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COMPOSITION OF ORGANIC ACIDS AND ANTI-NUTRITIONAL FACTORS OF PAPAYA (*CARICA PAPAYA* L. *VAR SOLO 8)* AT DIFFERENT STAGES OF MATURITY

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ABSTRACT

Papaya (*Carica papaya* L. *var solo 8*) is a fruit produced and widely consumed in Côte d'Ivoire is exported. To make better use of it, this study was conducted to determine its composition in organic acids, oxalates and phytates during four stages of maturity. Thus, these parameters were determined in three parts (skin, pulp and seeds) of the papaya. The contents of organic acids, oxalates and phytates in each studied part decrease from the immature state to the advanced state and present significant differences ($p < 0.05$). The most dominant organic acids in papaya are salicylic, tartaric and adipic acids. Salycilic acid is very present in the pulp with contents varying from 54.71±0.02 (A) to 14.07±0.09 g/Kg (D). Tartaric acid is predominant in the skin of papaya with contents varying from 39.70±0.03 (A) to 12.63±0.13 g/Kg (D). Adipic acid has high contents in the pulp ranging from 20.44±0.01 (A) to 0.54±0.07 g/Kg (D). Fumaric acid is non-existent in the three parts of the papaya during the four stages of maturity and oxalic acid in the skin and pulp. The oxalate and phytate contents are higher in the seeds and vary respectively from 15.46±0.47(A) to 5.84±0.06g/Kg (D) and from 10.54±0.05 (A) to 5.35±0.01g/Kg (D).

***KEYWORDS*:** Acids organics, Anti-nutritional factors, Papaya, Stages of maturity.

INTRODUCTION

Papaya (*Carica papaya* L), is one of the major fruit crops cultivated in tropical and sub-tropical zones. The papaya has a complicated reproductive system.[1] Plants are either male, hermaphrodite, or female. Flowers grow either singly (female and hermaphrodite plants) or in large clusters (male) in leaf axils.[1] Plants can produce flower and fruits continuously after flowering initiation and leaves generally senesce and abscise before fruits are harvested.[2] The papaya plant (*Carica papaya* L.) has been described with a large variety of adjectives, which acknowledge the structural and functional complexity and the high phenotypic plasticity of this giant tropical herb.[3] *C. papaya* , with a somatic chromosome number of 18, is the sole species of this genus of the Caricaceae, a family well represented in the Neotropics, that includes six genera with at least 35 species,[4,5] Most likely, papaya originated along the Caribbean coast of Mesoamerica[6] and spread to many tropical and subtropical regions around the world,[7] where its distribution is limited by chilling sensitivity,[8,9] Domestication eventually led to substantial changes in vegetative growth and sexual forms that distinguish wild populations from cultivated genotypes,[9] Because of its high yield, nutritional value, functional properties, and year-round fruit production, the importance of this crop around the world is undeniable.

The Solo varieties present a pear-shaped or oval appearance and are characterized by their small size (ranging between 400 and 600 g),[10] As the name suggests, a Solo papaya is about the right amount of fruit for one person. Papaya is a climacteric fruit, which grows year-round, is an elongated berry of various sizes with a smooth thin skin and a greenish-yellow color,[10,11,12] Its flesh is thick with a color ranging from yellow to red and offers a pleasant, sweet, mellow flavor,[12,13] Papaya fruits are berries and show high diversity in size and shape. Fruits from hermaphroditic plants tend to be elongated and vary from cylindrical to pear shaped, while fruits of female plants tend to be round. Different fruits have different periods as optimum maturity stages. Some researchers[14] identified different ripening/maturity periods for apple cultivars as early, mid and late stages.[15] noted four stages of ripeness for four tomato cultivars.Variation in fruits maturity stages brings about differences in color, taste, flavor, texture, fleshiness and other sensory properties..Maturity at harvest determines final fruit quality. Immature fruits are bitter, have inferior quality and when ripe and dried are more prone to shriveling (wrinkling) and mechanical damage.[16]

The objective of this work was to evaluate composition of papaya during its different stages of maturity.

MATERIAL AND METHODS

Biological material

Papayas (*Carica papaya var solo 8)* studied come from a plantation in Thomasset (Azaguié Ahoua) in south-east of Abidjan (Côte d'Ivoire). They were harvested at four stages of maturity: immature (A), 1/8 advanced (B), 1/4 advanced (C) and advanced (D). The study looked at the skin, the epicarp and the seeds of papayas.

Methods

Sampling

The sampled papayas were grouped into lots according to the stages of maturity. The skin, pulp and seeds were separated from each other using a knife, peeler and spatula. A quantity of 500g of each batch (skin, pulp and seeds) at different stages of maturity was dried in an oven at 45°C for 48 hours and then ground. The ground materials obtained were used for biochemical analyzes.

Organic acids determination

Method coupled with high performance liquid chromatography (HPLC) was used to identify and quantify organic acids.[17] Fifty (50) mg of powder was dissolved in 75 ml of distilled water. The mixture, homogenized by manual stirring for 2 min at room temperature (28°C), was centrifuged at 4000 rpm for 30 min at 4°C in a centrifuge (Sigma Aldrich 2-PK, France). Collected supernatant was filtered on Wattman #4 paper and then through a 0.45 µm millipore filter. Twenty (20) µl of filtered solution (clear) was analyzed by an HPLC system (Shimadzu Corporation, Japan) equipped with a binary pump (LC-6A) coupled to a UV-VIS detector (SPD-6A). Chromatographic separation of organic acids was carried out on an ICSep ICE ORH-801 column (30cm, Interchom, France) at a temperature set at 35°C. The eluent was sulfuric acid (0.004 N). The elution rate was 0.6 ml / min. The chromatograms obtained at 280nm were compared with those of organic acids standards. Peak areas have made it possible to quantify identified organic acids.

Oxalates determination

The titration method as described by[18] was performed. One (1) g of dried powdered sample was weighed into 100 mL conical flask. A quantity of 75 mL of sulphuric acid (3 M) was added and stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 mL of the filtrate was titrated while hot against $KMnO_4$ solution (0.05M) to the end point.

Oxalates (mg/100g) = (2.2×Veq×100)/me

Veq: volume (mL) of $KMnO_4$ equivalence. me : mass (g) of sample.

Phytates determination

The method described by[19] was used for determination of phytates content. A quantity (0.5 g) of dried powdered sample was mixed with 25 mL of trichloroacetic acid (3%, w/v) and centrifuged at 3500 rpm for 15 min. The supernatant obtained was treated with FeCl solution and the iron content of the precipitate was determined using spectrophotometric method at 470 nm. A 4 :6 Fe/P atomic ratio was used to calculate the phytic acid content.

Phytates (mg/100g) = (DO_{490} × 4)/(0.033×me)

Where, Calibration line: DO_{490} =0.033. Mass (µg) sodium phytate; R=0.99. m: mass (g) of the sample.

Statistical analysis

The data were brought to one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple-range test using SPSS (Version 21.0, SPSS Inc., Wacker Drive, Chicago, USA). Values expressed are means of triplicate determination± Standard deviation.

RESULTS AND DISCUSSION

The impact of organic acids was studied over four stages of maturity (A.B. C and D) of papaya (*Carica papaya var solo 8)* in three parts (skin, pulp and seeds) (Table 1). Organic acids content in each part studied drop from the immature to the advanced state of papayas. Most dominant organic acids in parts of papaya are salicylic, tartaric and adipic acids. Organic acid contents of different parts papaya present significant differences ($p < 0.05$) from one state of maturity to another. Maturity at harvest is very important to composition and quality of papaya. It determines the way in which papayas are handled, transported and marketed and their storage life and quality.[20] The characteristic flavour of a fruit is contributed mainly by the type and level of volatile compound present in tissue. However, the concentrations of sugars, organic acids as well as phenolic compound also give a significant contribution to the sensory component of the fruit.

Skin, pulp and seeds of papaya have high salicylic acid contents which are respectively (20.28±0.03-10.90±0.04 g/Kg), (54.71±0.02-14.07±0.09 g/Kg) (33.12±0.01-15.71± 0.09 g/Kg) according to the four stages of maturity (A, B. C and D). Skin and pulp don't contain oxalic acid during the four stages of maturity while oxalic acid contents of seeds varied from 0.07±0.01g/Kg (A) to 0.01±0.00 (D) and are less than 0.01g/Kg. Fumaric acid is non-existent (nd) in the skin, pulp and seeds of papaya. Butyric acid contents of the skin, pulp and seeds of the papaya are low and all less than 0.5g/kg.

Organic acids distributed in foods are digestible and provide a source of energy, improve the bioavailability of minerals by forming complexes, stimulate the secretion of endogenous enzymes through acidification, prevent the proliferation of microorganisms in foods.[21]

Organic acids have the capacity to complex the metal ions in the solution which degree depends on the particular organic acid, the concentration and the type of metal and Ph.[22]

Organic acids lower pH in the stomach thus reducing the growth of some pathogenic bacteria.[23,24] Organic acids distributed in the food are digestible and constitute a source of energy, they improve the bioavailability of the minerals by forming complexes; stimulate the secretion of endogenous enzymes through acidification.[25]

Acute toxicity of benzoic acid as a food additive is low. However, it was observed that in sensitive persons, intake of benzoic acid lower than 5 mg/kg of body weight per day, can cause non-immunological contact reactions (pseudoallergy).[26] Some studies suggested that very high intake of benzoic acid can cause adverse health effects such as metabolic acidosis, hyperpnoea and convulsions.[26]

Organic acids are one of the major phytochemicals in vegetables and responsible for food taste and odor. Different organic acids are analyzed in fruits and cereals, but least in vegetables and spices. Organic acids has been analyzed because of their high importance in the formation of other phytochemical and increased antioxidant activity.

Salicylic acid (SA) is an endogenous plant growth regulator ofphenolic nature. It plays some important roles in the regulationof plant growth development and enhances plant vigor under bio-tic and abiotic stresses.[27] SA plays an essential role in controlling berry quality such as color, flavor, astringency and bitterness[28] and enhance berry size.[29] weight[30] and berry firmness. Mainly, SA positively effects on reducing fruit respiration and ethy-lene biosynthesis weight loss, berry decay and softening rateduring storage and shelf-life.[31]

Table 1: Organic acids composition of three parts from papaya (*Carica papaya* L. *var solo 8*).

Carica papaya* L. *var solo 8					
Stages of maturity					
Organic acids (g/kg)	**Analyzed parts**	A	**B**	**C**	D
Oxalic acid	skin	nd	nd	nd	nd
	Pulp	nd	nd	nd	nd
	Seeds	0.07±0.01^d	0.05±0.01^c	0.02±0.00^b	0.01±0.00^a
Tannic acid	Skin	nd	0.17±0.02^b	0.13±0.01^b	0.06±0.01^a
	Pulp	0.44±0.05^d	0.35±0.04^c	0.12±0.03^b	0.04±0.01^a
	Seeds	1.16±0.06^g	0.91±0.08^f	0.72±0.03^e	0.50±0.01^d
Salicylic acid	Skin	20.28±0.03^d	19.47±0.05^c	14.88±0.06^b	10.90±0.04^a
	Pulp	54.71±0.02^g	40.78±0.01^f	39.37±0.06^f	14.07±0.09^b
	Seeds	33.12±0.01^e	17.66±0.02^c	15.71±0.09^b	nd
Tartaric acid	Skin	39.70±0.03^f	25.39±0.01^e	12.63±0.13^d	nd
	Pulp	26.99±0.09^e	17.06±0.03^d	3.24±0.02^b	1.19±0.02^a
	Seeds	19.82±0.02^d	8.66±0.01^c	3.69±0.03^b	3.07±0.06^b
Adipic acid	Skin	11.61±0.02^f	3.80±0.04^d	2.20±0.03^c	0.05±0.01^a
	Pulp	20.44±0.01^g	13.58±0.04^f	1.24±0.07^c	0.54±0.07^b
	Seeds	7.13±0.03^e	3.34±0.07^d	1.63±0.06^c	nd
Butyric acid	Skin	0.31±0.02^e	0.27±0.03^d	0.14 ± 0.02^c	0.03±0.00^a
	Pulp	0.32 ±0.02^e	0.17±0.04^c	0.13±0.01^c	0.03±0.00^a
	Seeds	0.09±0.03^b	0.04±0.00^a	0.02±0.00^a	nd
Fumaric acid	Skin	nd	nd	nd	nd
	Pulp	nd	nd	nd	nd
	Seeds	nd	nd	nd	nd
Citric acid	Skin	0.01±0.01^a	nd	nd	nd
	Pulp	0.24±0.02^c	nd	nd	nd
	Seeds	0.08±0.01^b	nd	nd	nd

Tests: n = 3; Means ± standard deviation, assigned different lowercase letters on the same line for each parameter are significantly different at p <0.05 according to Duncan's test. **A** (Immature) **B** (An advanced shift**). C** (An eighth advanced)**. D** (Advanced Analyse)**.**

Anti-nutritional factors are those substances or chemical compounds found in fruits and food substances in general. They are poisonous to humans or in some ways limit nutrients availability to the body.[32] Anti-nutritional factors are present in different food substances in varying amounts depending on the kind of food, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances. These anti-nutritional factors are known to interfere with metabolic processes such that growth and bioavailability of nutrients are negatively influenced.[33] The determination of the amount antinutrients in the samples is necessary because their reseearch can reduce essential nutrients bioavailability. Some of these antinutrients have been found to have protection against some diseases.[34]

Oxalate and phytate contents were determined in different parts (skin, pulp and seeds) of the papaya (*Carica papaya l var solo 8)* at four stages of maturity (Table 2). Oxalate and phytate contents in analyzed parts generally fall from one state of maturity to another. Papaya skin had high oxalate contents which varied from 9.07 ± 0.01 (A) to 2.18 ± 0.01 (D). Papaya seeds had high phytate contentcompared to skin and pulp papaya which varied from 10.54 ± 0.05 (A) to 5.35 ± 0.01g/Kg (D). Oxalates appear as end-metabolism products in many vegetable tissues. When they are consumed, oxalates can link calcium and other minerals.[35] The determination of antinutrients contents in samples is necessary because their reseearch can reduce essential nutrients bioavailability. Some of these antinutrients have been found to have protection against some diseases. Phytate level increases from 7.89±0.01% in unripe to 9.25±0.03% in ripe and then reduces to 6.54±0.01% in overripe. Oxalate in excess by exceeding the solubility limit results in the formation of calcium oxalate kidney stones.[36,37] However, the dietary contribution to excess oxalate was reportedly low.[37]

Oxalate content in differents samples (Table 2) was low compared to the range of mean values (42 to 469 mg/100 g) from two methods obtained for various types of nuts[38] The results showed that ripening could also be a way of reducing antinutrients in food samples. Oxalate content reduced from A to D in seeds (15.46±0.47- 5.84±0.06 mg/100g) and skin (9.07±0.01- 2.18±0.01 mg100g) samples. Generally, small amounts of oxalate may occur in many vegetables and fruits but do not pose nutritional problems.[39] determined oxalates contents in the peels (28.81±0.15%) and seeds (11.08±0.17%). This reported seed oxalate content is higher than that of papaya carica seeds at stage A (15,46 mg/100g) B (7.53 mg/100g), C (6.65 mg/100g) and D (5.84mg/100g) maturity. Recently,[40] reported that a daily intake of 450mg of oxalic acid has been reported to interfere with various metabolic processes.

Phytate and oxalates have the ability to form chelates with di- and trivalent metallic ions such as Cd, Mg, Zn, and Fe to form poorly soluble compounds that are not readily absorbed from the gastrointestinal tract thus reducing their bioavailability.[41] Phytate has strong binding capacity and forms insoluble complexes with multivalent cations, including Ca, Mg, Fe and Zn, and render them biologically unavailable.[42] Generally, small amounts of oxalate may occur in many vegetables and fruits but do not pose nutritional problems. Phytate contents of three parts from papaya **(***Carica papaya l var solo 8)* are not toxic because they are respectively less than 250 mg/100g,[43] 60 mg/100g.[44] Phytic acid has long been known as an anti-nutritional factor since it reduces bioavailability of several minerals due to its ability to chelate them.[45]

At present growing concern about phytic acid and their hydrolysis products has arisen from the finding that it might have beneficial effects such as antioxidant function, protecting against cancer risk.[46] In addition, they have been considered antinutritional components because they can react with certain essential amino acids, limiting their availability.[47] Phytate content decreased from A to D in differents parts (skin, pulp and seeds) of papaya. Phytate has strong binding capacity and forms insoluble complexes with multivalent cations, including Ca, Mg, Fe and Zn, and render them biologically unavailable.[42] Phytate has strong binding capacity and forms insoluble complexes with multivalent cations, including Ca, Mg, Fe and Zn, and render them biologically unavailable. The values obtained for phytate and oxalate are lower than the lethal dosage reported in other studies while the toxic effect of these anti-nutrients may not occur when these fruits are consumed because their levels are not enough to elicit toxicity. The antinutrients in mg/100g are phytate (7.89±0.02, 9.25±0.10 and 6.54±0.30), oxalate (0.32±0.00, 0.25±0.01 and 0.41±0.10) in pawpaw (*Carica Papaya*) seeds.[42] These contents are lower than those determined in *Carica papaya l var solo 8***.**

Table 2: Composition in antinutritional factors of three parts from papaya (*Carica papaya* L. *var solo 8)*.

Carica papaya* L. *var solo 8					
Stages of maturity					
Composition (mg/100g)	**Analysed parts**	**A**	**B**	**C**	**D**
Oxalates	Skin	9.07±0.01^{g}	6.45±0.09^{e}	3.89±0.07^{c}	2.18±0.01^{b}
	Pulp	1.63±0.08^{a}	2.03±0.48^{b}	2.35±0.09^{b}	3.81±0.05^{c}
	Seeds	15.46±0.47^{h}	7.53±0.04^{f}	6.65±0.02^{e}	5.84±0.06^{d}
Phytates	Skin	7.70± 0.03^{g}	6.59±0.06^{f}	3.37±0.05^{d}	2.01±0.01^{b}
	Pulp	3.77±0.01^{d}	3.66±0.04^{d}	1.78±0.03^{a}	2.43±0.05^{c}
	Seeds	10.54±0.05^{h}	6.90±0.03^{f}	5.91±0.01^{e}	5.35±0.01^{e}

Tests: n = 3; Means ± standard deviation, assigned different lowercase letters on the same line for each parameter are significantly different at p <0.05 according to Duncan's test. **A (**Immature**). B (**An advanced **shift). C (**An eighth advanced**). D (**Advanced**).**

CONCLUSION

Papaya (*Carica papaya* L.) is a popular and economically important fruit tree of tropical and subtropical countries. The fruit is consumed world-wide as fresh fruit and as a vegetable or used as processed products. The study revealed that at the four stages of maturity (A, B, C and D) compositions differ from one part to another. Organic acids contents decrease during the stages of maturity. The study also showed that pulp (edible part of papaya) is rich in salicylic, tartaric and adipic acids and devoid of oxalic and fumaric acids. Papaya skin, pulp and seeds had low antinutrient contents which could make it useful as alternative source of raw material in feed formulation. The results showed that ripening could also be a way of reducing antinutrients in food samples.

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