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EFFECTS OF DRYING AND BOILING ON SOME SPECIFIC DIETARY CAROTENOIDS PROFILES AND LEVELS OF PLANTAIN PULP (Batard cv.) PRODUCED IN CAMEROON

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ABSTRACT

In this study, the effects of drying and boiling on carotenoid contents and profiles of plantain pulps (Batard cv.) were evaluated. Three blanching methodologies (use of citric acid, application of boiled water and fruit precooking) were performed before drying meanwhile two boiling methods were investigated: boiling plantain pulps with and without peels. Some specific carotenoid profiles and contents of the derived products namely plantain flours and boiled plantain pulps were determined by High Performance Liquid Chromatography. The chromatograms of untreated plantain pulps, plantain flours and boiled plantain pulps showed that the principal carotenoids were β-carotene, α-carotene, lutein, 9-cis carotene and 13-cis carotene isomers. These five carotenoids were clearly identified meanwhile four others were detected but not identified and coded as UIC1, UIC2, UIC3and UIC4. Drying significantly ($p<0.05$) influenced the carotenoid profile and composition of plantain flour. Depending on the carotenoid, the average losses range between 88% and 58% respectively for the use of citric acid and fruit precooking. Boiling did not influence the carotenoid profile of plantain pulps (Batard cv.), but significantly ($p<0.05$) changes their concentrations. During boiling, the trends of variation of unidentified and identified carotenoid contents change considerably depending on the boiling method. Finally, fruit precooking should be recommended to flour processors. In order to better retain identified and unidentified specific dietary carotenoids, Batard pulps should be boiled for about 40 min.

Keywords: Drying, boiling, plantain pulps, carotenoid contents and profiles

1. INTRODUCTION

In general, carotenoids are classified in two great groups: carotenes, which are strictly hydrocarbons, and xanthophylls, which derive from the former and contain oxygenated

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functions. Nutritionally, their main physiological function is the capacity to act as precursors of vitamin A; therefore, they are said to have provitaminA value. However, this important quality has led to propose their classification according to nutritional activity (depending on their provitaminA character) and their biological activity (anti-ulcer, anti-cancer, immunological regulators, antenna photosynthetic pigments, etc.) [1]-[2]. Other health-benefiting effects of the carotenoid pigments are derived from their antioxidant action, which can protect against certain cancers and tumours related with the appearance of free radicals. They are substances of great dietetic importance not only as precursors of vitamin A but also as molecules that take part in cell protection and consumer attraction through the natural relation of colour and quality and vice versa. The predominant carotenoids found in human tissues are beta-carotene, alpha-carotene, lycopene, lutein, zeaxanthin, and beta-cryptoxanthin; their relative abundance depends on dietary intake [3]. Dietary beta-carotene is obtained from a number of fruits and vegetables, among the vegetables, the most important sources of carotenoids are carrots, spinach, peaches, apricots, and sweet potatoes [4]. In consequence, carotenoid content and composition are important factors in the nutritional evaluation of fruits, vegetables and foods in general [5].

Plantains and other cooking bananas are staple food crops for approximately 70 million of central and occidental African inhabitants[6]. They are produced throughout the year in Cameroon where their production was estimated at about 4 314 910 tons in 2016 [7].They are generally eaten cooked or boiled green/unripe and eaten as a vegetable, fried when ripe or unripe to make fried-ripe-plantains or chips, baked when ripe or green, mashed, etc. [8]-[9]. Plantains are always processed before eaten. Boiling, frying and drying are the common treatments applied by consumers or processors, the first being the most important. In Cameroon, although plantain pulps are always boiled after peeling, some tribes boil the entire fruit (pulpand peel) and the peels are removed at the end of the cooking process. What could be the effect of such processing technique on carotenoid content?

Drying is the most widespread and the oldest method of preserving perishable products. Traditional drying, known as open air, remains an economically viable solution to meet the expectations of processors. Indeed, to reduce the reactions involved in food deterioration, it is important to extract a significant portion of its water. Three fundamental parameters are essential for the control of the drying process: the thermal energy brought, the capacity of surrounding air and the speed of this air at the level of the product. The strong implication of traditional dryingis based on good local control, the absence of expensive tools and equipment, and good organoleptic qualities of the obtained product. The production and utilisation of flour from unripe cooking bananas and plantains has been reported in Nigeria and Cameroon [9]-[10]-[11].

In order to contribute to the reduction of postharvest losses of bananas and plantains and to diversify uses of plantain in Cameroon, flours derived from plantain pulps are sometimes produced using artisanal methodologies. The colour of these flours is often dark or black-like because of the activity of polyphenol oxidase. Therefore, to improve the flour's colour, plantain pulps are blanched before the heat drying process. The common costless blanching techniques are based on the utilisation of boiled water or the use of a solution of citric acid. Heat drying

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seriously damages some nutrient contents of food, and blanching has significant effect on approximate composition, mineral contents and pasting properties of whole Musa flour [12].

Few studies have revealed micronutrient composition of bananas and plantain pulps. In Micronesia, banana pulps were reported to be rich in carotenoids and minerals[13]. In Cameroon, a study [14] reported carotenoids significant differences between Musa cultivars according to their genotype with plantain type and plantain-like hybrid exhibiting the highest levels of Retinol Activity Equivalent (RAE) [(148 and 70) RAE•100 g–1] compared to dessert bananas (16 RAE•100 g–1). Also, during ripening, the concentrations of β- and α-carotene,as well as lutein changed significantly according to the Mua type. If it is clear that processing techniques (boiling, cooking, drying, etc.) influence carotenoid contents of green leafy vegetables, tomatoes, carrots, broccoli, spinach, cassava roots and sweet potatoes [15]-[16]-[17]-[18]-[19]-[20]-[21], very few information exists concerning banana and plantain pulps. Therefore, the objective of this work was to provide information on the effect of boiling time on carotenoid profiles and concentrations and the influence of blanching techniques on specific dietary carotenoid levels of plantain flour obtained from Batard cultivar, a local plantain mostly consumed in west and central African countries, this in furtherance of their potential industrial utilisation.

2.MATERIALS AND METHODS

2.1.Plant material

A local plantain cultivar namely Batard was investigated at unripe stage of postharvest maturation (green fruits at harvest). It was chosen based on its availability and high consumption, its consistent agronomic performance, postharvest qualities, farmers perception and its wildly distribution in west and central Africa. Samples were collected from the world's biggest plantain collection hosted by the African Research Centre on Bananas and Plantains (CARBAP), based in Njombé – Cameroon, located on latitude 4°35' N, longitude 9°39' E and 80m altitude above sea level. The soil is volcanic and slightly cuprous, but very organic. Rainfall is bimodal with an annual total of about 2550 mm. The ambient temperature varies between 21 and 35°C and the humidity of the air ranges between 85 and 90%.

2.2.Drying plantain pulps

Preliminary studies carried out in CARBAP (Postharvest Technology Laboratory) showed that unblanched plantain pulps gave a very dark colour derived flours. During hedonic tests, 95% of the consumers spontaneously rejected these flours compared to orange-colour flours derived from blanched pulps. These unpublished results confirmed that blanching is an essential step for plantain flour production. Therefore, three (03) blanching techniques were tested.

2.2.1.Blanching techniques

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Blanching using boiled water: After peeling, pulps are sliced into cubes of about 1 cm3. Boiled water at 95°Cto 100°C is poured on these pulp cubes. After 3 min, the pulps are drained and dried directly.

Blanching using citric acid: After peeling, pulps are sliced into cubes of about 1 cm3 and soaked in a citric acid solution (0.3%) at room temperature for 10 min, then drained and dried directly.

Blanching by precooking: Fruits are immersed in boiling water at about 100°C. Depending on the thickness of the skin, the fruit can remain between 5 and 15 min (10 min in this case). After this step, the fruits are quickly peeled and the pulp sliced and dried directly.

2.2.2. Flour production

Undamaged unripe green fruits of Batardfrom the second and third hands of the bunch were chosen and washed. Two batches were then peeled and pulps cut into cubes of about 1 cm3, resulting in "cossettes" which underwent blanching using boiling water and blanching using citric acid solution respectively as described above. Blanching was carried out on another batch by dipping whole fruit in boiling water at 100°C for 10 min before peeling and slicing.

These three techniques were implemented in order to inactivate an enzyme namely polyphenol oxidase (PPO) responsible for plantain pulp blackening when exposed to oxygen. Drying was carried out for 48 hours in an electric oven (Binder brand, model 708533) whose temperature was controlled at 50°C. The dried "cossettes" were milled using an ordinary stainless Chef Warring Blender (Blender 8010E, model 38BL40). The flour obtained was sieved using a 200 µm stainless steel sieve(photo 1, 2 & 3).The flour was kept in a hermetically sealed plastic box for further analysis of dietary carotenoids. Each blanching technique was repeated trice, thus 4 samples per blanching technique were obtained and kept in a cool room (12 – 13°C). Finally, the twelve flour samples obtained from four plantain bunches, were transported by airmail to CIRAD in Montpellier - France, for further carotenoid analyses.

Photo 1. Plantain flour (pulp blanched using citric acid)

Photo 2. Plantain flour (pulp blanched using boiled water)

Photo 3. Plantain flour (pulp blanched through fruit precooking)

Photo 4. Boiled plantain

2.3. Boiling plantain pulps

2.3.1. Boiling plantain pulps without peels

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Fruits from the 2nd and 3rd hands of the bunch were selected and randomized. Twenty-one (21) of these fruits were collected, washed and peeled. The pulps were temporarily conserved in cold water to avoid blackening. Three (03) pulps were considered for the cooking time T0 = 0 min. Three whole pulps were boiled at each cooking time (T1 = 10 min, T2 = 20min, T3 = 30min, T4 = 40min, T5 = 50min and T6 = 60min) in boiling water at about 100°C in an aluminum cooking pan placed on an ordinary gas-cooker. The three cooked pulps were removed and coded. This operation was repeated twice and the nine (09) boiled pulps obtained (photo 4) for each cooking time underwent a "quartering" process (split in the longitudinal direction, then the 2 parts obtained are cut at their median, the two diametrically opposed parts are then recovered) and cut into cubes of 1 cm3.

2.3.2.Boiling plantain pulps with peels

Fruits from the 2nd and 3rd hands of the bunch were selected and randomized. Twenty-one (21) of these fruits were collected and washed. Three (03) fruits were considered for the cooking time T0 = 0 min. Three whole fruits were boiled at each cooking time (T1 = 10 min, T2 = 20 min, T3 = 30 min, T4 = 40 min, T5 = 50 min and T6 = 60 min) in boiling water at about 100°C in an aluminum cooking pan placed on an ordinary gas-cooker. The three boiled fruits were removed, peeled and the pulps coded. This operation was repeated twice and the nine (09) boiled pulps obtained for each cooking time underwent a "quartering" process (as described above) and cut into cubes of 1 cm3.

2.3.3.Boiled plantain pulp samples

About 50 g - 100 g of these cubes were either lyophilized then kept in hermetically sealed plastic boxes and placed in the cold room, or packed in polyethylene sachets and cooled in the refrigerator for 2 hours before being stored at -20°C. These frozen samples were subsequently lyophilized and also kept in hermetically sealed plastic boxes and placed in the cold room (temperature between 11°C and 13°C).

2.4.Carotenoid extraction, qualitative and quantitative analysis

Reference compounds were from Extrasynthèse (Genay, France) and reagents as well as all solvents, which were of the highest analytical grade, were from Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France).

2.4.1.Carotenoid extraction

Carotenoid extraction was adapted from the method of [22].Tert-butyl-methyl-phenol (0.1% v/v) was added to all the extraction and HPLC (High Performance Liquid Chromatography) solvents. Under red light, powdered samples [(2–5) g] were mixed for 8 min in a glass tube with 10 mL of acetone and 150 mg of magnesium carbonate to neutralize acids. The mixture was then filtered with a sintered glass funnel (porosity 3 or 4). This operation was repeated thrice until the residue was colourless. The filtrate was transferred to a separatory glass funnel containing 10 mL

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petroleum ether for partition. The mixture was washed once with 10% sodium chloride and several times (3–5) with distilled water; the aqueous layer was discarded after 3 min of partition. The petroleum layer was then concentrated in a rotary evaporator at 32 °C.

Carotenoid extraction was also performed on non-treated pulp (undried or unboiled) in order to also visualise the effect of drying and boiling. Two fruits from each bunch were peeled and their pulps were quartered and frozen. 15 g of frozen pulps were pulverizedfor 3 min in liquid nitrogen with a Dangoumeau 300-ball mill (Prolabo, France). 2-5 g powdered samples then underwent carotenoid extraction as described above.

2.4.2.Carotenoid analysis (qualitative and quantitative)

Carotenoids were analyzed according to an already published method [23].The residue was dissolved in 1 mL [dichloromethane:methyl-buthyl-ether:methanol] (50:40:10,v/v/v). Samples were filtered through a0.45-µm polyvinylidenedifluoride (PVDF)filter and injected immediately into HPLC. The carotenoid analysis was performed with an Agilent 1100 series chromatograph. The column was a polymeric YMC-30 (250 mm ×4.6 mm, 5 µm particle size, YMC Inc., Wilmington) thermo stated at 25 °C, and the mobile phase was composed of distilled water, methanol and tert-methyl-buthyletherat a flow rate of 1 mL•min-1. A gradient was applied from (40:60:0) to (4:81:15) (v/v/v) over 10 min, then from (4:81:15) to(4:11:85) (v/v/v) over 50 min until the endof the run. A UV-visible photodiode array detector was used and chromatographs were analyzed at the wavelength of maximum absorption of the carotenoids in the mobile phase (λ= 450 nm). Carotenoids were identified according to their characteristic absorption spectra, and by comparing their retention times with a reference standardalpha+beta-carotene mixture, beta-carotene and lutein (Extrasynthèse-Genay-France). Quantification of carotenoids was achieved using calibration curves withβ-carotene.

2.5.Statistical analysis

For a bunch, three blanching techniques were applied and a control sample (fresh undried pulp) was collected. This operation was repeated thrice, thus four different bunches were investigated. Sixteen flour samples were submitted to carotenoid extraction and analyses were performed in triplicate. The results were expressed in mg Eq β-carotene/100 g dry weight (DW).For each boiling method (boiling pulps without and with peels) six boiling times were considered and applied trice. Also, three control samples (fresh unboiled pulp) were collected. Twenty-one boiled samples were submitted to carotenoid extraction and analyses were performed in triplicate. The results were expressed in µg Eq β-carotene/100 g fresh weight (FW).ANOVA and mean comparisons by the SNK test ($P < 0.05$) were performed with the general linear model procedure of GraphPad Prism 5 [24]and R i386 version 3.0.1 statistical software.

3. RESULTS AND DISCUSSION

3.1.Carotenoid profile of dried plantain pulps

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The chromatograms of undried plantain pulps and plantain flours obtained after applying three blanching techniques showed that the principal carotenoids were β-carotene, α-carotene, lutein, 9-cis carotene and 13-cis carotene isomers. The first two micronutrients being the major plantain pulp and flour carotenoids. These five carotenoids were clearly identified meanwhile four others were detected but not identified. They were further coded as UIC1, UIC2, UIC3 and UIC4 standing for unidentified carotenoid 1, 2, 3 and 4 as shown on figures 1 to 4.

In contrast to undried fresh pulps, no matter the blanching technique used, all dried pulps showed a number of peaks that would probably correspond to a multitude of unidentified isomers before the appearance of the lutein peak. These carotenoids were detected between 12 and 21 min after sample injection in the column. This observation enabledto conclude that drying causes a modification of carotenoid profile of plantain pulp (Batardcv.). Theincrease in the number of carotenoids could be attributed to the effect of heat characterised by isomerization of all-trans carotenoids. During thermal treatment, the total percentage of cis-isomers of provitaminA carotenoids is increased by 10 to 39% with canning [25].Also, during heat treatment of coloured fruits and vegetables, the predominant cis-isomer is the 13-cis- whereas for leafy vegetables, the 9-cis- and 13-cis-isomerspredominate, followed by an unidentified -cis-isomer and finally by the 15-cis-isomer [26]-[27].

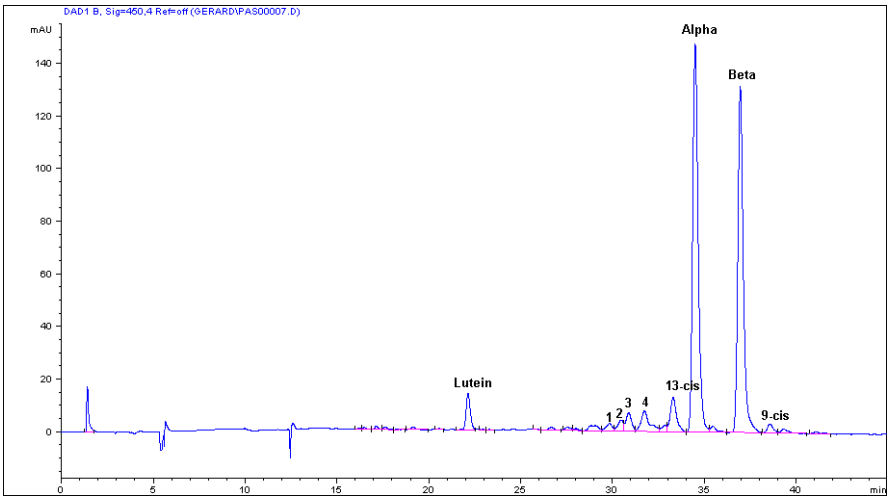

Figure 1. Carotenoid profile of untreated (undried and unboiled) plantain pulps (*Batard* cv.)

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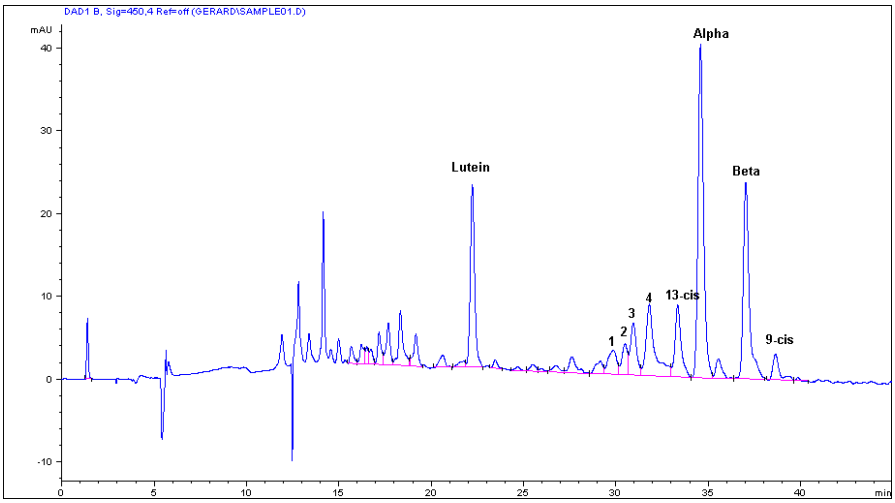

Figure 2. Carotenoid profile of dried plantain pulps blanched with boiling water (*Batard* cv.)

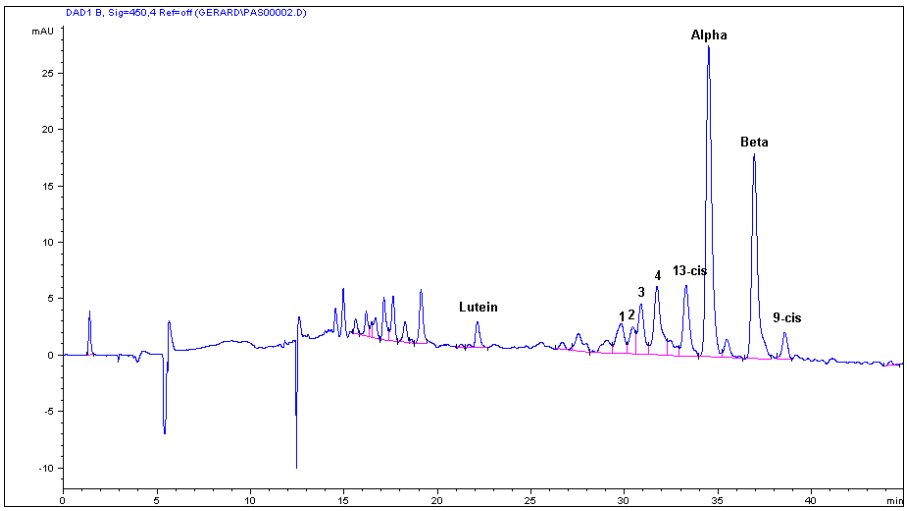

Figure 3. Carotenoid profile of dried plantain pulps blanched with citric acid (*Batard* cv.)

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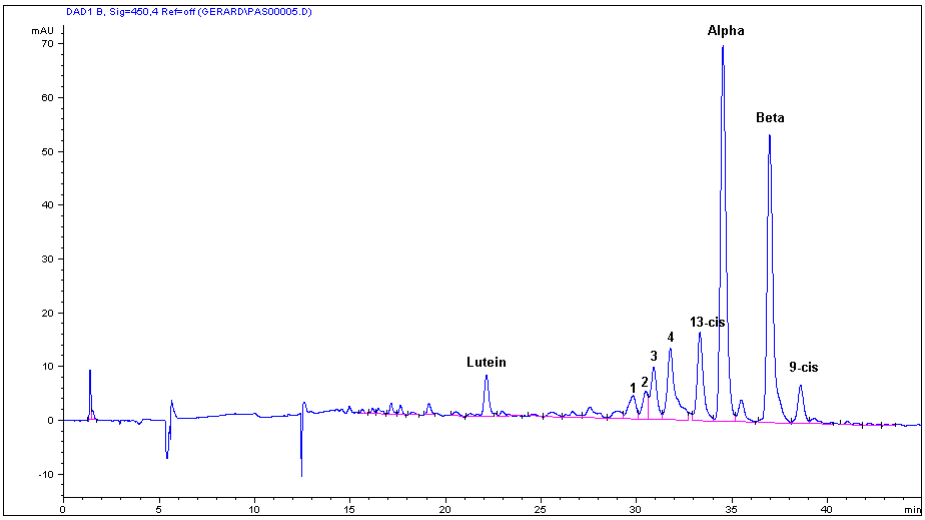

Figure 4. Carotenoid profile of dried plantain pulps blanched through fruit precooking (*Batard* cv.)

3.2.Carotenoid contents of dried plantain pulps

The average lutein content of Batardcv. pulp is 0.30 mg Eq. β-carotene Eq / 100 gdw. During drying, this value significantly decreases depending on the blanching techniques ($P < 0.0001$). Drying process causes 91%, 90% and 79% loss of initial lutein concentration respectively for boiling water treatment, citric acid utilisationandfruit pre-cooking respectively (Figure 5).

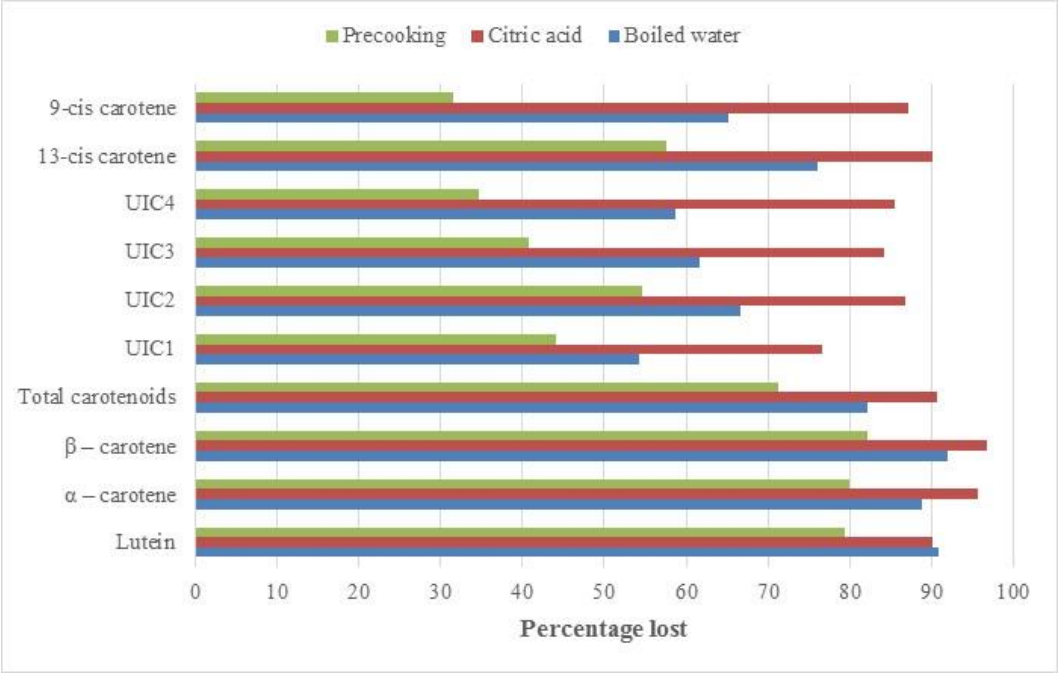

Figure 5. Losses encountered by carotenoid during plantain flour processing according to blanching technique

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The α-carotene levels of unripe plantain (Batard cv.) pulps were higher than those of lutein and β-carotene (Table 1). Fresh plantain pulp exhibits more than 4mg Eq. β-carotene/100 g dw, about 3.7 mg Eq. β-carotene/100 g dwand 10 mg Eq. β-carotene/100 g dw respectively for α-carotene, β-carotene and total carotenoids. Compared to Malaysian sweet potato flesh analysed by [28], these carotenoid contents are very high. During the drying process, they significantlydecrease according to blanching technique: precooking exhibited the lowest loss percentage meanwhile the utilisation of citric acid for blanching generally enabled highest losses of specific dietary carotenoids (Figure 5).Almost similar to lutein, 13-cis isomer and 9-cis isomer and as well as the unidentified carotenoids (UIC1, UIC2, UIC3and UIC4) presented low concentrations in undried plantainpulp (< 0.40 mg Eq. β-carotene Eq / 100 g dw).

Precooking enabled 9-cis and 13-cis-isomers as well asthe four unidentified carotenoids to lose less than 60% of their initial levels after the drying process, 9-cis isomer exhibited the lowest lost (32%). The utilisation of citric acid for blanching induces highest damages on plantain flour carotenoid compared to blanching using boiled water and fruit precooking. Based on the ten specific carotenoids analysed, the average losses range as follow: 88% (min = 77%- maxi = 97%), 74%(min = 54% - maxi = 92%) and 58% (min = 35% - maxi = 82%) respectively for the use of citric acid, the application of boiled water and fruit precooking as blanching techniques. Heat drying seriously damaged specific dietary carotenoids of plantain. The three blanching techniques applied in this study during flour processing significantly reduced carotenoid contents of the derived plantain product. Similar results were observed on proximate composition and some specific minerals of whole flour made from black Sigatoka resistant Musa hybrids pulp and peel mixture[12].

Table 1. Carotenoid contents of plantain flours obtained after drying (Batardcv.)

Treatment / Carotenoid	Undried pulp[1]	Boiled water[2]	Citric acid[3]	Precooking[4]
Lutein*	0.30^{a} ± 0.02	0.03^{b} ± 0.01	0.03^{b} ± 0.00	0.06^{c} ± 0.01
α – carotene*	4.08^{a} ± 0.35	0.45^{c} ± 0.01	0.17^{d} ± 0.01	0.81^{b} ± 0.16
β – carotene*	3.67^{a} ± 0.32	0.29^{c} ± 0.01	0.12^{d} ± 0.02	0.66^{b} ± 0.11
Total carotenoids*	10.03^{a} ± 0.83	1.78^{c} ± 0.23	0.93^{d} ± 0.08	2.89^{b} ± 0.39
13-cis carotene*	0.38^{a} ± 0.05	0.09^{c} ± 0.00	0.04^{d} ± 0.01	0.16^{b} ± 0.01
9-cis carotene*	0.10^{a} ± 0.01	0.03^{c} ± 0.00	0.01^{d} ± 0.00	0.07^{b} ± 0.01
UIC*$_1$	0.09^{a} ± 0.00	0.04^{c} ± 0.00	0.02^{d} ± 0.00	0.05^{b} ± 0.01
UIC*$_2$	0.11^{a} ± 0.01	0.04^{c} ± 0.00	0.01^{d} ± 0.00	0.05^{b} ± 0.00
UIC*$_3$	0.16^{a} ± 0.01	0.06^{c} ± 0.01	0.03^{d} ± 0.00	0.10^{b} ± 0.01
UIC*$_4$	0.29^{a} ± 0.03	0.12^{c} ± 0.02	0.04^{d} ± 0.01	0.19^{b} ± 0.01

*: results are expressed in mg Eq. β-carotene/100 g dry weight; 1: untreated fresh pulp or undried pulp; 2: dried pulp after blanching with boiled water; 3: dried pulp after blanching with citric acid; 4: dried pulp after fruit precooking; UIC: unidentified carotenoid.

Values in the same line with different superscript letters are significantly different at $p<0.05$.

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3.3.Carotenoid profile of boiled plantain pulps

In this section, the pulps obtained after cooking the whole fruit were called "pulp boiled with peels" or "pbwp" while the pulps obtained by direct cooking in boiling water were coded as "pulp boiled without peels" or "pbwop". According to [29], boiling is a cooking operation that involves heating a food at a level for a certain time and in a well-defined environment which is bubbling water. This action generally modifies the food and makes it suitable for specific purposes.

Figure 6 shows the carotenoid profile of uncooked plantain pulp. The detection of lutein occurred at the 21stminute of the sample flow, followed by the appearance of four (04) other unidentified carotenoids and labelled within the framework of this study asUIC1, UIC2, UIC3andUIC4. Five clearly identified carotenoids namely: 13-cis carotene, α-carotene, β-carotene and 9-cis carotene later appeared respectively at the 32nd, 34th, 36thand 37thminute of sample flow. The same carotenoid profile was observed on ten plantain cultivars analysed for their carotenoid contents during ripening[14].

The peak areas (and therefore the concentrations) of the different carotenoids do not disappear, but vary significantly according to boiling time, whether the plantain pulps are boiled with peels or not. Hence, the carotenoid profile of plantain pulps(Batard cv.) does not vary according to the boiling process and the boiling time. This is confirmed by the strong similarity of carotenoid profiles of pulp cooked for 40, 50 and 60 min (Figures 6 & 7). This result is contrary to those obtained during investigations on coloured fruits and vegetables which indicate an increase in the number of carotenoids, attributed to the effect of heat (all-trans isomerization in cis)[25].

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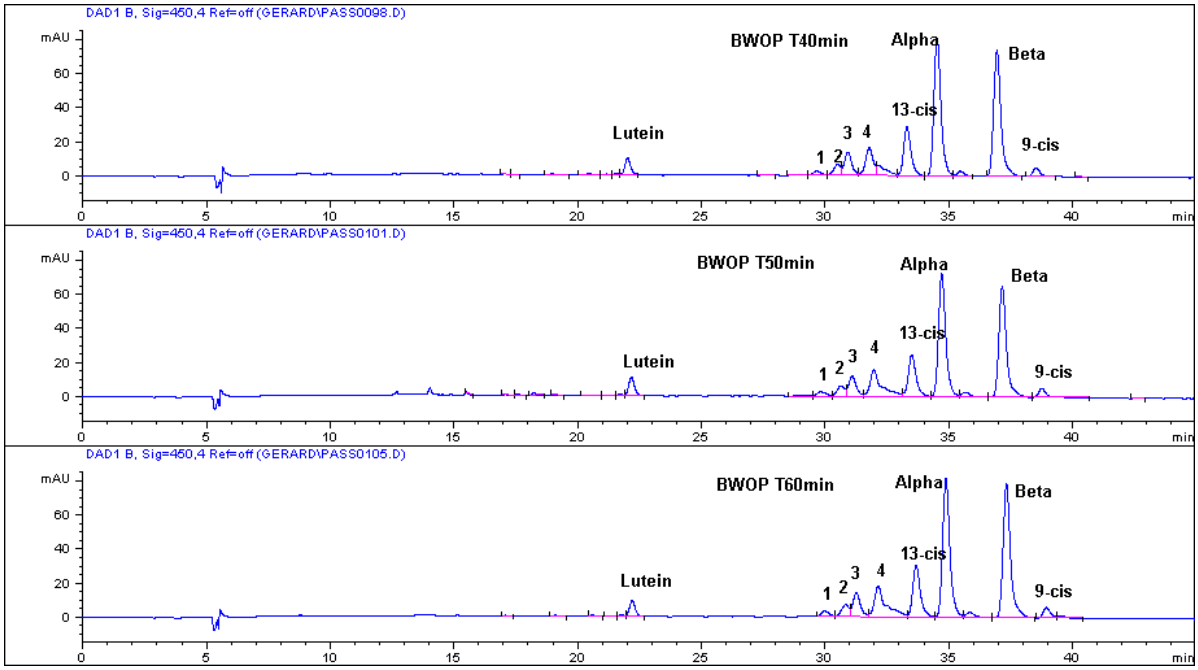

Figure 6. Carotenoid profiles of plantain pulps boiled without peels within 40, 50 and 60 min

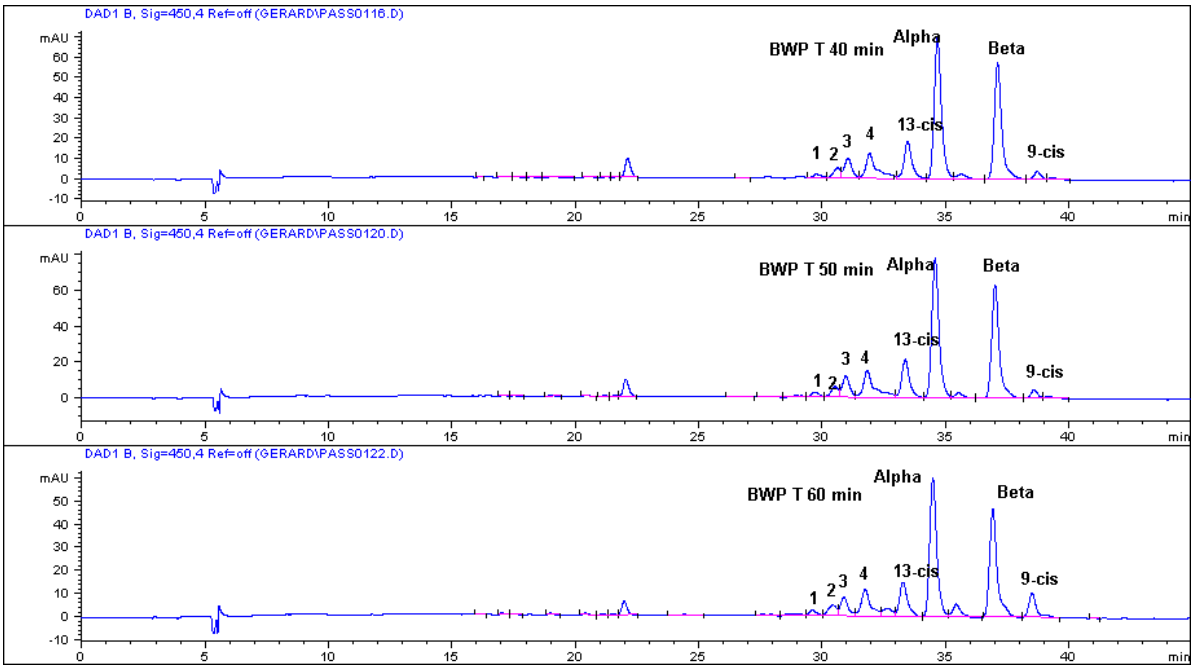

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Figure 7. Carotenoid profiles of plantain pulps boiled with peels within 40, 50 and 60 min

3.4.Carotenoid contents of boiled plantain pulps

Whether the pulps were boiled with or without peels, the four unidentified carotenoids showed significant concentrations during cooking. During this process, their contents increased sometime thrice compared to the value observedwith untreated fresh pulp. Boiling, therefore, enabled a significant increase of unidentified carotenoid levels. At 40 min, 50 min and 60 min of boiling, the variation of unidentified carotenoid contents was very low. Unripe plantain pulps are usually ready for consumption in Cameroon within these three boiling times. The cooking method (with or without peel) significantly influences the unidentified carotenoid contents (Table 2). In order to preserve maximum unidentified carotenoids or to enable their availability, it would be advisable for plantain consumers to boil them for 40 min and 60 min respectively with peels and without peels.

Table 2. Changes in unidentified carotenoid levels during plantain pulp boiling without and with peels

Cooking mode	Boiling time (min)	UIC$_1$*	UIC$_2$*	UIC$_3$*	UIC$_4$*
Pulps boiled without peels	0	13.78^{bc} ± 0.67	16.53^{d} ± 1.82	24.61^{e} ± 0.94	44.04^{d} ± 4.23
	10	10.69^{cd} ± 0.56	17.51^{d} ± 0.64	39.74^{d} ± 3.76	61.95^{cd} ± 17.15
	20	9.04^{d} ± 0.71	19.71^{d} ± 1.43	50.85^{c} ± 6.00	55.54^{cd} ± 4.76
	30	11.14^{cd} ± 2.89	26.24^{c} ± 2.27	62.06^{b} ± 4.56	78.95^{c} ± 5.95
	40	15.70^{ab} ± 4.50	33.52^{ab} ± 6.08	75.28^{a} ± 11.40	117.60^{b} ± 31.00
	50	16.06^{ab} ± 3.60	31.28^{bc} ± 3.04	61.60^{b} ± 4.19	123.74^{ab} ± 6.63
	60	19.69^{a} ± 1.30	37.22^{a} ± 1.50	72.72^{a} ± 4.92	145.41^{a} ± 10.36
Pulps boiled with peels	0	13.78^{ab} ± 0.67	16.53^{c} ± 1.82	24.61^{e} ± 0.94	44.04^{c} ± 4.23
	10	6.61^{c} ± 5.76	10.08^{d} ± 2.33	15.93^{f} ± 3.24	28.91^{c} ± 4.79
	20	9.83^{bc} ± 1.18	19.99^{b} ± 0.36	39.74^{d} ± 1.19	61.72^{b} ± 13.37
	30	7.12^{c} ± 1.28	19.78^{b} ± 1.43	43.32^{cd} ± 4.22	64.68^{b} ± 17.54
	40	14.35^{a} ± 0.15	27.16^{a} ± 1.17	55.92^{a} ± 2.94	100.80^{a} ± 6.28
	50	14.61^{a} ± 0.23	26.78^{a} ± 0.23	51.25^{b} ± 1.11	96.31^{a} ± 3.75
	60	13.18^{ab} ± 1.54	26.40^{a} ± 1.51	46.62^{c} ± 2.30	85.70^{a} ± 10.67

UIC*: concentration of unidentified carotenoid expressed in µg Eq. β-carotene/100 g fresh weight

Values in the same column with different superscript letters are significantly different at $p<0.05$.

The lutein content of unboiled plantain pulp was 37 µgEq β-carotene / 100 g FW. During the boiling process, it significantly increasesand decreases according to the boiling method. When

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plantain pulp is boiled without peel, a significant increase of lutein content is observed after 10 min. This same observation was made for pulp boiled with peel between 20 min and 30 min (Table 3). This difference in time may be due to the fact that, heat must pass through peel before reaching the pulp in the latter case.The increase of lutein concentration is due to the weakening of the cell walls which causes the release of carotenoids including lutein. Whether boiled with peels or not, the 13-cis and 9-cis isomers levels increase significantly during the boiling process, meanwhiletotal carotenoids, α-carotene and β-carotene concentrations decrease (Table 3). For all the boiling times, 13-cis-β-carotene isomer exhibited approximately 4 times 9-cis isomer contents. Boiling causes a very significant increase of 9-cis and 13-cis isomers levels. This could be attributed to the destruction of cell walls by heat with the release of these isomers as a direct consequence. In addition, this increase could be due to the isomerization of carotenoids under the effect of heat (all-trans isomerization to cis). Furthermore, a study showed a 10-39% increase of total cis-isomers under boiling conditions of canning[25]. In order to make available maximum 9-cis and 13-cis isomers, it would be advisable to boil plantain pulps withor without peels for 40 min (Table 3).

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Table 3. Changes in identified carotenoid levels during plantain pulp boiling without and with peels

Cooking mode	Boiling time (min)	Lutein*	13-cis β-carotene*	α-carotene*	β-carotene*	9-cis β-carotene*	Total carotenoids *
Pulps boiled without peels	0	$37.27^{a} \pm 2.41$	$57.35^{e} \pm 7.52$	$511.67^{a} \pm 44.51$	$460.06^{b} \pm 39.72$	$14.76^{d} \pm 1.57$	$1257.98^{abc} \pm 104.45$
	10	$59.17^{b} \pm 15.92$	$84.78^{d} \pm 7.16$	$512.10^{a} \pm 41.08$	$518.88^{a} \pm 40.33$	$27.86^{bc} \pm 4.46$	$1411.33^{a} \pm 143.71$
	20	$35.21^{a} \pm 4.95$	$109.93^{c} \pm 10.90$	$362.45^{cd} \pm 37.61$	$366.08^{cd} \pm 38.27$	$23.02^{c} \pm 6.03$	$1093.90^{ac} \pm 109.27$
	30	$42.03^{a} \pm 0.93$	$131.78^{b} \pm 6.38$	$416.23^{bc} \pm 10.28$	$408.75^{bc} \pm 13.62$	$33.50^{ab} \pm 1.80$	$1269.82^{abc} \pm 37.59$
	40	$43.38^{a} \pm 2.86$	$155.52^{a} \pm 18.92$	$428.32^{b} \pm 32.34$	$407.71^{bc} \pm 28.31$	34.83a ± 4.44	$1368.87^{a} \pm 154.29$
	50	$35.02^{a} \pm 4.20$	$131.29^{b} \pm 4.68$	$345.70^{d} \pm 19.19$	$332.79^{d} \pm 16.88$	$27.98^{bc} \pm 2.59$	$1155.64^{bc} \pm 59.10$
	60	$35.63^{a} \pm 1.33$	$155.79^{a} \pm 8.67$	$391.10^{bcd} \pm 20.79$	$386.08^{c} \pm 20.71$	$31.19^{ab} \pm 1.77$	$1319.71^{ab} \pm 77.88$
Pulps boiled with peels	0	$37.27^{b} \pm 2.41$	$57.35^{d} \pm 7.52$	$511.67^{a} \pm 44.51$	$460.06^{a} \pm 39.72$	$14.76^{bc} \pm 1.57$	$1257.98^{a} \pm 104.45$
	10	$29.32^{c} \pm 1.15$	$41.02^{e} \pm 1.19$	$383.97^{c} \pm 10.68$	$330.22^{c} \pm 8.92$	$11.60^{c} \pm 0.62$	$893.62^{d} \pm 30.38$
	20	$47.57^{a} \pm 0.74$	$86.22^{bc} \pm 3.11$	$438.10^{b} \pm 0.85$	$409.33^{b} \pm 1.69$	$22.16^{b} \pm 0.70$	$1189.15^{a} \pm 6.54$
	30	$46.50^{a} \pm 2.05$	$85.78^{c} \pm 8.85$	$376.84^{c} \pm 6.49$	$327.52^{c} \pm 4.60$	$19.41^{bc} \pm 1.67$	$1031.43^{bc} \pm 32.36$
	40	$37.29^{b} \pm 1.46$	$103.74^{a} \pm 5.27$	$360.94^{c} \pm 16.15$	$308.42^{c} \pm 13.77$	$21.85^{b} \pm 0.85$	$1082.22^{b} \pm 49.08$
	50	$30.75^{c} \pm 1.45$	$95.96^{ab} \pm 2.08$	$317.17^{d} \pm 9.44$	$271.30^{d} \pm 7.25$	$20.36^{bc} \pm 0.12$	$972.94^{cd} \pm 25.21$
	60	$21.73^{d} \pm 2.82$	$91.58^{bc} \pm 7.15$	$297.02^{d} \pm 3.71$	$259.73^{d} \pm 8.53$	$41.33^{a} \pm 14.93$	$945.13^{d} \pm 17.54$

*: concentration carotenoid expressed in µg Eq. β-carotene/100 g fresh weight

Values in the same column with different superscript letters are significantly different at $p<0.05$.

Unboiled plantain pulps exhibited significantly different levels of α-carotene and β-carotene (511 and 460 µgEq β-carotene / 100 g FW respectively). Whether boiled with peels or not, the concentrations of α-carotene and β-carotene significantly decrease till 40 min from where they stabilise between 300 and 400 µgEq β-carotene / 100 g FW.Unlike 9-cis and 13-cis carotene isomers whose contents increase significantly during boiling, the levels of α-carotene and β-carotene significantly decrease during this process. This may be due to theirisomerisation into cis isomers (probably 9-cis carotene and 13 cis carotene) under heat conditions. If it's true that cooking facilitates carotenoid extraction, it is not always true that it causes the increase of carotenoids reported by some authors. According to [26], this increase could be due to the loss of carotenoids through enzymatic activity,to the facility of carotenoids extraction from the cooked or treated food matrix, and/or to an unexplained water loss and to the leakage of soluble solids. However, exposure ofthe food matrix to drastic heat may lead to carotenoids deterioration. This seems to be the case of plantain pulp (Batard cv.) where serious damages were observed 40 min boiling with or without peel.

During cooking, the trends of variation of unidentified and identified carotenoid contents change considerably depending on the boiling method. The resulting equations attempt to predict the variation model of each specific carotenoid as a function of the boiling method (Tables 4 & 5). Generally, unidentified carotenoids and cis-isomers levels increase during boiling meanwhile α-carotene and β-carotene tend to decrease. It should be noted that the point cloud structure does not assume a linear or polynomial relationship between carotenoid concentration and boiling time. This is why an empirical and non-parametric approach is used, namely moving averages. The order of the moving average is chosen according to the approximation of the curve with the whole cloud of points.R is the degree of adjustment. The more R^2 is high and closer to 1, the more the relationship is well adjusted or more robust, thus justifying a better relation between the crossed parameters.

Table 6 presents the comparison data of the two boiling methods investigated within the framework of this study: boiling plantain pulps without peels and boiling plantain pulps with peels. Whether boiled with peel or not, no significant difference was observed regarding lutein and UIC1 levels. Furthermore, for each boiling time, the presence or not of peels influences significantly the concentrations of α-carotene, 9-cis carotene isomer and two unidentified carotenoids namely UIC2 and UIC4 ($P<0.05$); the levels of UIC3, 13-cis carotene, β-carotene and total carotenoids ($P<0.001$). Finally, plantain pulps boiled without peels better retained identified and unidentified carotenoids analysed.

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Table 5. Data related to variation model of carotenoid during plantain boiling (pulp boiled with peels)

N°	Component	Regression model	Equation	Degree of adjustment	Trend
1	UIC_1	Polynomial order 5	$y = -4\times10^{-10}x^5 + 6\times10^{-8}x^4 - 4\times10^{-6}x^3 + 0.0001x^2 - 0.0014x + 0.0137$	$R^2 = 0.5734$	± Stable
2	UIC_2	Linear	$y = 0.0003x + 0.0134$	$R^2 = 0.6978$	Increase
3	UIC_3	Linear	$y = 0.0005x + 0.0233$	$R^2 = 0.6516$	Increase
4	UIC_4	Linear	$y = 0.0011x + 0.0369$	$R^2 = 0.6645$	Increase
5	Lutein	Polynomial order 3	$y = -2\times10^{-7}x^3 - 6\times10^{-7}x^2 + 0.0005x + 0.034$	$R^2 = 0.6758$	Decrease
6	13-cis isomer	Linear	$y = 0.0008x + 0.0556$	$R^2 = 0.589$	Increase
7	9-cis isomer	Polynomial order 4	$y = 4\times10^{-8}x^4 - 4\times10^{-6}x^3 + 0.0001x^2 - 0.0012x + 0.0145$	$R^2 = 0.7494$	Increase
8	α-carotene	Linear	$y = -0.0031x + 0.4753$	$R^2 = 0.7786$	Decrease
9	β-carotene	Linear	$y = -0.0029x + 0.4259$	$R^2 = 0.7286$	Decrease
10	Total carotenoids	Polynomial order 5	$y = -5\times10^{-8}x^5 + 8\times10^{-6}x^4 - 0.0005x^3 + 0.0121x^2 - 0.1137x + 1.2532$	$R^2 = 0.7225$	Decrease

Table 6. Comparison of 2 cooking modes boiling plantain pulps with peel and boiling plantain pulps without peels (Batardcv.)

Nutritional Parameter	P value	(pbwop # pbwp)
Lutein	0.0799	ns
UIC_1	0.0637	ns
UIC_2	0.0177	*
UIC_3	0.0003	***
UIC_4	0.0311	*

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13-cis Isomer	<0.0001	***
α-carotene	0.0146	*
β-carotene	<0.0001	***
9-cis Isomer	0.0181	*
Total carotenoids	< 0.0001	***

ns : not significant ; * : significant at 5% threshold (P < 0.05) ;

** : significant at 1% threshold (P < 0.01) ; ** : significant at 10/00 threshold (P < 0.001) pbwop : pulp boiled without peel ; pbwp : pulp boiled with peel

4. CONCLUSION

β-carotene, α-carotene, lutein, 9-cis carotene and 13-cis carotene isomers, as well as four unidentified carotenoids, are plantain pulp principal carotenoids, the first two micronutrients being the major of plantain derived products (boiled pulp and flours). This study clearly demonstrates that heat drying seriously damages specific dietary carotenoids of plantain flour. The application of three blanching techniques before drying exhibited a negative effect on specific carotenoid contents, the degree of influence was different. Depending on the carotenoid, the average losses range as follows: 88%, 74% and 58% respectively for the use of citric acid, the application of boiled water and fruit precooking. Thus, fruit precooking should be recommended as the best pulp blanching technique (among the three investigated in this study) before drying in order to conserve maximum plantain flour carotenoids such as lutein, α-carotene, β-carotene, 13-cis isomer and 9-cis isomer as well as unidentified carotenoids coded as UIC1, UIC2, UIC3 and UIC4 in this study. Furthermore, fruit precooking enabled to avoid peeling difficulties, helped to reduce drying time and energy consumption because pulps were not in contact with water, thus increasing benefits of the processors.

Whether boiled with or without peels, plantain pulps presented the above nine specific dietary carotenoids detected within the framework of this study. During boiling, their profiles do not change meanwhile their concentrations varied significantly according to boiling methods and times. Generally, unidentified carotenoids (UIC1, UIC2, UIC3and UIC4) and cis-isomers levels increase meanwhile lutein, α-carotene and β-carotene tend to decrease during the boiling process. The trends of variation of unidentified and identified carotenoid contents differ depending on the boiling method. The variation model of each specific carotenoid changes as a function of the boiling method. Finally, boiling methods significantly influence the concentrations of α-carotene, β-carotene, 9-cis carotene and 13-cis carotene isomers, total carotenoids and three unidentified carotenoids namely UIC2, UIC3and UIC4. Because plantain pulps boiled without peels better retained identified and unidentified specific dietary carotenoids, 40 min is recommended for boilingBatard pulps.

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