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Effect of Ripening Stage an Organic Acid and Anti-Nutrient Contents of three *Canavalia* species

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A B S T R A C T

The objective of this study was to evaluate the anti-nutrient and organic acid content of the seeds of three legumes of the genus *Canavalia* according to their maturity stage. The biological material used for the laboratory operations consisted of seeds and pods at different stages of maturity harvested from a field in the Gbèkê region, central Côte d'Ivoire. Seeds of the three cultivars were harvested at different stages of maturity: 30 days (S1), 40 days (S2), 50 days (S3), 60 days (S4) and 80 days (S5) after fertilisation. The seeds are dried and ground to obtain the raw meal. The content of anti-nutrient compounds was studied using standard colorimetric methods, while the distribution of organic acids was carried out using HPLC analytical methods. The composition of anti-nutrients showed that their content decreased during ripening from stage S1 to stage S5 with the lowest contents, respectively for *Canavalia gladiata*, *Canavalia rosea* and *Canavalia ensiformis* being : 33.49 ± 0.06, 32.06 ± 0.06, 21.23 ± 0.06 mg/100g DM for phytates; 257.15 ± 0.06, 141.48 ± 0.07, 177.42 ± 0.06 mg/100g DM for oxalates; 1.39 ± 0.05, 1.67 ± 0.01, 1.09 ± 0. 01 mg/100g DM for saponin; 449.17 ± 5.54, 442.50 ± 5.38, 546.67 ± 2.98 mg/100g DM for L-dopamine and 98967.42 ± 3.27, 866.71 ± 3.19, 437.67 ± 1.99 trypsin units inhibited by trypsin inhibitors. With regard to the content of organic acids at all stages of maturity, it is noted that the predominant for the extracts of *C. gladiata* and *C. rosea* is lactic acid (0. 05 to 3.68 mg/kg DM and 0.03 to 4.02 mg/kg DM) followed by acetic acid (0.03 to 3.06 mg/kg DM and 0.01 to 2.11 mg/kg DM) and oxalic acid (0.01 to 2.76 mg/kg DM and 0.01 to 1.54 mg/kg DM).

Introduction

The world's population in 2010 was 6.9 billion, with approximately 5.7 billion living in developing countries (UNFPA, 2010). According to the Food and Agriculture Organization of the United Nations (2011), the number of undernourished people in the world at the beginning of the 21st century was approximately 855 million, of which 820 million were in developing countries, 25 million in countries in transition, and 10 million in developed countries. In addition, nearly 821 million people and over 150

million children were malnourished in 2017 (FAO, 2018). Sub-Saharan Africa still has the highest percentage of undernourished people. Development in Côte d'Ivoire relies on agriculture and food insecurity on the African continent is no exception. According to Akakpo *et al*., (2009), approximately 12.6% of rural households are food insecure. To meet their food needs, rural populations often turn to species such as cereals and legumes to supplement subsistence agriculture.

The composition of these species provides an important contribution to the diet in calories, vitamins, fiber, minerals, and protein (Tchumou, 2016). Beans are characterized by their richness in essential nutrients. They are particularly starchy and low in fat. In addition, they are also highly valued in human nutrition for their interesting protein content (Ait *et al*., 2016). These cereal legumes include the genus *Canavalia spp*. The genus *Canavalia* (Jack bean) comprises 48 species of underutilized native legumes widely distributed in tropical regions (Tiamiyu *et al*., 2016). It is rarely consumed by humans and has a total yield of up to 2.5 tons-ha-1 under optimal agronomic conditions (Okonkwo and Udedibie, 1991). The crude seeds of Canavalia contain about 600 g-kg-1 of carbohydrates (Rajaram and Janardhanan, 1992). Their minimum protein content ranges from 22.4% to 24.9%.

The protein of Canavalia seeds is higher than that of wheat (8.55%), parboiled rice (7.7%) and egg (12.6%) (Livsme-delsverk, 1988). *Canavalia spp*. seeds are an excellent source of minerals (sodium, potassium, magnesium, phosphorus, calcium...) fulfilling two thirds of the recommended dietary allowance of NRC/NAS (1989) similar to common legumes (Mo-han and Janardhanan, 1994). They therefore have great potential to replace conventional foods. As with other fruits and vegetables, the stage of harvest maturity can affect the nutritional quality of Canavalia seeds.

Considering the importance of organic acids, the present paper focuses on the acid organic profile and antinutrient contents of seeds of the three legume species *Canavalia gladiata*, *Canavalia rosea* (CR) and *Canavalia ensiformis* (CE) as a function of their ripening stage.

Materials and Methods

Study material

The biological material used for the realization of the manipulations in the laboratory is constituted of seeds and pods presented on the Figure 1 below harvested at various stages of maturation in a field elaborated in the "Gbèkê" region. The seeds used to create the field consisted of seeds of the three legume species *Canavalia gladiata* (CG), *Canavalia rosea* (CR) and *Canavalia ensiformis* (CE). Seeds of the three species were harvested at different stages of maturity: 30 days (S1), 40 days (S2), 50 days (S3), 60 days (S4) and 80 days (S5) after fertilization.

Cultivation system

The cultivation of the species *C. gladiata* (CG), *C. rosea* and *C. ensiformis* was conducted in the open field, in a block arrangement, during the 2019 - 2020 academic year, in Ahougnansou (Bouaké). The set-up, illustrated below, was installed in three blocks covering an area of 2760 m2 (60 m × 46 m). Each block has an area of 460 m2 (46 m x 10 m) and is formed by three lines of pits. Each row consists of 12 pits (i.e. 4 pits per species arranged randomly) and each pit contains three seeds of the same legume species. For all three species, the seeds were sown on the same day. In total, 36 pits per block and 108 for the whole field (three blocks) were sown.

The blocks are 10 m apart. Pockets are 3 m apart in a row and rows are 5 m apart, i.e. a distance of 3 m x 5 m. After the seedlings have emerged, a shelter made of simple wood about 1.5 m high was designed above the three rows of seedlings, to act as a stake. No insecticides or products were used in this field. Instead, it was carefully monitored on a daily basis and weeded in the traditional way (using a hoe).

Labelling of flowers and harvesting of pods

The evolution of the flowers was followed until the fertilization. A flower is said to be fertilized when the perianth falls. At this date, a tissue is attached to the base of each fertilized flower and the date is noted. Thus, different tissues of different colors were attached to the base of each flower after their fertilization. This fertilization date is noted on the flower label. For each of the cultivars (white, red and black), the evolution of the moisture content and more of the color of the pods and seeds were followed until the different dates of the harvests carried out at 30, 40, 50, 60 and 80 days after fertilization. Fifteen (30) days mark the beginning of seed emergence inside the pods and eighty (80) days, the physiological state of dried seeds inside the pods. These details were used to obtain the five stages of maturity by species. At each stage of maturity, 300 pods were harvested. A total of 1500 pods (300 pods × 5 maturity stages) were harvested per species.

Anti-nutritional factors analysis

Determination of oxalate

The titration method as described by Day & Underwood (1986) was followed. 1 g of sample was weighed into 100 mL conical flask. 75 mL 3 MH_2SO_4were added and stirred for 1 h with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25 mL of the filtrate were then taken and titrated while hot against 0.05 M $KMnO_4$ solution until a faint pink colour persisted for at least 30 s. The oxalate content was then calculated by taking 1 mL of 0.05 M $KMnO_4$ as equivalent to 2.2 mg oxalate (Chinma and Igyor, 2007).

Determination of phytates

Phytate was extracted according to the procedure described by Mohammed *et al.*(1986). 1.0 g Sample was extracted with 3% tri-chloro acetic acid (TCA) at 37°C for 45 min. with simple shaking followed by centrifugation and extractation by using anion exchange column. The extracted phytate (0.2 mL) was mixed with 4.6 ml of distilled water and 0.2 mL of chromogenic solution and the tubes were heated in a water bath at 95°C for 30 min, and then were allowed to cool. The developed color was read at 830 nm against blank. Standard phytate solution was prepared by dissolving sodium phytate in distilled water to prepare different phytate concentrations as described above in the tested samples. The amount of phytate in the tested samples was expressed as mg phytate/100 g sample.

Determination of Saponins

This was done by the double solvent extraction gravimetric method (A.O.A.C., 1990). Two grams (2g) of the processed sample were mixed with 100ml of 20% aqueous ethanol solution and incubation for 12hour at a temperature of 55 °C with constant agitation. After that, the mixture was filtered through whatman No 42 grade of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30 minutes and the extracts weighed together.

The combined extract was reduced to about 40 mL by evaporation and then transferred to a separating funnel and equal volume (40 mL) of diethyl ether was added to it. After mixing well, there was partition and the upper layer was discarded while the lower aqueous layer was re-extracted with the ether after which its pH was reduced to 4.5 with drop wise addition of NaOH solution. Saponin in the extract was taken up in successive extraction with 5% of NaCl solution and evaporated with a water bath in a previously weighed evaporation dish. The saponin was then dried in an oven at 60 °C (to remove any residual solvent), cooled in a desiccator and re-weighed. The saponin content was calculated as shown below:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W}$$

Where W = Weight of sample used

Determination of L-Dopamine

The L-dopa content was quantified according to the following protocol described by Vadivel and Pugalenthi (2008): 1 g of seed powder was put in a test tube, to which 5 ml of 0.1 NHCL was added. The whole mixture was put in a boiling bath for 5 min. After cooling, 5 ml of ethanol was added and shaken for 10 minutes.

The contents were then centrifuged at 5000 x g for 10 min. The supernatant was collected and made up to a known volume. From this extract, the L-dopa content was determined by measuring the absorption of ultraviolet light at 282 nm in a spectrophotometer (brand: Elico; model: SL-177) using L-dopa (Sigma chemicals) as a standard.

Determination of trypsin inhibitory activity

Trypsin inhibitory activity was determined according to the methodology described by Vadivel and Pugalenthi (2008) by taking 2 g of the seed sample which was shaken in 30 ml of 0.1 M sodium phosphate buffer (pH 7.6) for 4 h. The contents were centrifuged at 12,000 rpm for 20 min at 0°C.

The clear supernatant obtained was dialysed against 0.05 M sodium phosphate buffer (pH 7.6). The resulting dialysed extract was used for the trypsin inhibitor assay procedure, which consisted of a reaction mixture of an appropriate amount of enzyme, inhibitor extract and buffer (0.1 M sodium phosphate buffer, pH 7.6).

The mixture was incubated for 10 min at 37°C and the reaction was initiated by adding 1 ml of 2% casein solution (substrate). The reaction was stopped exactly after 20 min by adding 3 ml of 5% trichloroacetic acid solution. After standing for 20 minutes at room temperature, the solution was centrifuged at 2000 x g for 10 minutes. The resulting clear supernatant was analysed for residual enzyme activity. A trypsin inhibitor unit (TIU) is defined as the number of trypsin units inhibited per 1 ml of the extract and expressed as TIU/kg DM.

Extraction of Organic Acids

The organic acids of each dried sample of Canavalia were extracted according Hasib *et al*., (2002) method by grinding (Waring Blendor, Polychimie, Abidjan, Côte d'Ivoire) in distilled water (1:10, w/v) and clarified by centrifuging at 4000 rpm for 30 minutes. The supernatant was first filtered through Whatmann 4 paper, then through 0.45 µm filter (Millipore; Sartorius AG, Goëttingen, Germany) and stored at −20˚C prior further use.

HPLC Analysis of Organic Acids

The separation of the organic acids was carried out as previously reported Kouassi *et al*., (2016) by using a system consisting of an analytical HPLC unit (Shimadzu Corporation, Japan) in conjunction with a column heating device set at 35˚C with the aid of an oven Meta Therm TM (Interchrom, France), with an ions exclusion column ICSep ICE ORH-801 (40 cm × 5 µm, In terchom, France). The system was also coupled to a pump (Shimadzu LC-6A Liquid Chromatograph), a UV detector (Shimadzu SPD-6A UV Spectrophotometric Detector) and an integrator (Shimadzu Chromatopac CR 6A).

Elution was carried out isocratically with sulphuric acid 0.04 N, at a solvent flow rate of de 0.6 mL/min and detection was performed at 210 nm. Organic acids in mushroom extracts were identified by comparing the retention times and spectral data obtained from standards under the same conditions. Quantitation was performed by comparing the peak areas with those of the respective external standards.

Statistical analysis

All chemical analyses and assays were performed in triplicate, unless otherwise indicated. Results were expressed as mean values ± standard deviation (SD). Analysis of variance (ANOVA) followed by Duncan's test was performed to test for differences between means by employing XLstat 2019 statistical software. Significance of differences was defined at

the 5% level ($P<0.05$). Principal component analysis (PCA) was also used in order to discover relationships between independent variables.

Results and Discussion

Antinutritional factors

Anti-nutritional factors such as phytates, oxalate, saponins, L-dopamine and trypsin inhibitors were determined in *Canavalia* seeds at different stages of maturity and shown in Table 1. The phytate content in mg/100g DM decreased with the maturity stage. It varied from 33.49 ± 0.06 to 100.32 ± 0.03 mg/100g DM, from 32.06 ± 0.06 to 93.63 ± 0.03 mg/100g DM and from 21.23 ± 0.06 to 104.48 ± 0.04 mg/100 g DM for *C. gladiata*, *C. rosea* and *C. ensiformis*, respectively. Oxalate levels also changed in the same direction as phytate levels for all three Canavalia species studied. The last stage of maturation had the lowest levels of oxalates at 257.15 ± 0.06, 141.48 ± 0.07 and 177.42 ± 0.06 mg/100g DM for *C. gladiata*, *C. rosea* and *C.ensiformis* respectively. The phytate and oxalate levels obtained for these three samples are lower than the results found by Abeké *et al.*, (2008) for Lablab purpureus and for Lentinus sajor-caju mushrooms in the work of Oyeleke *et al.*, (2017). These levels are well above the levels recommended by the World Health Organization (22.10 mg/100g DM for phytates and 105 mg/100g DM for oxalates). Consumption of these three seeds from Canavalia species could result in micronutrient losses (Onomi *et al*., 2004). Indeed, phytates form insoluble complexes with minerals, especially essential minerals such as iron, zinc, magnesium and calcium, exhibiting a "chelating effect" and making them biologically unavailable for absorption, which can lead to severe mineral deficiencies in humans and animals (Thompson, 1993). Phytic acid also forms a complex with proteins and decreases the solubility of proteins. As a result, phytates reduce the activities of major digestive enzymes, such as lipase, α-amylase, pepsin, trypsin and chymotrypsin (Thompson, 1993). It is also reported that phytic acid binds to starch through phosphate bonds (Muzquiz *et al*., 2012) and can affect starch digestibility by forming a ternary protein-phytate carbohydrate complex (Thompson *et al.*, 1986). As for oxalate, its decrease during ripening could be explained by the enzymatic degradation (decarboxylation) of oxalate by decarboxylases. Like phytates, oxalates are also present at higher levels in the three legumes at different stages of maturity. Under these conditions, they can be anti-nutritional factors that can make the nutrients they contain unavailable. Therefore, it would be wise to apply treatments to these Canavalia species to reduce the content of these elements for consumption. With regard to saponin content, overall, all three seed powders of Canavalia species had low saponin contents. They varied in a decreasing manner from stage S1 to stage S5 for all three species, from 4.21 ± 0.04 to 1.39 ± 0.05 for *C. gladiata*, from 3.33 ± 0.04 to 1.67 ± 0.01 for *C. rosea* and from 4.14 ± 0.04 to 1.09 ± 0.01 mg/100g DM for *C. ensiformis*. Saponin has the ability to inhibit the absorption of lipoproteins or high density cholesterol required in the diet, hence their anti-nutritional action. The L-Dopa content of the seeds of the Canavalia species studied ranged from 442.50 ± 5.38 to 1785.00 ± 6.68 mg/100g DM for all species. These contents are not only lower than the recommended limit of consumption of 2000 mg (Versteeg *et al*., 1996), but also than the contents obtained by Tuleun *et al*., in 2008 on the species *Mucuna cochinchinensis* (4700 to 5900 mg/100g DM). Although these values obtained are not very high, it was important to evaluate them because L-dopamine is a pharmaceutically active compound, used in the treatment of Parkinson's disease for its function as a neurotoxic agent (Bell and Janzen, 1971), but potentially toxic from a nutritional point of view (Pugalenthi *et al*., 2007). Indeed, from a nutritional point of view, it has been found that the consumption of legumes containing an abundance of L-Dopa could cause gastrointestinal complications through limited digestive resorption and inhibition of protein and starch digestibility through competition between L-Dopa and many amino acids at the level of a common transport when crossing the intestinal barrier (Prada *et al*., 1984).

The presence of trypsin inhibitors, which are protease inhibitors, in the diet leads to a considerable decrease in the digestibility of dietary proteins due to the formation of irreversible complexes of trypsin and trypsin inhibitors. This is because they suppress the proteolytic activity of digestive enzymes and thus reduce the digestibility of proteins (Liener, 1976). They form stable complexes with trypsin, obstructing their binding sites and disrupting the enzymatic action. For all legume species of the genus Canavalia studied at five maturity stages, the seed powder harvested at maturity stage S1 contained the highest levels of inhibited trypsin units, namely 295449.00 ± 3. 46 TIU/100g DM for *C. gladiata*, 2828.91 ± 3.46 TIU/100g DM for *C. rosea* and 2288.24 ± 3.84 TIU/100g DM for *C. ensiformis*, these results expressing a significant difference at the 5% threshold with a decreasing evolution from the S1 to the S5 stage of ripening. The results obtained are much lower than those obtained by Udedibie and Carlini in 1998 on the species *Mucuna pruriens* (1186500 TIU/100g seeds). The work of Doss *et al.*, in 2011 also revealed higher results than ours for *C. ensifor*mis seeds (37830 TIU/100g).

Organic acids composition

Organic acids are known as food components, involved in the expression of taste and flavor, and determine the quality and safety of food (Yüksel *et al*., 2017). They have important effects on foods, such as sensory, antioxidant and acidifying properties(Tormo and Izco, 2004). Eight organic acids in the three species of Canavalia seeds were analyzed quantitatively, namely tannic acid, oxalic acid, formic acid, lactic acid, acetic acid, fumaric acid, citric acid and tartaric acid, and their organic acid content was expressed as mg/kg DM, see Table 2. In samples of the three canavalia species, large differences in organic acid content were observed between mature stages of the same Canavalia and between different species. Oxalic acid and lactic acid were measured at all maturity stages of *C. gladiata* and *C. rosea*.

Table.1 Antinutritional factors content as a function of ripening stage of three Canavalia species in mg/100g MS

Species	Stages	Phytates	Oxalates	Saponin	L-Dopamine	Trypsin Inhibitors
***Canavalia gladiata* (CG)**	S1	100.32±0.03	692.35±0.02	4.21±0.04	1728.33±5.92	295449.00±3.46
	S2	95.20±0.03	634.21±0.02	3.64±0.03	1409.17±6.84	265855.12±3.92
	S3	74.20±0.08	541.22±0.06	1.93±0.02	903.33±3.14	196095.45±2.07
	S4	68.68±0.06	447.43±0.03	1.75±0.01	889.17±6.26	157501.92±3.63
	S5	33.49±0.06	257.15±0.06	1.39±0.05	449.17±5.54	98967.42±3.27
***Canavalia rosea* (CR)**	S1	93.63±0.03	525.51±0.03	3.33±0.04	1499.17±5.92	2828.91±3.46
	S2	90.06±0.06	432.24±0.03	2.58±0.04	1406.67±5.30	2368.62±3.15
	S3	88.43±0.01	297.49±0.06	2.32±0.04	986.67±3.36	1736.80±2.18
	S4	66.85±0.06	281.48±0.02	1.79±0.04	771.67±5.50	1434.66±3.25
	S5	32.06±0.06	141.48±0.07	1.67±0.01	442.50±5.38	866.71±3.19
***Canavalia ensiformis* (CE)**	S1	104.48±0.04	670.49±0.02	4.14±0.04	1785.00±6.68	2288.24±3.84
	S2	98.09±0.02	590.66±0.04	3.09±0.05	1514.17±5.84	1871.32±3.42
	S3	80.50±0.04	364.14±0.06	2.02±0.02	1293.33±6.46	1332.12±3.73
	S4	60.34±0.06	304.15±0.04	1.84±0.01	761.67±9.24	926.82±5.12
	S5	21.23±0.06	177.42±0.06	1.09±0.01	546.67±2.98	437.67±1.99

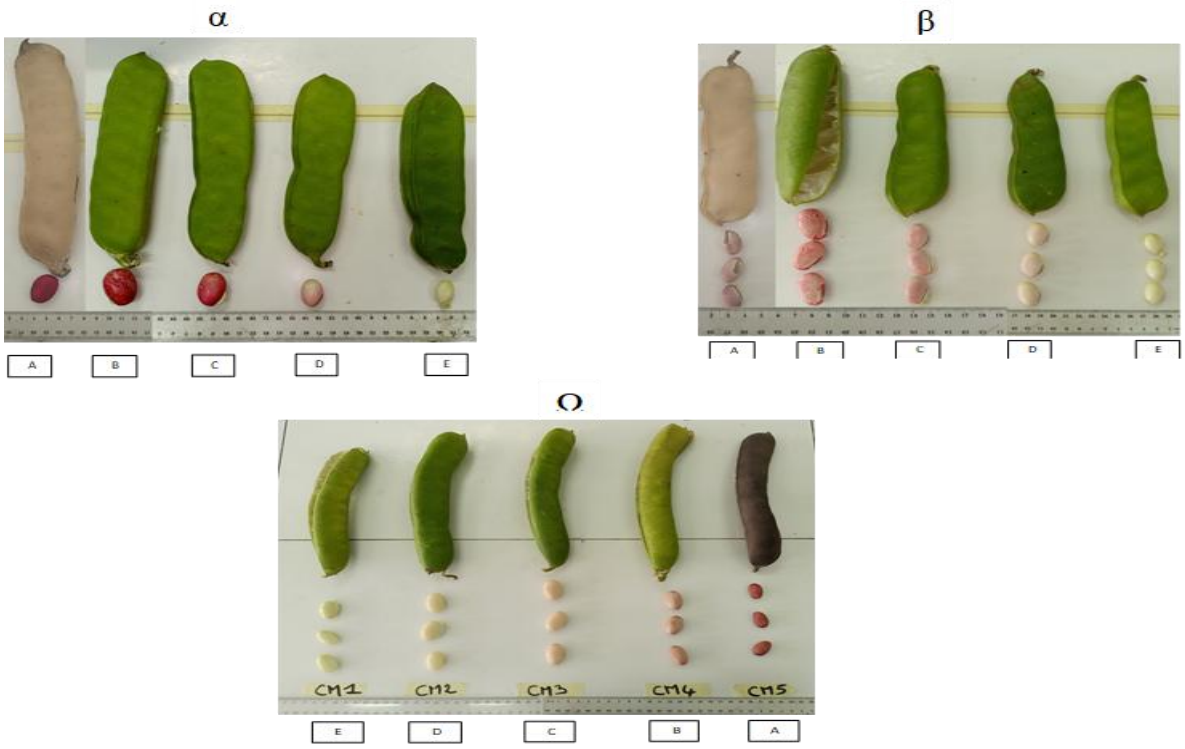
Table.2 Organic acids content as a function of ripening stage of three Canavalia species in mg/Kg MS

Species	Stages	Tannic ac.	Oxalic ac.	Formic ac.	Lactic ac.	Acétic ac.	Fumaric ac.	Citric ac.	Tartaric ac.
***Canavalia gladiata* (CG)**	S1	0.00±0.00	0.01± 0.00	0.00±0.00	0.05±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	S2	0.00±0.00	1.04± 0.01	0.00±0.00	0.43±0.01	0.00±0.00	0.09±0.00	0.00±0.00	0.00±0.00
	S3	0.00±0.00	1.47± 0.01	0.04±0.00	2.24±0.01	0.03±0.00	0.36±0.01	0.04±0.00	0.00±0.00
	S4	0.04±0.00	2.76± 0.01	0.06±0.00	3.68±0.01	3.06±0.01	0.38±0.01	0.09±0.00	0.01±0.00
	S5	0.00±0.00	2.13± 0.01	0.04±0.00	2.63±0.01	1.60±0.01	0.32±0.01	0.01±0.00	0.00±0.00
***Canavalia rosea* (CR)**	S1	0.00±0.00	0.01± 0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	S2	0.00±0.00	0.07± 0.00	0.00±0.00	0.03±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	S3	0.02±0.00	1.33± 0.01	0.00±0.00	0.29±0.01	1.08±0.01	0.24±0.01	0.00±0.00	0.00±0.00
	S4	0.68±0.01	1.54± 0.01	0.06±0.00	4.02±0.01	2.11±0.01	0.57±0.01	0.27±0.01	0.24±0.01
	S5	0.08±0.00	1.50± 0.01	0.01±0.00	2.58±0.01	1.93±0.01	0.53±0.01	0.19±0.01	0.00±0.00
***Canavalia ensiformis* (CE)**	S1	0.00±0.00	0.00± 0.00	0.00±0.00	0.00±0.00	0.05±0.00	0.02±0.00	0.00±0.00	0.00±0.00
	S2	0.00±0.00	0.00± 0.00	0.00±0.00	0.25±0.01	0.37±0.01	0.10±0.00	0.00±0.00	0.01±0.00
	S3	0.00±0.00	0.01± 0.00	0.01±0.00	0.68±0.01	1.49±0.01	0.22±0.01	0.09±0.00	1.03±0.01
	S4	0.04±0.00	0.08± 0.00	0.04±0.00	1.37±0.01	4.41±0.01	1.77±0.01	0.54±0.01	0.42±0.01
	S5	0.01±0.00	0.04± 0.00	0.01±0.00	1.05±0.01	1.78±0.01	0.88±0.01	0.37±0.01	1.13±0.01

Table.3 Pearson correlation coefficient for organic acids and antinutritional factors of Canavalia species sample

Variable	Phy	Oxa	Sap	L-Do	FlnTr	AcTan	AcOxa	AcFor	AcLac	AcAcé	AcFum	AcCit	AcTar
Phy	1,00000												
Oxa	0,84986	1,00000											
Sap	0,85049	0,87715	1,00000										
L-Do	0,92487	0,87958	0,92009	1,00000									
FlnTr	0,22311	0,54124	0,34856	0,20833	1,00000								
AcTan	-0,13970	-0,29352	-0,24015	-0,27025	-0,20111	1,00000							
AcOxa	-0,38048	-0,30849	-0,47386	-0,58843	0,28298	0,26969	1,00000						
AcFor	-0,43429	-0,34083	-0,60781	-0,59840	0,08289	0,53608	0,67957	1,00000					
AcLac	-0,59040	-0,49678	-0,67147	-0,72751	0,04531	0,60135	0,80470	0,90477	1,00000				
AcAcé	-0,58707	-0,64276	-0,66517	-0,66435	-0,28565	0,27429	0,32115	0,65426	0,58712	1,00000			
AcFum	-0,58278	-0,56940	-0,58589	-0,62274	-0,26764	0,18782	0,02371	0,48538	0,36279	0,85114	1,00000		
AcCit	-0,59392	-0,59486	-0,56013	-0,57250	-0,34432	0,33477	-0,08797	0,42802	0,35634	0,80606	0,95038	1,00000	
AcTar	-0,41893	-0,44049	-0,45143	-0,25666	-0,33410	0,02911	-0,35782	0,01853	-0,02522	0,35821	0,43164	0,56559	1,00000

Fig.1 Seeds and pods of three *Canavalia species* (α : *Canavalia gladiata* (CG), β: *Canavalia rosea* (CR), Ω : *Canavalia ensiformis* (CE)) at different stages of maturation



A = harvested at 80 days after fertilization (S5)
B = harvested at 60 days after fertilization (S4)
C = harvested at 50 days after fertilization (S3)
D = harvested at 40 days after fertilization (S2)
E = harvested at 30 days after fertilization (S1)

Fig.2 Diagram of the experimental device of the culture of the cultivar of *Canavalia gladiata* (CG), *Canavalia rosea* (CR) and *Canavalia ensiformis* (CE) created in Ahougnansou (Bouaké) : (Dimensions : 60 m x 46 m)

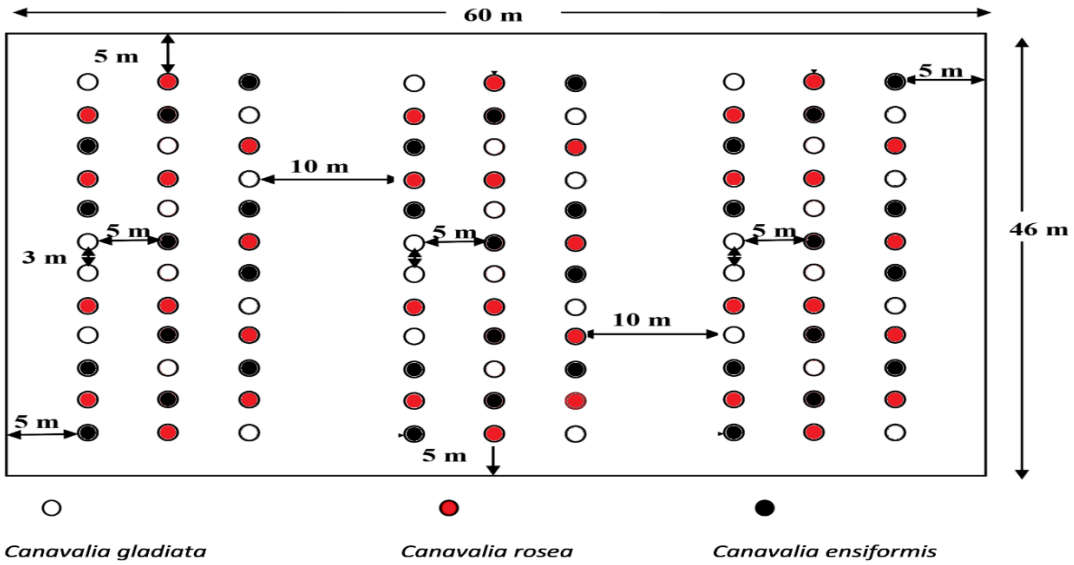


Fig.3 Circle of correlation of organic acids parameters and antinutrional factors of three Canavalia species samples at ripening stages

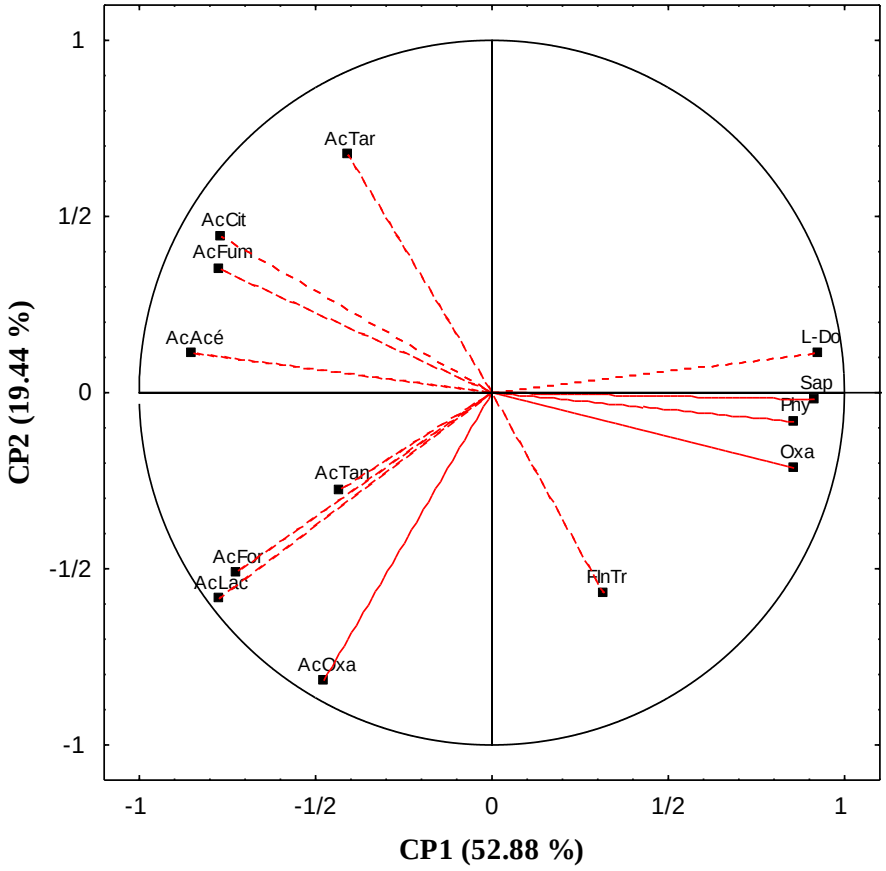
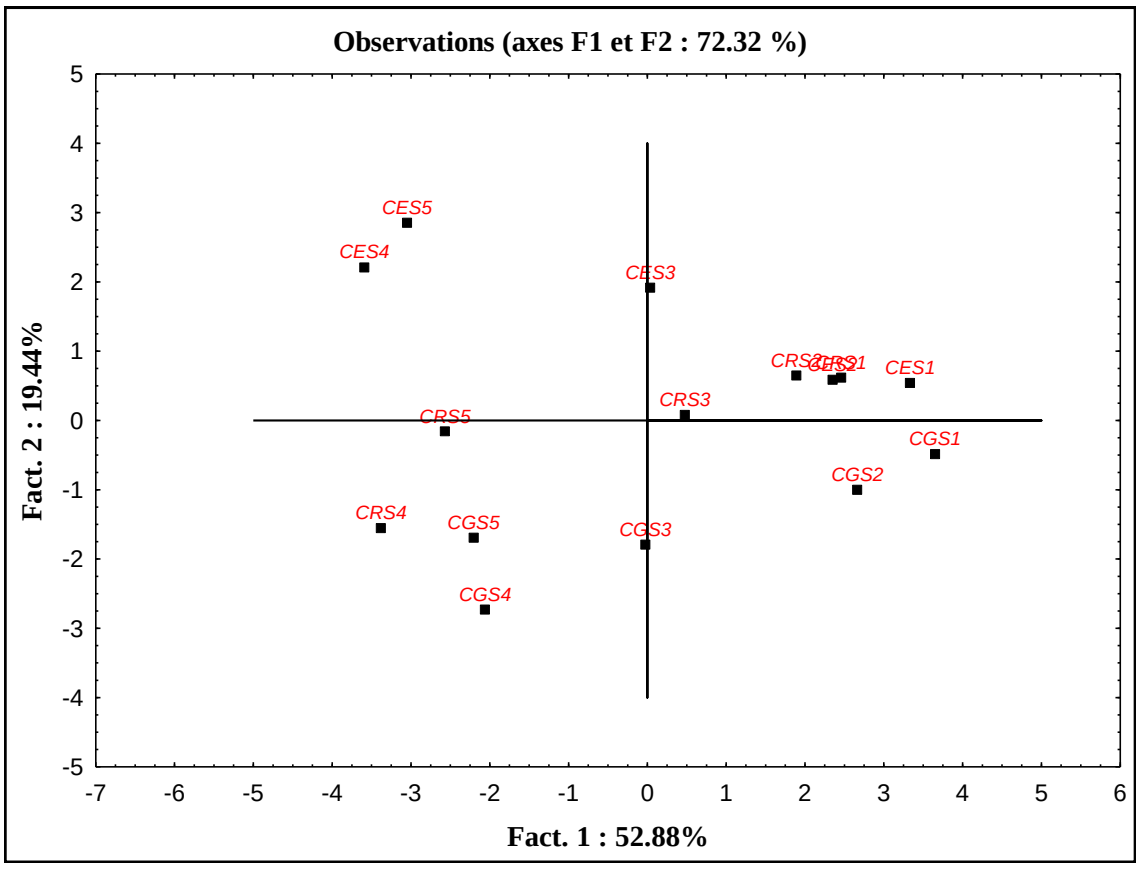


Fig.4 Sample plot of principal components 1 and 2 of Canavalia species at ripening stages



However, for *C. ensiformis*, only acetic and fumaric acids were measured at all stages of ripening. As for the others, they were determined at least at one stage of maturity of each Canavalia. However, all organic acids tested were elevated at maturity stage 4 for the species studied. Concerning the extracts of *C.gladiata* and C. *rosea*, it should be noted that in terms of organic acids, the predominant was lactic acid (0.05 to 3.68 mg/kg DM and 0.03 to 4.02 mg/kg DM), followed by acetic acid (0.03 to 3.06 mg/kg DM and 0.01 to 2.11 mg/kg DM) and oxalic acid (0.01 to 2.76 mg/kg DM and 0.01 to 1.54 mg/kg DM). As for *C. ensiformis*, the most important was acetic acid (0.05 to 4.41 mg/kg DM), followed by fumaric acid (0.02 to 1.77 mg/kg DM) and lactic acid (0.25 to 1.37 mg/kg DM). Lactic acid in foods is characterized as a natural, mild and persistent flavor enhancer (Jarrett, 2012). Acetic acid accompanies the metabolism of food, improving the quality and safety of the product. It also has an antiseptic effect, thus capable of prolonging the shelf life of the three Canavalia studied. Fumaric acid and oxalic acid have antimicrobial, antioxidant, antibacterial, anti-inflammatory and neuroprotective properties. Therefore, organic acids have a significant impact on the acceptability, nutrition, and stability of foods (Duru *et al*., 2019). The changes in organic acid content observed in this study suggest that maturity stage has a significant effect on organic acids in the three Canavalia studied.

Analysis PCA

Principal component analysis of the seeds of three Canavalia species by stage of maturation in relation to eight organic acids and five anti-nutritional factors revealed two axes explaining most of the variability, namely axes 1 (CP1) and 2 (CP2). These axes expressed 72.32% of the total variability

observed, with 52.88% and 19.44% for axes 1 and 2 respectively. The correlation circle (Figure 3) revealed three groups of many distinct variables. The parameters phytates, oxalates, saponins and L-dopamine contributed 10.64%, 10.72%, 12.14% and 12.46% respectively to the formation in the positive part of the axis (CP1). This axis is a good indicator of the composition of anti-nutritional factors in samples of Canavalia species at different stages of maturation. Citric acid, fumaric acid and acetic acid constituting the second group were expressed in the order of 8.57%, 8.69% and 10.50% in the negative part of the axis (CP1). Oxalic acid (26.52%), formic acid (10.37%) and lactic acid (13.55%) have a significant negative score in axis 2 (CP2). Otherwise, the correlation matrix (Table 3) showed that the first group consisting of the parameters Oxalates, phytates, saponins and L-dopamine were positively correlated with each other. This group of variables is negatively correlated (<0.05) with the other two groups of variables (positively correlated with each other at $p<0.05$) consisting of citric acid, fumaric acid and acetic acid for one; and oxalic acid, formic acid and lactic acid for the other. Principal component analysis allowed us to bring together samples from three species of Canavalia at different stages of maturation (Figure 4). The first group consisted of the sample at maturation stages S4 and S5 for all species. They generally expressed the highest organic acid score. The graph also showed that the second group consisted of the sample at maturation stages S1 and S2 for the species studied in general. These positions explain the high presence of anti-nutritional factors in these samples. The third group consisted of the sample at maturation stage S3. It showed intermediate levels of the different parameters studied.

The present study showed that the content of the anti-nutrient compounds phytates, oxalates, saponin, L-dopamine and trypsin inhibitors increases up to the S5 stage for the three Canavalia legume species studied. However, as these values are globally well above the standard safety limits, it would be wise for their consumption if these studied legumes underwent some treatment to reduce their anti-nutrient content. With regard to the organic acid content, at all stages of maturity, it should be noted that the preponderance for the extracts of *C. gladiata* and *C. rosea* is lactic acid followed by acetic acid and oxalic acid. As for *C. ensiformis*, the most preponderant is acetic acid followed by fumaric acid and lactic acid.

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