Evaluation of anti-nutritional factors and profiles (vitamin, organic) of the seeds of five clones of rubber tree (*Hevea brasiliensis*) commonly grown Ivory Coast

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abstract

This study was conducted to evaluate the antinutritional factors and the organic acid and vitamin profile of the kernels of five rubber tree clones commonly grown in *Ivory Coast*. The kernels of these seeds were analyzed for their antinutritional factors (saponins, alkaloids, trypsin inhibitors, L-dopamine) and their organic acid and vitamin profiles (organic acids and vitamins). The results showed that the kernels of the five clones contained saponins (1.45-4.33 mg/100 g), trypsin inhibitors (3.27-10.54 TiU/mg), and alkaloids (1.94-5.18 mg/100 g). The organic acid profile revealed the presence of four main acids: tannic acid (35.5-57.30 mg/100 g), citric acid (31.28-61.12 mg/100 g), fumaric acid (42.10-61.12 mg/100 g) and tartaric acid (43.10-54.44 mg/100 g). In terms of vitamins, the almonds from the five clones were rich in vitamins B2 (28.40-31.34 mg/100 g dry matter), B6 (39.74-41.18 mg/100 g dry matter), B9 (11.10-12.28 mg/100 g dry matter), and E (3.50-3.78 mg/100 g dry matter). Given their profiles, almonds from these five clones could have beneficial effects in several areas.

Keywords: profiles, organic acids, Hevea brasiliensis, vitamins, seeds, clones.

Introduction

Rubber cultivation has become an important economic activity for Côte d'Ivoire because it generates income through the sale of its rubber product (Rodrigo *et al*., 2012). Rubber trees are cultivated for their latex, which is their raw material. In Côte d'Ivoire, it covers an area of 479,985 hectares, only 60% of which is in production (FIRCA & APROMAC, 2024). Due to its economic profitability, this crop is being cultivated in new areas that were previously unsuitable for rubber cultivation, known as marginal areas. Ivory Coast's natural rubber production is estimated at 1,980,113 tons in 2024, making it the leading producer in Africa and the third largest producer of natural rubber in the world (FIRCA & APROMAC, 2024). This production is largely carried out by village plantations. In addition to natural rubber, rubber trees produce seeds every year in August and November. Seed production varies between 150-200 kg ha-1 year-1. These seeds are used in nursery production (rootstock) with less than 25% (Okoma *et al*., 2018). The rest are left on the plantations, losing their potential. According to several studies, these seeds are rich in lipids (40-50%), proteins (19-23%) and bioactive compounds (polyphenols, flavonoids and tannins) (Oluodo *et al*., 2018; Piccolella *et al*., 2023). These bioactive compounds have antioxidant properties that can help combat oxidative stress while reducing the risk of chronic diseases in the body (Rincón-Cervera *et al*., 2020). They are used as a source of oil in the production of biodiesel and paint (Ramadhas *et al*., 2005; Ahmad *et al*., 2014; Onoji *et al*., 2016). As for their protein content, these seeds are used in animal feed (Oluodo *et al*., 2018). However, these seeds are rich in hydrocyanic acid, which limits their use in human food. Hydrocyanic acid is toxic to humans when its content exceeds 100mg/kg. However, there are technologies that can significantly reduce the hydrocyanic acid content, such as cooking, roasting, and combined methods Agbai *et al*., 2021. The availability of these seeds makes them a valuable raw material, but this requires knowledge of their antinutritional factor content and their vitamin and organic profiles, which is the objective of this study.

Materials and methods

The plant material used in this study consists of seeds from five clones of Hevea brasiliensis commonly used in Côte d'Ivoire (GT1; PB 217; IRCA 41; IRCA 230; IRCA331). Sampling was carried out according to the method described by Okoma et al., 2025 with modifications. The rubber seeds from the five clones were collected from the monoclonal plantations of two rubber companies: the Société Africaine de Plantation d'Hévéa (SAPH) located in Toupah (5° 19′ N, 4° 34′ W) in the Grands Ponts region, approximately 63 km from Abidjan, and EXAT-AGRICULTURE in Prikro (7° 38′ N, 3° 59′ W) in the Iffou region. On the site, three one-hectare monoclonal plots producing rubber were selected by clone, taking into account their age at planting. In these monoclonal plots, five elementary blocks of twelve trees were regularly defined within the lines of one-hectare plots, taking care to leave two border lines (Figure 1). Harvesting was carried out manually every two days to prevent the seeds from rotting or germinating. The harvested seeds were sorted to remove any damaged seeds (germinated or tarnished), then purified using the criteria set out by (Michel *et al.*, 2020), dried in the sun on racks for a week, and then shelled. The kernels obtained were ground for analysis.

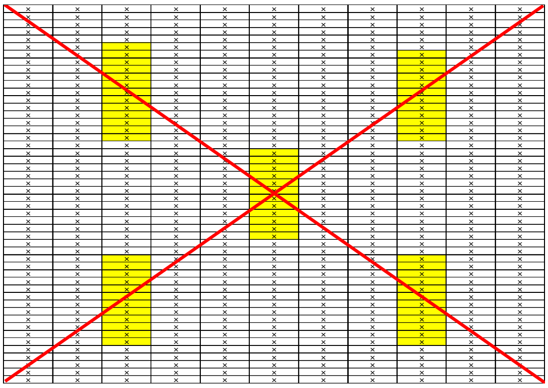

Figure 1: Experimental setup

Methods

Alkaloid assay

The assay was performed using the spectrophotometric method described by (Sreevidya & Mehrotra, 2003). A 5 mL sample of the extract solution was taken and the pH was maintained between 2 and 2.5 using diluted HCl. Two milliliters of Dragendorff's reagent was added, and the precipitate formed was centrifuged. Complete precipitation of the centrifugate was verified by adding Dragendorff's reagent. The centrifuged mixture was decanted completely. The precipitate was washed with alcohol. The filtrate was discarded and the residue was then treated with 2 mL of disodium sulfate solution. The brownish-black precipitate formed was then centrifuged. The completion of precipitation was verified by adding 2 drops of disodium sulfate. The residue was dissolved in 2 mL of concentrated nitric acid, with heating if necessary. This solution was diluted to 10 mL with distilled water. Then 1 mL of this diluted solution was taken and 5 mL of thio-urea solution was added to it. The absorbance was measured at 435 nm. The standard curve was made from a stock solution of atropine. The absorbances were read at 435 nm using a spectrophotometer against the blank tube prepared under the same conditions, replacing the extract with distilled water. The alkaloid content of the extracts was evaluated from the calibration curve and expressed in milligrams of atropine equivalent per 100 g of almonds.

Trypsin inhibitor

Trypsin inhibitors are determined by measuring the hydrolysis of a synthetic peptide substrate: TAME (Tosyl-L-Arginine Methyl Ester) (Udensi *et al.*, 2007). This method is based on the decrease in the hydrolysis rate of porcine trypsin caused by the presence of inhibitors in the samples. The activity of uninhibited trypsin is then measured in the presence of TAME, which releases toluene sulfonyl with a maximum absorption at 410 nm. For extraction, 0.1 grams of almond powder from the sample was stirred in Erlenmeyer flasks with 50 mL of 0.1 mol NaOH. The pH of the mixture was adjusted to 9.8 with NaOH (0.1 mol). The solution was stirred under a fume hood at room temperature for 3 hours. It was then transferred to a cold room at 4°C overnight. Afterwards, it was centrifuged at 3500 rpm for 20 minutes at 20°C. Finally, the supernatants obtained were used for the assay of trypsin inhibitors.

Saponin

Saponins were quantified according to the method described by Makkar *et al*. (2007). 1.5 g of finely ground, defatted almonds were added to 30 mL of 80% aqueous ethanol and mixed for 16 hours, followed by centrifugation at 3,000 rpm for 20 minutes. The supernatant obtained was collected and the residue resuspended in 80% aqueous ethanol and treated in the same manner as before. The two supernatants were combined and filtered through coffee filter paper to remove any particles that might be floating on the surface. The ethanol was then evaporated at 42-45°C in a Rotavapor. The aqueous phase was centrifuged to remove water-insoluble material before being transferred to a separating funnel for two equal-volume extractions to collect the pigments. The precise final volume of each extract was noted. The calibration curve was constructed using a saponin standard (Quillaja bark, Sigma, USA). The absorbance was read against a blank at 544 nm on a visible UV spectrophotometer (UV/VIS Spectrophotometer SP8001, Axiom, Germany). The results were expressed in mg of saponins/100g of dry matter.

Dopamine

L-Dopa was determined using a modified method (Vadivel & Pugalenthi, 2008). First, 0.1 g of crushed almond samples were extracted with 20 mL of HCl (0.5N) in 50 mL centrifuge tubes for 2 hours and centrifuged at 3000 rpm for 20 minutes. Next, 0.1 mL aliquots of the extracts, diluted to 1 mL with distilled water, were used for estimation. The quantitative estimation of L-DOPA concentration was extrapolated from a standard curve prepared from 1 mL aliquots of standard L-Dopa solutions at different concentrations. The absorbance was read against a blank at 283 nm on a visible UV spectrophotometer.

Organic acid profile

The method of (Ergönül & Nergiz, 2010) using an HPLC system (Shimadzu Corporation, Japan) consisting of a pump (Shimadzu LC-6A liquid chromatograph, Japan), a UV detector (Shimadzu SPD-6A UV spectrophotometric detector, Japan) and an integrator (Shimadzu CR 6A Chromatopac, Japan). Organic acids were extracted from 1 g of sample with 50 mL of 80% methanol saturated with NaCl. All separations were performed in isocratic mode with an ICSep ICE ORH-801 ion exclusion column (40 cm x 5 μm, Interchrom, France) or stainless steel column (40 cm x 5 μm) maintained at 35°C using a Meta ThermTM oven (Interchrom, France). The levels of organic acids in the samples were obtained by comparing the retention times of the eluted compounds with the retention times of the reference solutions.

Vitamin profile

Water-soluble vitamins (B, C) were determined by HPLC according to AOAC method 992.04 / 986.27. The extraction of water-soluble vitamins was carried out by weighing 2 g of crushed almond sample in an amber centrifuge tube, then adding 10 mL of a hot extraction buffer: for B vitamins (thiamine, pyridoxine, riboflavin), a 0.1 M HCl solution preheated to 90-95°C was used, while for vitamin C, a 1% oxalic acid solution supplemented with 0.5% metabisulfite or ascorbic acid was used to prevent oxidation. The mixture is then heated to 95°C for 30 minutes with gentle stirring; alternatively, enzymatic digestion can be performed by adding takadiastase and incubating at 37°C for 30 to 60 minutes when the matrix requires the degradation of starches or proteins. After heating, the tube is rapidly cooled on ice and then centrifuged at 4,000 × g for 10 minutes at a temperature between 4 and 10°C. The supernatant is collected, the extraction is repeated a second time, and the aqueous phases are combined. The final extract is filtered through a 0.22 µm PVDF filter into an amber HPLC vial. The extracts are stored at 4°C and injected quickly into the HPLC to prevent degradation. Subsequently, 20 µl of each sample prepared under the above conditions is injected for chromatographic analysis.

Vitamins A, D, E, K

Water-soluble vitamins were determined according to ISO 20633:2015 and ISO 27533:2023. The extraction of fat-soluble vitamins begins with preliminary degreasing, which is essential for lipid-rich matrices: 2 g of sample are defatted with three successive extractions of 10 mL of hexane under agitation for 15 minutes, then the organic phase is filtered and the residual powder is dried in air under a fume hood, the hexane extract being stored if necessary. The defatted powder is then transferred to a flask or test tube for saponification, which is recommended for vitamins A and K: 10 mL of ethanol and 1 mL of 10% ethanolic KOH are added, then the mixture is heated to a gentle reflux between 60 and 80°C for 30 to 45 minutes, protected from light, in order to hydrolyze the esters and release the vitamins, adding 0.1% BHT to protect vitamin E. After cooling, 10 mL of distilled water is added, then the free vitamins are extracted by three successive washes with 10 mL of hexane or n-hexane, vortexing for one minute and centrifuging at 3,000 × g for 5 minutes to recover the upper organic phase. The combined organic phases are then dried under a stream of air and reconstituted in 1 to 2 mL of methanol/hexane mixture in the column used containing 0.01% BHT. The final extract is filtered through a 0.22 µm PTFE filter into an amber HPLC vial, avoiding exposure to light, and then stored at −20°C for prolonged storage.

Statistical analysis

The tests were performed in three replicates. One-way analysis of variance (ANOVA) was performed using XLSTAT 2019 software on all the results obtained in order to determine whether there were any significant differences between the various values calculated. The different means were compared using Duncan's test at a 5% probability threshold. The correlation test was performed to determine the relationships between anti-nutritional factors and the organic acid profile.

Results and discussion

Results

Antinutritional factors

Table 1 shows the antinutritional factor content of the almonds from the seeds of the five clones studied. The results show that there is a significant difference between the antinutritional factors of the almonds from the five clones. Saponin contents ranged from 1.45 to 4.33 mg/100gDM. The highest value was obtained with clone GT1 (4.33 mg/100g DM) and the lowest with clone IRCA 331 (1.45 mg/100g). The different clones had L-dopamine contents ranging from 0.07 to 0.16 mg/100g DM. However, there was no difference between clones GT1, PB217 (0.140.16 mg/100g DM) and IRCA 41 (0.160.16 mg/100g DM), which had the highest contents. In terms of trypsin inhibitor content, the clones showed a significant difference at the 5% threshold. Clone GT1 (10.54 TiU/mg) had the highest content, followed by clone IRCA 41 (8.33 TiU/mg) and clone (3.27 TiU/mg) with the lowest content among the five clones. In terms of alkaloids, the clones had values of 5.18, 2.64, 4.73, 1.94, and 2.11 mg/100 g for clones PB217, GT1, IRCA41, IRCA230, and IRCA 331, respectively. Clone PB217 (5.18 mg/100g) had the highest value and clone IRCA 230 (1.94mg/100g) had the lowest value.

Table 1: Antinutritional factor composition of almonds from the five clones

	Rubber tree clones				
Antinutritional factors	GT1	PB217	IRCA 41	IRCA 230	IRCA 331
Saponin (mg/100g)	4,33± 0,02^{a}	2,55± 0,02^{c}	3,76± 0,03^{b}	2,02± 0,01^{d}	1,45± 0,04^{e}
L-dopamine (mg/100g)	0,14± 0,04^{a}	0,14± 0,03^{a}	0,16± 0,01^{a}	0,07± 0,02 ^{b}	0,13± 0,02^{a}
Trypsin inhibitor (TiU/mg)	10,54± 0,04^{a}	5,15± 0,03^{c}	8,33± 0,02^{b}	4,59± 0,03^{d}	3,27± 0,02^{e}
Alkaloids (mg/100g)	5,18± 0,03^{a}	2,64± 0,02^{c}	4,73± 0,04^{b}	1,94± 0,4^{e}	2,11± 0,03^{d}

The means ± standard deviation assigned different letters at each parameter on each line are significantly different at $p < 0.05$ according to Duncan's test.

Organic acid profile

The organic acid content of the almonds is shown in Table 2. The chromatographic profile reveals that tannic, citric, fumaric, and tartaric acids were the most abundant in the different almonds of the five clones. The tannic acid content of the almonds showed a significant difference at the 5% threshold. The highest contents were 57.30, 46.27, and 41.28 mg/100g for clones GT1, PB217, and IRCA331, respectively. Citric acid content ranged from 31.28 (GT1) to 41.65 mg/100g (IRCA331). Fumaric acid content was 42.10± 0.04; 58.23± 1.02; 48.05± 0.31; 49.47± 0.02 and 61.12± 2.01 mg/100g respectively for clones GT1, PB217, IRCA41, IRCA 230 and IRCA 331. The clones PB217 (58.23± 1.02mg/100g) and IRCA331 (61.12± 2.01mg/100g) had the highest values. Ascorbic acids were the lowest among all organic acids detected, with contents ranging from 2.10± 0.01 to 2.20± 0.01 mg/100g. The highest content was obtained with clone PB217 (2.20± 0.01 mg/100g) and the lowest with IRCA 41 (2.10± 0.01 mg/100g). As for the glycolic, phthalic, and sulfanilic acid contents, there was a significant difference between the almonds of the different clones. The contents varied from 16.58± 0.01 to 19.15± 0.03mg/100g; 14.11± 0.00 to 18.14± 0.01 mg/100g and 15.20± 0.01 to 19.20± 0.01 mg/100g for glycolic, phthalic and sulfanilic acid content, respectively. For the three acids below, the IRCA 331 clone gave the highest values for glycolic acid (19.15± 0.03mg/100g), phthalic acid (18.14± 0.01 mg/100g) and sulfanilic acid (19.20± 0.01 mg/100g). The lowest values were 16.58± 0.01 mg/100g for glycolic acid, 14.11± 0.00 mg/100g for phthalic acid and 15.20± 0.01 mg/100g for sulfanilic acid obtained with the IRCA230 clone. As for tartaric acid content, values ranged from 43.10± 0.05 to 54.44± 0.04mg/100g. As with the three previous acids (glycolic, phthalic, and sulfanilic), the IRCA 331 clone produced the highest tartaric acid content (54.44± 0.04 mg/100g) and the lowest for the IRCA 41 clone (43.10± 0.05mg/100g).

Table 2: Organic acid composition of the kernels of five rubber tree seed clones

Organic acids (mg/100g)	**Rubber tree clones**				
	GT1	**PB217**	**IRCA 41**	**IRCA 230**	**IRCA 331**
Tannic acid	57,30± 0,01^{a}	46,27± 0,04^{b}	35,5± 0,41^{e}	38,54± 0,03^{d}	41,28± 0,21^{c}
Citric acid	31,28± 0,04^{e}	40,81± 0,09^{b}	36,5± 0,01^{d}	39,21± 0,04^{c}	41,65± 0,07^{a}
Fumaric acid	42,10± 0,04^{d}	58,23± 1,02^{b}	48,05± 0,31^{c}	49,47± 0,02^{c}	61,12± 2,01^{a}
Ascorbic acid	2,17± 0,01^{b}	2,20± 0,01^{a}	2,10± 0,01^{c}	2,11± 0,02^{c}	2,19± 0,01^{ab}
Glycolic acid	17,88± 0,01^{d}	19,10± 0,02^{b}	18,10± 0,01^{c}	16,58± 0,01^{e}	19,15± 0,03^{a}
Phthalic acid	16,13± 0,02^{c}	17,25± 0,01^{b}	15,40± 0,01^{d}	14,11± 0,00^{e}	18,14± 0,01^{a}
Sulfanilic acid	17,44± 0,01^{c}	18,23± 0,04^{b}	16,44± 0,02^{d}	15,20± 0,01^{e}	19,20± 0,01^{a}
Tartaric acid	50,17± 0,81^{c}	52,20± 0,91^{b}	43,10± 0,05^{d}	49,29± 0,06^{c}	54,44± 0,04^{a}

The means ± standard deviation assigned different letters at each parameter on each line are significantly different at $p < 0.05$ according to Duncan's test.

Vitamin profile of almonds

The content of water-soluble vitamins (Table 3) and fat-soluble vitamins (Table 4) expressed in mg/100g. The B vitamins consist of vitamins B1, B2, B3, B5, B6, and B9. The vitamin B1 content of the five clones ranged from 7.28 (GT1) to 8.15 mg/100g (PB217). Vitamin B2 is the second most abundant vitamin in almonds, with levels of around 30mg/100g; only clone PB217 had a level below 30mg/100g. Vitamin B3 levels ranged from 7.62 to 8.71mg/100g. Vitamin B5 was below 2mg/100g in all samples. However, the IRCA331 clone (1.96mg/100g) had the highest content and the GT1 clone (1.75mg/100g) had the lowest. Vitamin B6 was the most abundant of the B vitamins in each clone. Its content ranged from 39.74 to 41.18 mg/100 g, with the highest levels found in clones GT1 (41.18 mg/100 g), IRCA 41 (41.16 mg/100g) and IRCA 331 (41.15 mg/100g), which obtained statistically identical values. Vitamin B9 levels ranged from 11.10 (GT1) to 12.33 mg/100g (PB217). Vitamin C content ranged from 2.29 to 2.89 mg/100g. The clones with the highest values were IRCA331 and PB217, with respective contents of 2.89 and 2.77 mg/100g.

Table 3: Water-soluble vitamin content of the kernels of the five rubber tree clones

Vitamin profile (mg/100g DM)	Rubber tree clones				
	GT1	PB217	IRCA 41	IRCA 230	IRCA 331
Vitamin B1	7,28± 0,00^{d}	8,15± 0,00^{a}	7,37± 0,01^{c}	8,13± 0,01^{b}	7,38± 0,00^{c}
Vitamin B2	31,34± 0,01^{a}	28,40± 0,01^{e}	30,49± 0,02^{c}	31,30± 0,01^{b}	30,39± 0,01^{d}
Vitamin B3	8,41± 0,01^{b}	7,62± 0,01^{d}	8,71± 0,01^{a}	8,32± 0,01^{c}	7,63± 0,00^{d}
Vitamin B5	1,75± 0,00^{e}	1,90± 0,00^{b}	1,87± 0,00^{c}	1,80± 0,01^{d}	1,96± 0,01^{a}
Vitamin B6	41,18± 0,01^{a}	40,25± 0,02^{b}	41,16± 0,01^{a}	39,74± 0,01^{c}	41,15± 0,03^{a}
Vitamin B9	11,10± 0,00^{d}	12,28± 0,01^{a}	11,10± 0,00^{d}	12,22± 0,01^{b}	11,33± 0,00^{c}
Vitamin C	2,52± 0,01^{c}	2,77± 0,02^{b}	2,29± 0,01^{e}	2,49± 0,01^{d}	2,89± 0,00^{a}

The means ± standard deviation assigned different letters at each parameter on each line are significantly different at $p < 0.05$ according to Duncan's test.

Fat-soluble vitamin composition (A, E, K)

For vitamins A, E, and K, the levels were significantly different at the 5% threshold for all clones (Table 4). The levels of vitamin K (0.13-0.18 mg/100g), E (3.50-3.78 mg/100g) and A (0.37-0.43 mg/100g) were significantly different at the 5% threshold (Table 4). The highest levels of vitamin E (3.78 mg/100g), A (0.43 mg/100g) and K (0.18 mg/100g) were obtained with clone PB217. Clone GT1 had the lowest vitamin E (3.50 mg/100g) and K (0.13 mg/100g) contents, while the lowest vitamin A content was obtained with clone IRCA41 (0.37 mg/100g).

Table 4: Fat-soluble vitamin content of almonds from the five clones

Vitamin profile (mg/100g DM)	**Rubber tree clones**				
	GT1	**PB217**	**IRCA 41**	**IRCA 230**	**IRCA 331**
Vitamin E	3,50± 0,00^{e}	3,78± 0,00^{a}	3,70± 0,00^{c}	3,57± 0,01^{d}	3,76± 0,00^{b}
Vitamin K	0,13± 0,00^{b}	0,18± 0,01^{a}	0,15± 0,02^{b}	0,15± 0,00^{b}	0,18± 0,01^{a}
Vitamin A	0,38± 0,00^{c}	0,43± 0,00^{a}	0,37± 0,01^{c}	0,41± 0,00^{b}	0,40± 0,01^{b}

The means ± standard deviation assigned different letters at each parameter on each line are significantly different at $p < 0.05$ according to Duncan's test.

Pearson's correlation matrix

Pearson's correlation matrix (Table 5) was used to establish the relationships between antinutritional factors and organic compounds. This correlation shows that the alkaloid content is strongly and positively correlated with the trypsin inhibitor and saponin contents (r=0.97) but negatively correlated with citric acid (r=0.89) and fumaric acid (r=0.75). Trypsin inhibitors are negatively correlated with citric acid (r=0.96) and fumaric acid (r=0.83) but positively correlated with saponins (r=0.99). Saponins are strongly and negatively correlated with citric acid (r=0.91) and fumaric acid (r=0.83), but fumaric acid is strongly and positively correlated with citric acid (r=0.92). When the ascorbic acid content increases, the glycolic (r=0.76), phthalic (r=0.87), sulfanilic (r=0.88), and tartaric (r=0.85) acid contents increase in the same direction. Similarly, if the glycolic acid content increases, the phthalic acid (r=0.95) and sulfanilic acid (r=0.93) contents also increase. There is a strong positive correlation between the phthalic acid and sulfanilic acid contents (r=1.00).

Table 5: Correlation matrix between antinutritional factors and organic acids

Variables	Dopa	Alca	I.try	Sap	Ac.tan	Ac. citr	Ac.fum	Ac.asc	Ac.glyc	Ac.phta	Ac. sulf	Ac. tar
Dopamine (Dopa)	1,00											
Alkaloids (Alca)	0,67	1,00										
Trypsin inhibitor (I.try)	0,49	**0,97**	1,00									
Saponin (sap)	0,55	**0,97**	**0,99**	1,00								
Tannic acid (ac.tan)	0,22	0,43	0,53	0,48	1,00							
Citric acid (Citr.ac)	-0,29	**-0,89**	**-0,96**	**-0,91**	-0,61	1,00						
Fumaric acid (Fu.ac)	-0,02	**-0,75**	**-0,87**	**-0,83**	-0,36	**0,92**	1,00					
Ascorbic acid (Asc.ac)	0,28	-0,21	-0,23	-0,24	0,58	0,23	0,54	1,00				
Glycolic acid (Glyc.ac)	0,69	-0,04	-0,21	-0,16	0,14	0,36	0,67	**0,76**	1,00			
Phthalic acid (Phta.ac)	0,53	-0,15	-0,29	-0,28	0,27	0,35	0,68	**0,87**	**0,95**	1,00		
Sulfanilic acid (Sulf.ac)	0,54	-0,10	-0,24	-0,24	0,33	0,28	0,63	**0,88**	**0,93**	**1,00**	1,00	
Tartaric acid (Tar.ac)	-0,22	-0,59	-0,55	-0,60	0,40	0,43	0,61	**0,85**	0,44	0,67	0,68	1,00

Discussion

The trypsin inhibitor recorded during this study ranges from 3.27 to 10.57 (TiU/mg) and is lower than that obtained from raw rubber tree seeds (18.87%) (Oluodo *et al*., 2018c) and on the seeds of three Canavalia legumes at different stages of maturity. These levels are within the same range as those found in green chickpeas (4.41 to 10.44 TUI/mg), with the exception of the GT1 clone (Nikarthil Sudhakaran *et al*., 2024). The presence of these trypsin inhibitors in food, which are actually protease inhibitors, reduces protein digestibility because they form irreversible complexes of trypsins and trypsin inhibitors. According to (Pan *et al*., 2021), these trypsin inhibitors act on the proteolytic activity of digestive enzymes, thereby affecting protein digestibility. These relatively low levels in rubber tree seeds could be due to factors that may influence the condition of the seeds, namely the ecological zone of the seed, climatic conditions, genetic modifications, and storage (Oluodo *et al*., 2018). The consumption of these almond powders would have less impact on protein digestibility when used in livestock and poultry feed. However, several methods exist to reduce this trypsin inhibitor content, including heat treatments that can reduce it by 100%, as demonstrated by (Assam *et al*., 2022). Saponins are steroidal glycosides naturally produced by plants (Bashir *et al*., 2022). Biologically, saponins are considered antinutritional factors because of their physiological effects on the body (decreased feed intake, protein digestibility, and inhibition of growth rate in monogastric animals) and when their content reaches a certain threshold, they become toxic (Oluodo *et al*., 2018). Saponins also have a bitter taste and reduce palatability in livestock. The levels obtained in these seed kernels (1.45 to 4.33 mg/100 g DM) are low compared to those in raw seeds (24.89 mg/100 g DM) (Udo *et al*., 2018). As the saponin content of the kernels is lower than that of the raw products, this means that solar drying has had an effect on reducing it. Indeed, heat treatment has been recognized as effective in reducing saponin content (Anya, 2012). In warm-blooded mammals (humans), the toxicity of saponin depends on certain conditions, including the method of administration, the source, its concentration, and its composition (Arthur, 2019). Low saponin concentrations give almonds cholesterol-lowering, immunostimulant, and anticarcinogenic properties (Liener, 1974); (Samtiya et al., 2020). Alkaloids are compounds that have biological effects on humans, even in small quantities (Kurek, 2019). The levels obtained in this study (1.94 to 5.18 mg/100g) compare favorably with those of (Agbai *et al*., 2021) on processed rubber tree seeds in Nigeria (0.97 to 4.54 mg/100g). The GT1 clone tends to have a more bitter taste than the other clones because alkaloids are known for their bitter taste and may have beneficial effects such as anti-inflammatory, analgesic, antimicrobial, and antifungal properties (Kurek, 2019). That said, if their levels become significant, they can be responsible for certain dysfunctions, including disruption of nerve impulse transmission and cellular toxicity (Rajput *et al*., 2022). The presence of L-dopamine in food is important because it allows its properties to be evaluated. In pharmaceuticals, L-dopamine is used in the treatment of Parkinson's disease because of its neurotoxic function, but it is toxic in terms of nutrition (Bell & Janzen, 1971; Pugalenthi & Vadivel, 2007). The L-dopamine levels in this study are relatively very low, even trace levels, and well below the maximum recommended level of 2000 mg (Versteeg *et al*., 1996). Indeed, from a nutritional point of view, the abundance of L-dopamine can lead to gastrointestinal complications and inhibit the digestibility of proteins and starch (Da Prada *et al*., 1984). Variations in L-dopamine content depend on genetic variation and geographical location (Sridhar & Bhat, 2007). The main organic acids present in almonds were tannic acid, citric acid, fumaric acid, and tartaric acid. Organic acids are food compounds whose presence is beneficial to the population. They have effects on the taste, quality, and flavor of foods and possess intrinsic properties (antioxidant, acidifying) (Tormo & Izco, 2004). Tartaric acid has properties that reduce iron (III) to iron (II) in wine. It also plays a role in the taste of food by reducing the pH, which creates an environment that is not conducive to the proliferation of microorganisms (Li *et al*., 2023). According to these authors, tartaric acid production is influenced by many factors such as light, temperature, and growth regulators. These factors contribute to the taste and flavor of fruit (Walker & Famiani, 2018). In human nutrition, tartaric acid is used as a food additive in cereals and bread, where it acts as an acidifying and firming agent (Redmond, 2024). It is considered a good laxative and reduces the intestinal transit of food (Spiller *et al*., 2003). Ascorbic acid promotes the absorption of heme iron thanks to its reducing and inhibitory properties. It is considered a natural antioxidant present in fruits and vegetables that reduces and traps free radicals responsible for cellular aging (Teucher *et al*., 2004). A high tannic acid content of 35.5± 0.41 to 57.30± 0.01mg/100g has been observed in rubber tree seed kernels. These kernels are believed to have antibacterial, antimicrobial, and antioxidant properties, leading to their widespread use in both the food and non-food industries (Zhang *et al*., 2023). The antimicrobial property of tannic acid can be explored in food processing to increase shelf life (Chung et al., 1998). The abundant presence of this tannic acid in these kernels could be extracted for use in the areas mentioned above. Recent studies have shown that tannic acid can be used in the manufacture of biopolymer-based food films and that its combination with ferrous ions helps to promote rapid healing of diabetic wounds (Zhang *et al*., 2023; Xu et al., 2023). As for fumaric acid in almonds, the levels were high (42.10± 0.04 to 61.12± 2.01mg/100g). According to (Barros *et al*., 2013), fumaric acid is important because of its antioxidant, antimicrobial, and acidifying properties. In chickens, a diet rich in fumaric acid improves nutrient digestibility while promoting growth (Reda *et al*., 2021). Fumaric acid also has anti-inflammatory and anti-arthritic properties, as demonstrated by (Xaviera *et al*., 2024) in their work on rats. These powders could be used as insecticides, disinfectants, and cleaning agents because the fumaric acid they contain has bacteriostatic properties (Diomande *et al*., 2021). Citric acid affects the organoleptic quality of food through the action of titratable acidity. In addition, there is a positive correlation between citric acid content and titratable acidity. The citric acid content (31.28± 0.04-41.65± 0.07mg/100g) in almonds could probably be due to the increased activity of citrate synthase and the decrease in the activities of mitochondrial aconitase and nicotinamide adenine dinucleotide (NAD)-dependent isocitrate dehydrogenase, involved in its degradation (Jia *et al*., 2023). Vitamins are organic compounds that are essential for the body to function properly. The vitamin profile of the almonds from the five clones revealed the presence of vitamins B (B1, B2, B3, B6, and B9); A, K, E, and C. The B vitamins were provided by vitamin B1 (7.28 to 8.15 mg/100 g), B2 (28.40 to 31.34 mg/100g), B3 (7.62 to 8.71 mg/100g), B6 (39.74 to 41.18 mg/100g) and B9 (11.10 to 12.28 mg/100g). These vitamin B1, B2, and B3 contents are higher than those reported by (Agbai *et al*., 2021) for rubber tree seeds in Nigeria, where the contents were 0.30; 0.52 and 0.77mg/100g respectively for vitamins B1, B2 and B3, and higher than the reference levels for B1 (1.1-1.3mg/day); B2 (1.1-1.4 mg/100g), B6 (1.3 mg/day or 1300 µg/day of MS0) that an adult individual must consume to meet their daily requirements FAO, 2002 (Strohm *et al*., 2016). The high vitamin B content of almonds could have beneficial effects and advantages for consumers. Vitamin B plays a role in energy synthesis. Thiamine (B1) plays a key role in aerobic metabolism for energy production. It is also necessary for carbohydrate synthesis. Thiamine deficiency leads to mitochondrial dysfunction and focal degeneration of the thalamus, resulting in Wernicke's encephalopathy or Wernicke-Korsakoff syndrome (Mrowicka *et al*., 2023). Riboflavin (B2) plays a role in the synthesis of niacin, folic acid, and vitamin 6. Its antioxidant effect is crucial for cellular respiration and immune system function. Folic acid (B9) plays a role in the production and synthesis of nucleic acids and red blood cells. It also participates in the conversion of homocysteine to methionine, which is essential for hematopoiesis and the prevention of megaloblastic anemia. These water-soluble vitamins perform several physiological functions (metabolism, energy production, cellular integrity, growth, and development) by combating beriberi (a disease caused by thiamine deficiency) and pellagra (a disease caused by niacin deficiency). They are coenzymes that play an essential role in the single carbon (1-C) metabolic network (Strohm *et al*., 2016). These hevea seed kernels could be recommended in the human diet as a potential source of thiamine, riboflavin, and niacin, given that their content exceeds the levels recommended by the FAO/WHO. Vitamin C (ascorbic acid) is a vitamin that is not synthesized by humans and is generally obtained from fruits and vegetables (Buxeraud & Faure, 2021). It is a powerful antioxidant that combats oxidative stress caused by environmental pollutants and participates in several biological reactions (Hansen *et al*., 2014). Vitamin C deficiency can cause mood and cognitive disorders (Plevin & Galletly, 2020). Certain diseases can also be caused by vitamin C deficiency in humans, such as scurvy, which manifests itself as fatigue, and in children as growth deficiency (Halcrow *et al*., 2014). Vitamin C also contributes to the formation of red blood cells by enhancing iron absorption to prevent anemia (Ponka *et al*., 2016). The vitamin C content is well below the daily dose for an adult (60mg/day) and that of vitamin E, which is 15mg/day. In short, these two vitamins are found in lower levels in rubber tree seeds and therefore cannot be considered a source of vitamins C and E. This low vitamin C content could be due to the drying process that rubber tree seeds undergo, as factors such as temperature, light, and oxygen influence the degradation of vitamins in fruits and vegetables during storage (Muhamad, 2005). Vitamins A and K are the two lowest vitamins in these nuts, with contents of 0.13± 0.00 to 0.18± 0.01 and 0.37± 0.01 to 0.43± 0.01mg/100g for vitamins K and E, respectively. This vitamin A content could be due to the low carotenoid content in almonds, as carotenoids contain provitamins A, which are precursors of vitamin A. In addition, there is a positive correlation between carotenoid content and vitamin A content, such that if the carotenoid content is high, the vitamin A content is also high. Vitamin A is essential for vision, healthy skin and mucous membranes, and proper immune system function (N'zebo *et al.*, 2019). Like vitamins D and C, vitamin K contributes to bone health, blood clotting, and vascular health (Tsugawa & Shiraki, 2020). The recommended daily allowance (RDA) or adequate intake (AI) of vitamin K is intended to ensure normal blood clotting (Sato *et al.*, 2020). There are several variations in this intake quotient depending on the different organizations, such as the US National Academy of Medicine (120 µg/day for adult men and 90 µg/day for adult women) (Del Valle *et al.*, 2011). The World Health Organization and the Food and Agriculture Organization of the United Nations (65 µg/day for men and 55 µg/day for women) and the European Commission have established a recommended daily intake of 75 µg/day (Communities, 2008). In 2012, the Italian Society of Human Nutrition (SINU) suggested a stratified vitamin K intake according to age (140 or 170 µg/day for 18-59 year olds and >60 year olds, respectively) (Fusaro *et al.,* 2020). Regardless of the organization, the levels obtained in almonds are higher than those of its organizations. These almonds will cover vitamin K requirements while combating osteoporosis and blood clotting problems.

Conclusion

This study focused on rubber tree seeds commonly found in Côte d'Ivoire, with the aim of evaluating their antinutritional factors and profiles (organic acids and vitamins). The analyses revealed that these almonds are rich in organic acids (fumaric, citric, tartaric, and tannic) and vitamins (B2, B6, B9, and E). The IRCA 331 clone had the highest content of these most abundant organic acids, and GT1 had the highest content of antinutritional factors. This content of organic acids and vitamins opens up avenues for the use of these almonds in a number of fields (food, pharmaceuticals, etc.) due to their intrinsic properties. These seeds, once considered agricultural waste used only for seed production, can now be used in a number of areas. The PB217 clone is rich in fat-soluble vitamins (A, E, K).

Conflicts of interest

The authors declare that there are no conflicts of interest with respect to this article.

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