

Kinetics of thermal degradation of carotenoids related to potential of mixture of wheat, cassava and sweet potato flours in baking products

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Abstract

Background: The consumption of foods such as sweet potato and cassava with high levels of carotenoids is a possible solution to reduce vitamin A deficiency. In this study, we evaluated the kinetics of thermal degradation of carotenoids. The content of carotenoids was quantified by high-performance liquid chromatography, first in fresh material, then in flour and finally in bakery products using mixtures of wheat, sweet potato and cassava. The degree of acceptance of the bakery products by children was also assessed through a sensory acceptance test.

Results: The study found that the degradation of carotenoid compounds in sweet potato followed first-order kinetics and fitted the Arrhenius equation with correlations of $R^2 > 0.9$. The retention rates of all-*trans*- β -carotene were 77%, 56% and 48% at cooking temperatures of 75, 85 and 95 °C respectively, during a cooking time of 20 min. The concentrations of all-*trans*- β -carotene, after baking, for bread, cookies and cake were 15, 19 and 14 $\mu\text{g g}^{-1}$ db, respectively. In a sensory acceptance test carried out in a school, 47.6% of the boys and 79.2% of the girls rated the cookies made from a mixture of cassava, sweet potato and wheat flour with the indicator *I like it a lot*.

Conclusion: The content of carotenoid compounds was reduced by exposure to high temperatures and long cooking times. The combinations of cooking time and temperature which minimized degradation of all-*trans*- β -carotene occurred at 75 °C–20 min and 95 °C–10 min. All-*trans*- β -carotene retentions for bread, cookies and cake were 25%, 15% and 11% respectively. The mixture of wheat, sweet potato and cassava flour can be considered in the development of cookies with positive contributions of all-*trans*- β -carotenes and with a good acceptance by children between 9 and 13 years old.

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Keywords: all-*trans*- β -carotene; sensorial analysis; vitamin A deficiency; biofortification; consumer acceptance

INTRODUCTION

Deficiencies in micronutrients such as vitamin A, iron and zinc have significant consequences for human well-being and socio-economic development of communities around the world. Malnutrition due to lack of micronutrients can lead to blindness, lethargy, decreased learning ability, less resistance to infections, mental retardation and, in some cases, death.¹ An estimated two billion people suffer from micronutrient malnutrition and the majority of those affected are in developing countries.²

Crop biofortification offers a favorable alternative for overcoming some of the difficulties and consequences associated with micronutrient deficiencies. Biofortified crops are being used as a strategy to reduce malnutrition and the deficiency of micronutrients such as vitamin A, iron and zinc. In addition, yellow cassava and sweet potato crops are a viable way to reach rural families

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who have limited access to fortified foods and nutritional supplements that are typically distributed in food aid programs.^{3–5} The intake of foods with high content of pro-vitamin A carotenoids (pVACs), particularly all-*trans*- β -carotene (TBC), can overcome or at least alleviate deficiency of vitamin A. Therefore, biofortification programs have developed and promoted the use of sweet potato and cassava clones with high pVAC contents and adequate levels of dry matter. These two crops are commonly cultivated and consumed where poverty and malnutrition are prevalent problems. Sweet potato (*Ipomoea batatas* L.) is the fifth most important food in developing countries, due to its nutritional and culinary characteristics.^{6,7} Cassava (*Manihot esculenta* Crantz) clones with high pVAC contents have been developed⁸ by cassava breeding program at CIAT (International Center for Tropical Agriculture). Sweet potato and cassava can be consumed in various forms, for instance boiled or fried, or transformed into long-shelf-life products such as semolinas (gari, attieke) and flours. In turn, these flours can be used to produce a variety of nutritionally enhanced bakery products, either as main ingredient or in partial replacement of wheat flour.^{9,10} An issue with biofortified crops however is the degradation of carotenoids during processing into final products, which reduces their nutritional value. Milder processing conditions (e.g. temperatures not higher than 110 °C, high moisture content of the product) can help mitigate this phenomenon.¹¹ Processing conditions may also be adjusted to increase the antioxidant effect of naturally present compounds (e.g. anthocyanins), in order to better protect carotenoids.¹² Therefore, the purposes of the study reported here were to: (1) evaluate the thermal degradation kinetics of carotenoid compounds; (2) analyze the functional properties of sweet potato and cassava flours and find alternatives for their use in the elaboration of bakery products combined with wheat flour; and (3) assess the retention of carotenoids in the steps from fresh material to final product.

MATERIALS AND METHODS

Materials

Cassava genotype GM3523-25 and sweet potato genotype CIP440287 were planted and harvested in CIAT, Palmira, Colombia (3°30'17" N, 76°21'24" W). For the kinetics of thermal degradation of carotenoids in fresh sweet potato roots, we generated transverse slices of genotype CIP440287 of approximately 1 cm in thickness and 4 cm in diameter. The wheat flour used in breadmaking was a commercial type. Cassava and sweet potato flours were produced by peeling, washing and chopping in a food processor (Siemens, Paei, Brazil). The pieces were dried in a convection oven at 45 °C for approximately 24 h. Then, a hammer mill and a 150 μ m mesh sieve were used to obtain fine flour for each material evaluated. The statistical program JPM Pro 14.1 (SAS, USA) was used to generate 14 formulations. For this analysis an experimental design of mixtures was carried out. In the formulations, sweet potato flour, cassava flour and wheat flour were restricted in a range of 5–45%, 5–10% and 50–90%, respectively (Table S1). Protein, ash, ethereal extract and crude fiber content were determined for these formulations. These mixtures were used to make products such as bread, cookies and cake, which were evaluated according to a global assessment with a hedonic scale of 1–10 (baking test), with parameters such as color, texture, crumb development (bread), flavor and good baking in each of the bakery products. To select the best mixture, parameters such

as high carotenoid content and higher evaluation of the baking test were considered.

Kinetics of thermal degradation of carotenoids during cooking

In a first experiment, the kinetics of thermal degradation of carotenoids (TBC, lutein, zeaxanthin, β -cryptoxanthin and total carotenoid content (TCC)) was evaluated in sweet potato slices at temperatures of 75, 85 and 95 °C during times of 0, 10 and 20 min of cooking in water. Quantification of carotenoids was performed by high-performance liquid chromatography (HPLC).⁸ Thermal degradation kinetics parameters were calculated from Eqns (1)–(6).¹³ Data obtained from thermal degradation kinetics were analyzed by regression analysis. The kinetics of carotenoid degradation in fresh cassava slices was not evaluated due to their low carotenoid content compared to sweet potato.

$$\frac{C_t}{C_0} = \exp(-k \times t) \quad (1)$$

$$k = A \exp\left(-\frac{E_a}{RT}\right) \quad (2)$$

$$Q_{10} = \left[\frac{k_2}{k_1}\right]^{10/(T_2-T_1)} \quad (3)$$

$$t_{0.5} = -\frac{\ln 2}{k} \quad (4)$$

$$Z = 10 \frac{\ln(10)}{\ln Q_{10}} \quad (5)$$

$$\%R = \frac{\text{carotenoid per gram of cooked sample}}{\text{carotenoid per gram of fresh sample}} \times 100 \quad (6)$$

where C is the concentration of carotenoids ($\mu\text{g g}^{-1}$); k is the first-order rate constant (min^{-1}); t is the baking time (s); E_a is the activation energy (kJ mol^{-1}); R is the gas constant ($\text{kJ K}^{-1} \text{mol}^{-1}$); T is the absolute temperature (K); A is the pre-exponential constant; Q_{10} is the change in the rate constant of a reaction with temperature increasing by 10 °C; $t_{0.5}$ is the time required to reduce 50% the original concentration of carotenoid (min); Z is the thermal resistance constant (°C); $\%R$ is the percentage retention of carotenoids.

Characterization of cassava, sweet potato and wheat flour

The second experiment evaluated sweet potato flour; cassava flour; a flour mixture for bread composed of 25% sweet potato flour, 5% cassava flour and 70% wheat flour; and a flour mixture for cookies and cake composed of 45% sweet potato flour, 5% cassava flour and 50% wheat flour. The bread mixture and cookies mixture were selected based on preliminary tests of 14 mixtures (experimental design of mixtures), using the criteria of baking performance (acceptability of the resulting bread or cookies) and carotenoid content (as high as possible among the recipes with good baking performance). In the proximate analysis of the flours, dry matter (AOAC 925.09 of 1995), protein (AOAC 955.04 of 1995), crude fiber (AOAC 978.10 of 1995), ash (AOAC 942.05 of 1995) and ethereal extract (AOAC 920.39 of 1995) were determined.

The apparent viscosity profiles were obtained with a Rapid Visco Analyzer model RVA-4 Series (Newport Scientific, Warriewood, Australia). In this analysis the behavior of flours was measured when subjected to heating or cooling.¹⁴ Concentration of 10% (w/v; dry basis, db) flour combined with a solution of silver nitrate (AgNO_3 , 0.002 mol L^{-1}) was used. The parameters measured were

pasting temperature, peak viscosity, trough, final viscosity, setback and breakdown.

Farinograph and alveograph analysis

The dough mixing and stretching properties of the different wheat–sweet potato–cassava flour mixtures were studied using farinograph and alveograph instruments (Brabender, Duisburg, Germany). The measurements were conducted according to AACC International Approved methods 54-21 and 54-30A (1995). From the farinograph curves, water absorption (percentage, mL (100 g flour)^{−1}), dough development time (min), stability (min) and level of decay of dough (UF) were determined. The parameters obtained from the alveograph curves were expressed as: force (J), extensibility (mm) and tenacity (mmH₂O).

Breadmaking test

In the preparation of bread, a formulation of 500 g of flour mixture composed of 25% of sweet potato flour, 5% of cassava flour and 70% of wheat flour was used. Also 30 g of butter, 25 g of sugar, 200 mL of water, 15 g of yeast (*Saccharomyces cerevisiae*), 10 g of cooking salt, 75 mL of milk and 1 egg were used. The ingredients were mixed for 10 min and kneading was performed for 20 min. The resting of the dough was carried out for 1 h at 35–38 °C. Baking was done at 180 °C for 15 min. In the recipe for cookies, a formulation of 500 g of flour mixture composed of 45% of sweet potato flour, 5% of cassava flour and 50% of wheat flour was used. Also, 150 g of butter, 150 g sugar, 5 g of yeast (*S. cerevisiae*), 5 g of baking powder, 8 g of salt, 5 mL of vanilla essence and 1 egg were used. The baking of cookies was done at 180 °C for 12 min. In the preparation of cakes, a formulation of 500 g of flour mixture composed of 45% of sweet potato flour, 5% of cassava flour and 50% of wheat flour was used. In addition, 500 g of butter, 500 g of sugar, 10 g of baking powder, 10 mL of vanilla essence, 700 mL of milk and 6 eggs were used. Baking of the cake was done at 180 °C for 45 min. A GFO oven (GFO-1X1C, Series A11010, Colombia) was used for baking all products.

Quantification of carotenoids in fresh sweet potato and cassava roots, flours and bakery products

In the quantification of the content of carotenoids, the following amounts were taken in duplicate as test samples: 5 g of fresh cassava tissue, 1 g of fresh sweet potato tissue and 1 g of each type of flour.⁸ For the quantification of carotenoid content in bakery products,¹⁵ 2 g of each bakery product was weighed and 5 mL of Milli-Q water was added. Then, 20 mL of cold acetone was added and allowed to stand for 10 min. The sample was homogenized in an Ultra-Turrax for 30 s and then filtered using a sintered glass filter with vacuum pump suction. The organic phase was extracted, and the solids were passed back to the Eppendorf tube to repeat the extraction. The extractions were performed three times. The extract obtained was taken to a separatory balloon where 20 mL of petroleum ether was added with 0.1% BHT. An amount of 150 mL of 0.1 mol L^{−1} NaCl saline solution was then added to the separating flask. The extract was poured into an Eppendorf tube and centrifuged at 805 × g for 10 min at 10 °C and the aqueous phase was extracted with a pipette. Bakery products were saponified with 10% KOH in methanol for 4 h. After saponification, the volume of the extracts was adjusted to 15 mL with petroleum ether with 0.1% BHT.

The carotenoid content of all the samples was determined by HPLC. Aliquots (15 mL) were dried with a nitrogen evaporator (Nevap 112, Organomation Associates, Berlin, MA, USA). Immediately

before injection, the dry extract was totally redissolved in 2 mL of (1:1) methanol and methyl *tert*-butyl ether (HPLC grade). After sonication (10 s) and agitation in a VWR multi-tube vortex (Avantor, New York, NY, USA) (1451 g; 60 s), the extract was filtered through a 0.22 µm polytetrafluoroethylene filter. Separation and quantification of carotenoids were achieved using a YMC Carotenoid S-5C30 reversed-phase column (4.6 × 150 mm; particle size, 5 µm), with a YMC Carotenoid S-5 guard column (4.0 × 23 mm; YMC America Inc., Allentown, PA, USA) in an Agilent HPLC system (Agilent Technologies 1200 series, Waldbronn, Germany), using a diode array detector with wavelength at 450 nm (for colored carotenoids). Peaks were identified by comparing retention time and spectral characteristics against a pure standard from CaroteNature GmbH, Lupsingen, Switzerland: β -cryptoxanthin no. 0055 HPLC 97%; lutein no. 0133 HPLC 97%; β -carotene no. 0003 HPLC 96%; violaxanthin no. 0259 HPLC 95% and zeaxanthin no. 0119 HPLC 97%. The quantity of each carotenoid was determined by integration of peak area against respective standard curves. The retinol activity equivalent (RAE) considers that 1 RAE unit corresponds to 1 µg of retinol or 12 µg of TBC or 24 µg of other pVACs.¹⁶

Sensory acceptance test

A comparative test was carried out with 45 untrained evaluators, children between 9 and 13 years old belonging to socioeconomic strata 1 and 2 (scale from 1 lowest stratum to 6 highest stratum) as determined by the municipal government of Palmira, Colombia, considering low strata that include people with fewer economic resources and access to services. The cookies product was chosen for evaluation in the sensory acceptance test because it had the highest content of TBC. The evaluators tested the control sample (a cookie made with 100% wheat flour) and the product to be evaluated (a cookie made with 45% sweet potato flour, 5% cassava flour and 50% wheat flour). A 5-point hedonic scale was used to evaluate overall acceptability: 1 = I dislike it a lot, 2 = I dislike it, 3 = Neither like nor dislike, 4 = I like it, 5 = I like it a lot.

Statistical analysis

Statistical analysis was performed with JMP® 14.1 software (SAS, NC, USA) and SAS version 9.1 software (SAS Institute Inc., Cary, NC, USA). Data were analyzed using a completely randomized design. A 5% probability was considered to indicate statistical significance for the analyses. Each trait value was analyzed using analysis of variance.

RESULTS

Thermal degradation kinetics of carotenoid compounds

Thermal degradation followed first-order kinetics for all carotenoid compounds. Higher temperature and cooking time decreased the content of TBC, lutein, zeaxanthin, β -cryptoxanthin and TCC present in sweet potato slices (Table 1). In the thermal degradation kinetics, there was a significant effect of time for the compounds TBC (0.0001), zeaxanthin (0.0002), β -cryptoxanthin (<0.0001) and TCC (0.0022). There was also an effect of temperature for TBC (0.0061), zeaxanthin (0.0004), β -cryptoxanthin (0.0067) and TCC (0.0455). For zeaxanthin there was a significant effect of time by temperature interaction (0.0134). The degradation rates of lutein, zeaxanthin, β -cryptoxanthin and TBC gradually increased with the same increasing order for all temperatures and cooking time, namely $k(\beta\text{-cryptoxanthin}) > k(\text{TBC}) > k(\text{TCC}) > k(\text{lutein})$ (Table 2). Among the carotenoids, the strongest activation energy was decreased for TBC (54.6 kJ mol^{−1}), followed by

Table 1. Effect of temperature and cooking time on carotenoid content in boiled sweet potato root slices

Time (min)	T (°C)	TBC		Lutein		Zeaxanthin		β -Cryptoxanthin		TCC	
		($\mu\text{g g}^{-1}$, fw)	%R	($\mu\text{g g}^{-1}$, fw)	%R	($\mu\text{g g}^{-1}$, fw)	%R	($\mu\text{g g}^{-1}$, fw)	%R	($\mu\text{g g}^{-1}$, fw)	%R
0		113.8	100	0.3 ^c	100	0.6 ^f	100	558	100	127.1	100
10	75	93.5	82.1	0.2 ^c	79.4 ^d	0.4 ^f	71.7	4.2 ^h	75.6	103.6	81.5
	85	75.3 ^a	66.1 ^b	0.2 ^c	76.6 ^d	0.3 ^f	55.3 ^g	3.4 ^h	60.3	83.6	65.8 ⁱ
	95	73.1 ^a	64.2 ^b	0.2 ^c	64.5 ^e	0.3 ^f	44.8	3.1 ^h	55.0	86.5	68.1 ⁱ
20	75	87.1	76.6	0.2 ^c	76.6 ^d	0.4 ^f	64.7	4.0 ^h	71.1	96.8	76.2
	85	64.3	56.5	0.2 ^c	72.6	0.3 ^f	53.8 ^g	2.7 ^h	49.2	71.5	56.3
	95	54.1	47.6	0.2 ^c	66.1 ^e	nq	nq	2.3 ^h	40.4	64.2	50.5

Note: Mean values ($n = 2$). Values with the same superscript letter in each column indicate that there are no statistically significant differences between them.

Abbreviations: fw, fresh weight; %R, percentage of carotenoid retention; nq, not quantifiable; TBC, all-*trans*- β -carotene; TCC, total carotenoid content; T, temperature.

β -cryptoxanthin (52.5 kJ mol^{-1}) and TCC (49.3 kJ mol^{-1}), while that of lutein (26.7 kJ mol^{-1}) was the lowest. The time required for the initial quantity of carotenoid to decrease by half is called the degradation half-life period ($t_{0.5}$). Higher temperatures were associated with shorter half-lives.

On the other hand, the retention percentages of TBC (the most important vitamin A precursor) after 20 min cooking time were 77%, 56% and 48% for temperatures of 75, 85 and 95 °C respectively. The degradation of TBC fitted the Arrhenius equation with correlations of $R^2 > 0.93$ ($y = -6565.8x + 14.63$). The first-order rate constant (k) increased with increasing temperature from 75 to 95 °C. Similarly, $t_{0.5}$ decreased with cooking temperature: 52, 24 and 19 min at 75, 85 and 95 °C, respectively. Another kinetic parameter obtained was the change in reaction rate constant with temperature increasing by 10 °C (Q_{10}) equal to 2.1. The thermal resistance constant (Z) was 30 °C, which indicated

the number of degrees that the temperature can be increased to reduce the thermal degradation one logarithmic cycle.

Evaluation of flours and bakery products

According to the results obtained in the characterization of the 14 types of flour mixtures, two formulations were selected, the first one for cookies and cakes (45% sweet potato, 5% cassava and 50% wheat), this formulation being selected according to the TBC content (Fig. S1), and it presented the best baking test for these products. The second formulation was for bread (25% sweet potato, 5% cassava and 50% wheat); this was selected according to the baking test (Fig. S2), because only this formulation presented crumb development and good baking. When comparing the two mixtures, it can be observed that mixture 1 (cookies and cakes) had a higher fiber content, while mixture 2 (bread) had a higher content of ethereal extract, both mixtures having similar protein and ash contents.

Table 2. Kinetic parameters of carotenoids in boiled sweet potato root slices

Temperature (°C)	Kinetic parameters	TBC	Lutein	β -Cryptoxanthin	TCC
75	k (min^{-1})	0.01 ^a	0.02 ^a	0.02 ^a	0.01 ^a
	R^2	0.93 ^b	0.94 ^b	0.88	0.92 ^b
	RMSE	0.05 ^c	0.06 ^c	0.09	0.06 ^c
	$t_{0.5}$ (min)	51.73	43.32	40.77	50.97
	D	171.83	143.91	135.45	169.31
85	k (min^{-1})	0.03 ^d	0.02 ^d	0.04 ^d	0.03 ^d
	R^2	0.94 ^e	0.79	0.94 ^e	0.94 ^e
	RMSE	0.11 ^f	0.16	0.12 ^f	0.11 ^f
	$t_{0.5}$ (min)	24.24 ^g	31.51	19.53	24.07 ^g
	D	80.51	104.66	64.86	79.95
95	k (min^{-1})	0.04 ^h	0.01 ^h	0.05 ^h	0.03 ^h
	R^2	0.98 ⁱ	0.77	0.97 ⁱ	0.99 ⁱ
	RMSE	0.08	0.10	0.12	0.04
	$t_{0.5}$ (min)	18.63	52.12	15.27	20.27
	D	61.90	173.13	50.72	67.33
	E_a	54.56	26.7	52.50	49.34
	Q_{10}	2.13 ^j	1.38	2.09 ^j	2.12 ^j
	Z	30.37	72.31	31.27	30.69

Note: Values with the same superscript letter in each row indicate that there are no statistically significant differences between them.

Abbreviations: RMSE, root mean square error; TBC, all-*trans*- β -carotene; TCC, total carotenoid content.

Table 3. Chemical characterization and functional parameters obtained by RVA from sweet potato, cassava and wheat flour, and from baking mixes of these flours

Parameter	Sweet potato	Cassava	Wheat	Bread flour mixture	Cookies and cake flour mixture
Dry matter (% wb)	84.5 ^a	84.2 ^a	87.9	91.5 ^b	92.6 ^b
Protein (g kg ⁻¹ , wb)	4.8	1.8	13.0	10.5 ^c	11.0 ^c
Crude fiber (g kg ⁻¹ , wb)	87.8	23.1 ^d	1.4	24.7 ^d	77.6
Ethereal extract (g kg ⁻¹ , wb)	80.7	21.0	2.3	62.3	54.4
Ash (g kg ⁻¹)	53.3	18.1	0.7	19.7 ^e	19.5 ^e
Peak viscosity (cP)	1581.0	2480.7	140.8	364.4	651.3
Trough (cP)	1311.3	2257.0	117.4	285.4	517.3
Final viscosity (cP)	1616.3	2702.8	158.6	505.4	826.3
Pasting temperature (°C)	74.0	68.0	83.9	79.1 ^f	78.5 ^f
Breakdown (cP)	269.7	223.7	23.3	79.0	134.0
Setback (cP)	305.0 ^g	445.8	41.2	220.0	309.0 ^g

Note: Mean values ($n = 2$). wb, wet basis. Bread flour mixture: 25% sweet potato flour, 5% cassava flour and 70% wheat flour. Cake and cookies flour mixture: 45% sweet potato flour, 5% cassava flour and 50% wheat flour. Values with the same superscript letter in each row indicate that there are no statistically significant differences between them.

The results of proximal analysis and functional parameters are reported in Table 3. The protein content was higher in wheat flour, followed by mixtures of flours, sweet potato flour and finally cassava flour with the lowest protein content. In relation to the pasting properties of sweet potato, cassava, wheat flour and their mixes, the apparent viscosities are reported in Table 3. Significant differences were observed among the flour samples in their behavior during heating and cooling in excess water (Fig. 1). Peak viscosity was highest for cassava flour (2481 cP), followed by sweet potato (1581 cP) and the lowest peak viscosity was for wheat flour (141 cP).

The pasting temperature presented values of $84 > 79 > 78 > 74 > 68$ °C for wheat, bread flour mixture, cookies and cake flour mixture, sweet potato and cassava flours, respectively. Maximum

viscosity, minimum viscosity, final viscosity and setback were significantly higher in cassava flour, while wheat flour presented the lowest values for all functional properties obtained with the RVA. The mixes of wheat flour with sweet potato and cassava resulted in higher RVA properties than the control wheat flour. Significant differences were found in the breakdown and setback values between the flours evaluated.

Results from farinography and alveography are presented in Table 4. The mixture of cookies and cake flour showed the highest water absorption capacity with the wheat flour showing the lowest. It was observed that the development time required for wheat flour dough was greater than for the compound wheat–sweet potato–cassava dough, both for bread, and cookies and cake flours. The dough formed by the mixtures of flours

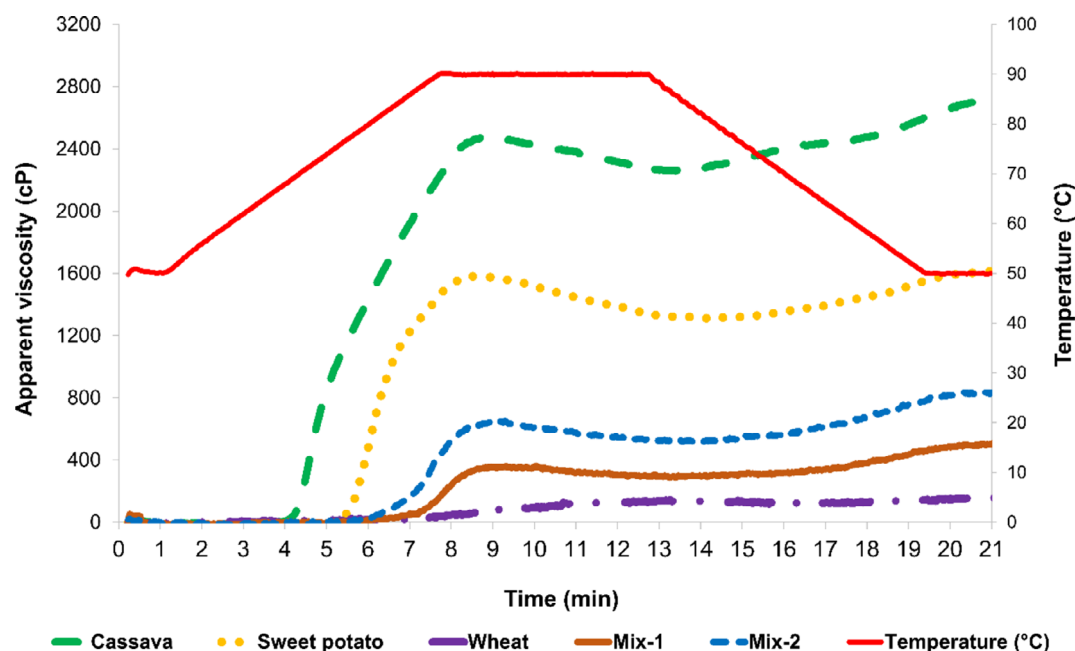
**Figure 1.** RVA profiles of sweet potato, cassava and wheat flour, and of baking mixes of these flours. Mix-1, bread flour mixture; mix-2, cake and cookies flour mixture.

Table 4. Farinograph and alveograph results for bakery products

Characteristic	Parameter	Wheat flour 100% (control)	Bread flour mixture	Cookies and cake flour mixture
Farinograph	Water absorption (mL (100 g of flour) ⁻¹)	67.1 ^a	67.6 ^a	69.2 ^a
	Development time (min)	11.2	7.6	1.9
	Stability (min)	15.2	9.3	4.4
	Level of decay (UF)	30.0	55.0	17.0
Alveograph	Force, <i>W</i> (J)	396.8	262.6	54.2
	Toughness, <i>P</i> (mmH ₂ O)	114.4	38.0	29.2
	Extensibility, <i>L</i> (mm)	95.6	46.3	37.5
	<i>P/L</i>	1.2 ^b	0.8 ^b	0.8 ^b

Note: Bread flour mixture: 25% sweet potato flour, 5% cassava flour and 70% wheat flour. Cake and cookies flour mixture: 45% sweet potato flour, 5% cassava flour and 50% wheat flour. Values with the same superscript letter in each row indicate that there are no statistically significant differences between them.

Table 5. Carotene content in fresh sweet potato, fresh cassava, flour and bakery products, RAE and retention

Processing	pVACs (µg g ⁻¹ , db)							Lutein (µg g ⁻¹ , db)	TCC (µg g ⁻¹ , db)	RAE (µg g ⁻¹ db)	Retention (%)
	Viola	Zea	β-Crypto	TBC	15-c-βC	13-c-βC	9-c-βC				
Sweet potato raw	nq	18.8	nq	375.8	13.1	nq	nq	nq	413.2	31.5	66.1
Sweet potato flour	nq	1.6	nq	248.4	6.0	nq	nq	nq	281.2	21.1	
Cassava raw	3.3	nq	nq	22.1	3.0	16.4	10.5	4.6	40.5	2.8	73.5
Cassava flour	0.2	nq	0.2	16.3	1.0	5.9	5.3	0.7	29.9	2.2 ^c	
Bread flour mixture	nq	0.3 ^a	2.9	62.6	1.4	2.4	0.3	0.5	72.5	5.6	24.8
Bread	nq	0.3 ^a	nq	15.5	1.6	4.9	0.8	nq	23.0	1.7	
Cookies and cake flour mixture	0.4	0.7	6.4	128.2	1.7 ^b	3.6	nq	0.6	144.9	11.3	15.2
Cookies	nq	0.3 ^a	nq	19.4	1.7 ^b	4.7	0.6	nq	30.0	2.2 ^c	
Cake	nq	0.3 ^a	nq	14.0	1.2	4.0	0.4	nq	20.4	1.4	11.0

Note: Mean values (*n* = 2). Bread flour mixture: 25% sweet potato flour, 5% cassava flour and 70% wheat flour. Cake and cookies flour mixture: 45% sweet potato flour, 5% cassava flour and 50% wheat flour. Values with the same superscript letter in each column indicate that there are no statistically significant differences between them.

Abbreviations: db, dry basis; nq, not quantifiable; pVACs, provitamin A carotenoids; RAE, retinol activity equivalents; TCC, total carotenoid content; viola, violaxanthin; zeax, zeaxanthin; β-crypto, β-cryptoxanthin; TBC, all-trans-β-carotene; 15-c-βC, 15-cis-β-carotene; 13-c-βC, 13-cis-β-carotene; 9-c-βC, 9-cis-β-carotene.

Table 6. Acceptability of sweet potato, cassava and wheat mixed flour cookie by children

Indicator	Boys (percentage of acceptance)		Girls (percentage of acceptance)	
	Wheat-sweet potato-cassava cookie	Wheat cookie (control)	Wheat-sweet potato-cassava cookie	Wheat cookie (control)
5. I like it a lot	47.6	90.5	79.2	83.3
4. I like it	38.1	9.5	12.5	16.7
3. Neither like nor dislike	14.3	0.0	8.3	0.0
2. I dislike it	0.0	0.0	0.0	0.0
1. I dislike it a lot	0.0	0.0	0.0	0.0

composed of wheat-sweet potato-cassava reached a consistency of 500 UF faster, because it required less mixing and kneading time. In other words, the greater the amount of sweet potato and cassava flour, the lower the stability of the dough and less tolerance to kneading. The degree of decay was greatest for the bread dough of wheat-sweet potato-cassava flours. The alveograph parameters (Table 4) showed a decrease of the resistance to deformation or toughness (*P*), baking strength or force (*W*)

and configuration ratio (*P/L*), and the extensibility (*L*) in the mixture of wheat-sweet potato-cassava flours.

The contents of different carotenoid compounds of the fresh samples, flours and products are reported in Table 5. No significant contributions of carotenoids were found from eggs and butter to bakery products. The retention of carotenoids from fresh sweet potato to sweet potato flour was 66% while the retention of fresh cassava to cassava flour was 73%. The final product retention in

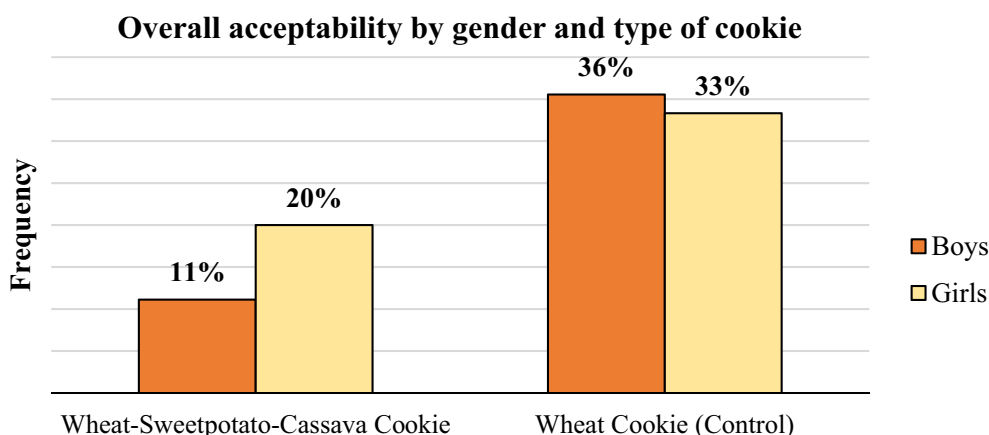


Figure 2. Overall acceptability results of sweet potato, cassava and wheat cookie and control cookie by gender of children.

bread was 25%, in cookies 15% and in cake 11%. Vitamin A expressed as RAE units was highest for fresh sweet potato followed by sweet potato flour and lowest for cake (Table 5).

Sensory acceptance test

The sensory acceptance test showed that 47.6% of boys and 79.2% of girls qualified the flour mixture cookies (cassava, sweet potato and wheat) as *I like it a lot* (Table 6). In addition, the sensory acceptance test showed that for overall acceptability, 20% of girls and 11% of boys preferred the wheat–sweet potato–cassava cookie, while 36% of boys and 33% of girls preferred the control cookie (Fig. 2).

DISCUSSION

A comparison of R^2 values for each temperature indicated that the degradations of TBC, TCC, β -cryptoxanthin and lutein were described by a first-order kinetic model, which is consistent with a previous report.¹⁷ The degradation rates of carotenoid compounds increased gradually with the same increasing order for all temperatures. All root mean square error values were very small (Table 2), suggesting that the quality of the linear model established was adequate. In addition, the R^2 values were high, which further confirmed that the model was adequate to explain the thermal degradation of carotenes. This has also been reported by Xiao *et al.*¹⁸ who found that the degradation of lutein was faster than for other compounds. This means that lutein was more sensitive to heating than carotenoids, but less than β -cryptoxanthin and zeaxanthin. The decrease in TBC concentration with increasing cooking times and boiling temperatures was similar to that reported for the Chinese sweet potato variety Yanshu No. 5.¹⁹ Similarly, the concentration of TBC decreased from $108 \mu\text{g g}^{-1}$ of dry weight to $73 \mu\text{g g}^{-1}$ of dry weight in sweet potato (variety SPK004-1) cooked at boiling temperature for 20 min.²⁰ The content of TBC in sweet potato variety CIP440287 was reduced by exposure to high temperatures and long cooking times in water. The combinations of cooking time and temperature for which the least degradation of TBC occurred were 75°C –20 min and 95°C –10 min.

The evaluation of flours and bakery products showed that fiber content increased the absorption of water in bakery products made of various proportions of flours from wheat and roots and tubers.²¹ This was reflected by the higher absorption of water in

the composite flours compared to wheat flour. Also, the use of a greater amount of sweet potato and cassava flour reduced the stability of the dough and its tolerance to kneading compared to dough made from only wheat flour. This lower stability was also observed by Bressiani and co-workers.²¹ The degree of decay was higher for the dough of wheat–sweet potato–cassava, which classifies them as weak flours, because wheat flour presents greater resistance to breaking by force, conferred by the gluten.^{22,23}

Based on results of the alveograph, the reduction in the strength of wheat–sweet potato–cassava flour mixes decreased the resistance of the dough to gas pressure, resulting in a weak and porous structure that allowed part of the gas produced during fermentation to escape. Wheat flour was classified as high strength and the wheat–sweet potato–cassava flour was classified as showing low extensibility, a feature that makes it more suitable for biscuits and cakes.^{22,24}

The pasting temperature of wheat flour was higher compared to the other flours evaluated, indicating that the starch granules of cassava flour, sweet potato flour and mixtures swelled earlier than those of wheat flour. The peak viscosity of wheat flour was low compared to those of sweet potato and cassava flour, which explains the low peak viscosity of the mixes. In this case, the protein content of wheat flour directly affected the viscosity peaks of the mixes. Olkku and Rha²⁵ reported that protein forms complexes with the starch granule surface, preventing the release of exudates and reducing peak viscosity. Therefore, it seems advantageous to combine sweet potato or cassava flour with wheat flour to optimize some functional parameters. Addition of hydrocolloids such as carrageenan²⁶ or other ingredients (e.g. legume protein flour²⁷) can be considered for further studies, in order to mitigate the less desirable viscosity behavior and gas retention of the wheat–sweet potato–cassava mixtures compared to pure wheat flour and improve the baking performance of the mixtures. In the case of cassava flour, it is possible that starch granules exhibit strong swelling power, which makes them easily reach their maximum viscosity, and they are likely to break easily due to their weak intermolecular strength, thus becoming more sensitive to shear strength as temperature increases.²⁸ During breakdown, the swollen granules further disintegrate and the amylose molecules generally leach out into solution.²⁹ Breakdown was lower in the 70% wheat flour in the bread flour mixture, compared to cookies and cake flour mixture, indicating that bread flour mixture was more resistant to heat and shear.

The bread flour mixture (25% sweet potato, 5% cassava and 70% wheat) showed the highest paste stability, as indicated by the lower breakdown, which is explained by the higher percentage of wheat. This mixture therefore may have potential as an ingredient for foods exposed to high-temperature heat treatment and mechanical agitation. Wheat flour presented the lowest values of RVA viscosity parameters. Ragae and Abdel-Aal³⁰ found similar behavior in durum wheat flour, which is related to the lower rate of water absorption and swelling of wheat starch granules, as well as lower starch content in wheat flour compared to flours with low protein content such as cassava and sweet potato. Bread flour mixture also presented lower values of setback compared to cookies and cake flour mixture, indicating a lower rate of amylose retrogradation.

The content of TBC was higher in fresh tissues, compared to flour and bakery products. The above results were expected, given that carotenoid compounds are easily degraded by heat treatment, light exposure and oxidation.^{7,31} Other studies reported that carotenoid content was reduced throughout the processing stages of making bread, cookies and pasta.³² In addition, in the kneading stages there was a limited degradation, while cooking had a stronger influence in the loss of carotenoids.³² The degradation of carotenoids is mainly related to their susceptibility to heat.³³ The stability of β -carotene during the baking of bread made from wheat and sweet potato has also been reported by Nzamwita *et al.*³⁴ Those authors concluded that the degradation of TBC occurred in the kneading process by oxidation of carotenoids, but also occurred during the cooking process due to the susceptibility of carotenoids to temperature increase.

On the other hand, the consumption of sweet potato and cassava with enhanced carotenoid content in the diet is a possible solution to reduce vitamin A deficiency. This strategy needs to take into account that exposure to high temperatures for long cooking times during preparation reduces the carotenoid content. The main form of consumption of sweet potato is fresh, boiled or fried, while cassava is more often consumed after more complex processing such as fermentation, grating and toasting, among others. In this study, the mixture of wheat flour, cassava flour and sweet potato flour in the preparation of bakery products is considered as an alternative for nutritional improvement in bakery products. Transforming sweet potato or cassava that is not accepted in the local fresh market into flour may improve the income of the producing families, extend the shelf life of the product and reduce the use of imported wheat flour.

The suggested daily intake of RAE for girls aged 9–13 years is 420 μg while boys the same ages require a daily intake of 445 μg .³⁵ A 20 g slice of bread made with 25% sweet potato flour, 5% cassava flour and 70% wheat flour would contribute 34 μg of RAE, a 100 g portion of cake made with 45% sweet potato, 5% cassava flour and 50% wheat flour would contribute 144 μg of RAE while a 20 g cookie would contribute 43 μg of RAE. Therefore, a positive nutritional contribution is evidenced in the content of vitamin A precursor for the diet of children.

In the finished product (bread, cookies and cakes) there was a decrease of carotenoids. In the case of violaxanthin and β -cryptoxanthin they were not quantifiable, because the concentrations of these pigments were very low, but detectable. While pigments 9-, 13- and 15-*cis*- β -carotene increased, this increase being explained by isomerization and hydroxylation of carotenes at high temperatures,³⁶ suggesting that pigment degradation is not limited to enzymatic and/or non-enzymatic oxidation, as already mentioned by some authors.^{37,38}

The concentration of TBC for bread, cookies and cake was 15, 19 and 14 $\mu\text{g g}^{-1}$, respectively. The mixture of 45% sweet potato flour, 5% cassava flour and 50% wheat flour to make cookies had a positive acceptance by children aged between 9 and 13 years. Therefore, this option can be considered for its potential contributions of TBC (precursor of vitamin A) for this demographic.

AUTHOR CONTRIBUTIONS

Maria A Ospina: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Jhon Larry Moreno:** Formal analysis (supporting); methodology (equal); writing – original draft (equal). **Thierry Tran:** Formal analysis (equal); investigation (equal). **Angélica M. Jaramillo:** Formal analysis (supporting). **Sonia Gallego-Castillo:** Investigation (supporting); methodology (supporting). **Bernardo Ospina:** Funding acquisition (supporting); investigation (supporting); supervision (equal). **Dominique Dufour:** Funding acquisition (supporting); investigation (supporting); project administration (lead); supervision (equal); writing – review & editing (equal).

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CONFLICT OF INTEREST

All the authors declare that they have no conflict of interest.

ETHICS STATEMENT

Research described in this paper (from laboratory through consumer preferences interviews and surveys) has been previously and formally approved by the competent authorities of the Secretary of Education of Palmira and the Pablo Sexto school in Colombia. Written informed consent was obtained from the parents for all study participants and is available.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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