeria, it is common practice to pre-heat crude palm oil before cooking (usually above 150°C) to enhance the flavour and taste of dishes (10). Unfortunately, nutrient losses accompany this flavour and taste enhancement due to epoxidation and isomerization reactions that occur during palm oil pre-heating (11, 15) resulting in the loss of carotenoids (1).

In the present study we modelled the beta-carotene retention in commonly consumed palm oil soups in Ibadan-North LGA, south-west Nigeria, using experimental data from multiple cooking simulations.

### MATERIALS AND METHODS

## Participant Selection, 24-Hr Dietary Recall and Soup Preparation.

The study was registered by the University of Ibadan/University College Hospital Ethics Committee with registration number UI/EC/20/0101. Seventy-five participants were randomly selected from Agbowo and Bodija areas of Ibadan-North LGA in Oyo State, south-west Nigeria. A pre-validated 24-hour Dietary Recall questionnaire was administered to participants, to obtain information on the different palm oil dishes consumed. Participants provided information about the recipes used to cook the soups. In addition, the durations of palm oil pre-heating and total cooking time were specified. Based on the frequency of consumption of the recalled dishes, three most consumed palm oil soups – Palm Oil Stew, Vegetable Soup (efo riro), and Melon Soup (equsi soup) were selected for cooking simulation and beta-carotene retention estimation. Palm Oil and other ingredients required for the preparation of the selected soups were purchased from multiple stores in Bodija market, in Ibadan North LGA. The ingredients were sorted into three portions, based on the recipe for each soup and the weight of each ingredient was obtained before cooking. Edible portions of ingredients were weighed with a weighing scale (Kerro, India) at a precision of 0.1g and a total capacity of 3000g. Volume of palm oil preheated was measured with a conical flask (Pyrex, United States) while pre-heating and cooking temperatures of palm oil were measured using a mercury thermometer (OEM service, China). Selected soups were cooked by strictly following the recipes provided by participants.

# Cooking Simulation, Sample Preparation and Laboratory Analyses

The duration of palm oil pre-heating and the overall cooking time for the soups were extracted from all questionnaires and the average duration used by participants was applied in cooking the soups during the cooking simulation. To simulate palm oil pre-heating, oils from three different markets (Agbowo, Bodija and Telemu) in south-west Nigeria were homogenized and heated in an aluminium pot for 5 minutes, 15 minutes and 30 minutes at 190°C. Samples were later cooled to room temperature, wrapped in opaque foils and stored at -25°C before carotenoid analyses at the Department of Human Nutrition, Wageningen University, the Netherlands. All analyses were conducted in duplicates.

To simulate beta-carotene retention during cooking, the nutrient compositions of cooked ingredients were subtracted from that of raw ingredients using nutrient values from the West African Food Composition table. This was followed by the calculation of yield factors. The following formulas were applied in calculating yield factors, betacarotene content of raw ingredients, beta-carotene content of cooked soups and the beta-carotene loss during soup preparation (16-18).

- i. Yield Factor = <u>Amount of composite cooked soup</u> Amount of Raw ingredients
- ii. β-Carotene content of raw ingredients (μg/100g)
   = values of β-carotene content of raw ingredient
   (μg) obtained from the West African Food
   Composition Table.
- β-carotene content of cooked soup (µg) =total
   β-carotene content of cooked ingredients (µg) \*
   Yield Factor of each Soup.
- iv.  $\beta$ -carotene content of cooked soup ( $\mu$ g/100g) = [total  $\beta$ -carotene content of cooked ingredients ( $\mu$ g/100g)] \* retention factor; for ingredients with given retention factors in the West African Food Composition Table.
- ν. β-carotene loss during soup preparation (µg/100g) = β-carotene content of raw ingredients (µg/100g) - {[β-carotene content of cooked ingredients (µg/100g)] \* yield factor}
- vi.  $\beta$ -carotene loss during soup preparation (%) = [ $\beta$ -carotene loss during cooking  $\mu$ g/100g) /  $\beta$ -carotene content of raw ingredients ( $\mu$ g/100g)] \* 100

Beta-carotene in palm oils were analysed using the HPLC (Thermo Scientific Accella LC system; Thermo Fisher Scientific Massachusetts, USA). Two grams of oil was mixed with 0.2g magnesium carbonate, 5mL

deionized water, and 1000mL ethanol (with added retinyl acetate as internal standard) and extracted 3 times with 20mL methanoltetrahydrofuran (1:1 vol:vol%), using a rod mixer (Polytron PT 20 OD; Kriens/Luzern) until the residue was colourless. Extracts were filtered on a glass funnel with filter paper (Whatman grade 1, GE healthcare life Sciences); the combined filtrates were transferred to a 50-mL volumetric flask and made up to volume with methanoltetrahydrofuran (1:1 vol:vol%). Then, 4 ml filtrate with 1mL 10% NaCl solution was transferred to a 10-ml glass stoppered centrifuge tube (Kimax, Kimble Chase), and beta-carotene were extracted 3 times with 1.5ml petroleum-ether containing 0.01% butylated hydroxytoluene. The combined ether fractions were evaporated under nitrogen at 358°C. The residue was dissolved in 2 mL methanol/butanol (60/40 vol:vol%), and 1mL was injected into the HPLC system. Carotenoids were separated on a Vydac 201TP52 column by gradient elution and monitored at 450 nm on a photodiode array detector. Runtime was 20 min per sample. The summed content of trans and cis βcarotene, the predominant provitamin A carotenoid in palm oil was measured.

#### **Development of Mathematical Models**

An exponential decay curve of beta-carotene concentration in  $\mu$ g/100g versus time in minutes (equation 1) was used to calculate beta-carotene loss in palm oils during pre-heating, using the average pre-heating durations obtained from participants. A decay curve (Figure 1.0) was fitted using a non-linear regression model (19) in R-Studio version 4.3.2. The formula in equation 1 represents the equation of the curve which follows a first order degradation.

y=a\*e-<sup>k\*t</sup> ..... equation 1

Where

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y = \text{concentration of beta-carotene in palm oil at time t in <math>\mu g/100g
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a = initial concentration of beta-carotene at time t

k = palm oil decay rate constant

t = time in minutes

Goodness of fit test was conducted by linearizing the decay curve and calculating the regression

coefficient between the dependent and independent variables. The model equation was used to predict beta-carotene loss at different pre-heating durations.

To calculate beta-carotene retention during cooking, we fitted a polynomial model using data from Dongho et al (20) where palm oil was heated at 120°C temperature for up to 2hrs. This model was chosen because it mimicked the cooking temperature of the soups.

### RESULTS

In this study, we modelled the retention of betacarotene in three commonly consumed Nigerian soups using a non-linear regression and a polynomial model from simulated cooking experiments, following the recipes provided by participants.

Fifteen palm oil dishes were recalled by participants using the 24-hour recall questionnaire administered. The most consumed soups were palm oil stew which comprised about 40% of total dish consumed, followed by vegetable soup (efo Riro) (16.7%); melon (egusi) soup (5.0%), and okra Soup (5.0%); gbegiri soup (3.3%); oha soup (1.7%); ogbono soup (1.7%); bitter-leaf soup (1.7%).

(figure 2) Palm oil provided between 53% and 82% of beta-carotene content of the cooked soups (Tables 2-5). The average pre-heating duration and temperature for palm oil was 5 minutes and 200°C respectively. Beta-carotene concentration was highest in melon soup (Egusi) with a concentration of 13,822 $\mu$ g/100g (Table 2), followed by vegetable soup (efo-riro), with a concentration of 12,297 $\mu$ g/100g (Table 3). Palm oil stew contained the lowest amount of betacarotene with a concentration of 5,880 $\mu$ g/100g (Table 4). Similarly, beta-carotene nutrient retention was highest in melon soup (22%), followed by vegetable soup (18%), and palm oil stew (9%) (Table 5).

The kinetics of decay for trans and cis- $\beta$ -carotene followed a first order degradation, with a half-life of 2.19 minutes and rate constant 0.3164

(figure 1, Table 1). The estimates for betacarotene retention in palm oil after heating for 5 minutes at 190°C are presented in Table 1. Goodness of fit test showed an adjusted regression coefficient of 0.97 (p=0.012).



Figure 1.0: Exponential decay (A) and polynomial curves (B) used to model pre-heating and cooking of palm oil soups

Table	1:	Estimates	for	<b>Beta</b>	-carotene	Loss	during	palm e	oil	pre-heating	g
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Coefficients (β)	Estimate	Standard Error	t-value	P-value	
Intercept	11.3576	0.5832	19.474	0.00263	
Time	-0.3164	0.0344	-9.197	0.01162	

Adjusted R-squared = 0.9654; F-statistic: 84.59 on 1 and 2 DF; p=0.0116



Figure 2: Percentage consumption of soups by participants during the 24-Hr Dietary Recall

Raw ingredients	Amount of	β-carotene	β-carotene	Amount of	Yield	β-carotene content	β-carotene content	β-carotene content
	raw	content of raw	content	composite	factor	of cooked	of cooked soup	of cooked soup
	ingredients	ingredients	of raw	cooked soup	(%)	ingredients	(6 <i>r</i> /)	(hg/100g)
	(B)	(b <i>n</i> )	ingredients (µa/100a) <sup>1</sup>	(B)		(µg/100g)		
Elaies guineensis (Palm	139.30	95,671	59,954			24,874 <sup>2</sup>		
Oil)								
Cucumeropsis mannii	99.80	0	0			0		
(Egusi)								
Gardus morhua	31.70	0	0			0		
(Stockfish)								
Cambarus spp.	14.50	0	0			0		
(Crayfish)								
Capsicum spp. (Atarodo)	90.20	577	640			554		
Capsicum spp. (Bawa)	99.50	637	640			611 <sup>1</sup>		
Allium cepa (Onion)	92.30	0	0			0		
Cucurbita pepo	191.40	4,402	2,300			4,173 <sup>1</sup>		
(Pumpkin Leaves)								
Parkia biglobasa (Locust	31.40	0	0			0		
Bean)								
Maggi (knorr)	12.90	0	0			0		
Salt	5.95	0	0			0		
Gadus chalcogrammus	62.15	0	0			0		
(Panla)								
Bos taurus (Ponmo)	202.85	0	0			0		
Water	812.80	0	0			0		
Total	1,886.8	101,287	63,534	1,224.4	0.65	30,214	19,639	13,822
<sup>1</sup> Value obtained from the M	Vest African Foo	d Composition Da	itabase (16).					
<sup>2</sup> Value estimated from exp	onential decay	and polynomial m	odels					

Table 2: Estimated Beta-Carotene Content of Melon (Egusi) Soup

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Raw Ingredients	Amount of raw	β-carotene content of raw	β-carotene content of raw	Amount of composite	Yield factor	β-carotene content of	β-carotene content of	β-carotene content of	
	ingredients (g)	ingredients (µg)¹	ingredients (μg/100g)¹	cooked soup (g)	(%)	cooked ingredients (µg)	cooked soup (µg)	cooked soup (µg/100g)	
Elaies guineensis (Palm Oil)	98.65	67,753	59,954			16,938²			
Spinacia oleracea	276.20	16,682	6,040			12,843 <sup>1</sup>			
Capsicum spp.	205.10	1,313	640			1,260 <sup>1</sup>			
(Atarodo) Parkia biglobasa	53.10	0	0			0			
(Locust Bean) Gardus morhua	31.45	0	0			0			
(otockrish) Cambarus spp.	11.45	0	0			0			
(Crayrısh) Allium cepa (Onion)	95.55	0	0			0			
Maggi (Knorr)	8.50	0	0			0			
Salt	5.40	0 0	0 0			0			
Bos taurus (Ponmo) Gadus	235.90 155.20	0 0	0 0			0 0			
chalcogrammus (Panla)									
Lyopersicon	121.75	760	624			935			
esculentum									
(Iomatoes) Water	798.50	0	0			0			
Total	2,096.75	86,508	67,333	1,121.4	0.53	31,976	16,947	12,297	
<sup>1</sup> Value obtained from t <sup>1</sup> Value obtained from t <sup>2</sup> Value estimated from	he West African Fo he West African Fo exponential decay	od Composition D od Composition D v and polynomial r	atabase (16). atabase (16). models						1

Raw ingredients	Amount of raw ingredients (g)	β-carotene content of raw ingredients (μg) <sup>1</sup>	β-carotene content of raw ingredients ( <i>u</i> g/100g) <sup>1</sup>	Amount of composite cooked soup (g)	Yield factor (%)	β-carotene content of cooked ingredients	B-carotene content of cooked soup ( <i>u</i> g)	B-carotene content of cooked soup (µg/100g)
Elaies guineensis	238.80	164,008	59,954			11,481 <sup>2</sup>		
Capsicum spp.	247.45	1,584	640			1,520 <sup>1</sup>		
(Aldrodo) Allium cepa (Onion)	183.15	0	0			0		
Parkia biglobasa	27.50	0	0			0		
(Locust Bean) Lyopersicon	649.10	4,050	624			4,985 <sup>1</sup>		
esculentum								
(10matoes) Capsicum spp. (Bawa)	158.45	1,014	640			9741		
Capsicum annuum	105.00	2,415	2300			2,6571		
(Tatase)								
Gadus	139.70	0	0			0		
chalcogrammus								
(Panla)								
Bos taurus (Beef)	115.45	0	0			0		
Maggi (Knorr)	4.20	0	0			0		
Salt	18.00	0	0			0		
Water	795.00	0	0			0		
Total	2,681.80	173,071	64,158	1,685.75	0.63	21,617	13,618	5,880
<sup>1</sup> Value obtained from the	e West African Foo	d Composition Do	atabase (16).					
<sup>1</sup> Value obtained from th	e West African Foo	od Composition D	atabase (16).					

Table 4: Estimated Beta-Carotene Content of Palm Oil Stew

<sup>2</sup>Value estimated from exponential decay and polynomial model

Variable	Melon (Egusi) Soup	Vegetable Soup (Efo Riro)	Palm Oil Stew
Retention factor	0.22	0.18	0.09
Yield factor	0.65	0.53	0.63
Beta-carotene content of raw	63,534	67,333	64,158
ingredients (μg/100g)			
Beta-carotene content of cooked	13,822	12,297	5,880
soup (μg/100g)			
Beta-carotene loss during soup	49,712	55,036	58,287
preparation ( $\mu$ g/100g)			
Beta-carotene loss during soup	78.24	81.74	90.84
preparation (%)			

 Table 5: Estimated Beta-Carotene Content of Palm Oil Soups Based on Yield Factor and

 Retention Factor

### DISCUSSION

The results showed that although palm oil was a major contributor to the beta-carotene content of the soups, the pre-heating duration of the palm oil was a major contributor to nutrient loss, leading to a high loss of beta-carotene. Pre-heating of crude palm oil before cooking is a common practice and this was reported by most of the participants who often heated the oil up to 190°C for an average duration of 5 minutes. Following the rate constant estimated in this study, it would take about 3 minutes to lose half of the carotenoid content of palm oil. These findings corroborate previous findings on carotenoid loss during food handling and thermal processing (1), (3), (11-15), (17-18).

Interestingly, the estimates for beta-carotene concentrations in the soups were comparable with previous experimental study by Sanusi et al (21) who reported that melon soup (egusi), vegetable soup (efo-riro) and palm oil stew contained 13,047 $\mu$ g/100kg, 12,976  $\mu$ g/100kg and 7,872  $\mu$ g/100kg respectively. In the present study, the modelled estimates were 13,822  $\mu$ g/100kg, 12,297 $\mu$ g/100kg and 5,880 $\mu$ g/100kg (21).

The models from this study can be applied to calculate beta-carotene retention in melon soups (egusi) vegetable soups (efo-riro) and palm oil (obe ata) soups cooked with similar recipes. It offers a novel approach of accounting for beta-carotene loss during the entire cooking process, most especially during the pre-heating process of palm oil. However, there is need for more work focusing on other carotenoids in order to account for all provitamin A carotenoids. This would lead to more accurate estimations of retinol active equivalents (RAE) from Nigerian soups.

The multi-ethnic and multi-cultural nature of Nigeria

often reflects in the various cooking habits of the Nigerian people. For example, prolonged heating of palm oil is a more common practice in the southwest compared to the eastern part of the country and this contributes to the variations seen in the (21). Indeed, such variations in cooking habits will have implications for beta-carotene content of soups from these regions and in principle, can lead to wide variations in vitamin A nutrient intake. Therefore, the application of validated models can be useful for capturing individual differences in cooking habits and can lead to a more accurate assessment of vitamin A intake. Moreover, most Nigerian studies on dietary vitamin A intake assessment often assume similar cooking habits/recipes which are potential sources of bias during vitamin intake assessment.

Numerous studies on nutrient composition of Nigerian traditional soups rarely provide standardised recipes for analysed soups, thus leading to increasing difficulty in the estimation of the vitamin A content of these soups (22). Therefore, nutrient intake assessment is usually conducted using available values of food composition data from limited sources. In practice, different individuals adopt different food preparation methods which is almost impossible to capture using a static food composition table. Hence, mathematical models which individualise nutrient intake estimation by serving as a predictive tool for measuring nutrient retention from various cooking methods proves useful and sufficient for a quick and economical nutrient intake estimation. Thus, the results of this study will enable a near accurate estimation of beta-carotene intake from the three soups, once the recipe and palm oil pre-heating duration are provided by individuals during

quantitative dietary assessment.

Although this study has numerous strengths, it was limited by a reduction in the number of participants. Only 52% of study participants consumed at least one palm oil dish per day, thus reducing the population size planned for this study. Secondly, this study only catered for estimating beta-carotene retention in three soups, which is a very small fraction of palm oil soups consumed in the country. Thirdly, only four data points were used, which can affect the accuracy of the model. Fourthly, the model equation from which beta-carotene retention estimates were computed assumed a first-order reaction with no vertical nor horizontal shifts. This in principle could reduce the accuracy of the estimates, although we think this may not be too significant.

Future studies should focus on other palm oil dishes and should include more experimental data to improve the accuracy of future models.

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