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Effect of maturation stage on the phytonutrient content of seeds of three species of *Canavalia* cultivated in the Gbèkê region (Côte d'Ivoire)

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Abstract

Legumes are plants with high nutritional values. It is necessary to control the nutritional quality of legumes at different stages of maturity for better popularization. This study aims to contribute to food security through determination of the phytochemical and antioxidant characteristics of the seeds of three legumes of the *Canavalia* genus according to their stage of maturity. Seeds of different stages collected in a field in the Gbèkê region served as biological material for this study. The seeds of three species at different stages of maturity: 30 days (S1), 40 days (S2), 50 days (S3), 60 days (S4) and 80 days (S5) after fertilization were collected and then dried in the sun and finally ground to obtain raw flour which was analyzed according to standard procedures. The results revealed that the contents of total phenolic compounds, flavonoids and tannins, suffered a decrease during seed maturation with the highest contents observed at the S1 stage. On the other hand, at the level of carotenoids, the peak was observed at stage S4 for all the seeds of the legumes studied. Overall, it appears that the best seed harvest stage for all the legumes studied is the S4 stage. This stage corresponds to the 60th day after fertilization of the flowers for species of the *Canavalia* genus. These seeds are potential sources of protein that can contribute to the fight against protein-energy deficiencies.

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Introduction

Acute food insecurity quadrupled between 2019 and 2022, going from ten million to nearly forty million people experiencing severe food and nutrition insecurity (CILSS, 2022). Around 870,000 people in Côte d'Ivoire, or 4 percent of the population, faced high levels of acute food and nutrition insecurity from March to May 2023 (CILSS *et al.*, 2023). The increase in the world population in the coming decades unfortunately risks making this situation worse. The state's ultimate ambition is therefore to fill the imbalance between population growth and food production. This is why the search for local products with high nutritional potential is sought.

In Côte d'Ivoire, several studies have shown that neglected minor plants can reduce the problems of food deficiencies and malnutrition (Tchumou *et al.*, 2018 ; Bediakon *et al.*, 2019 ; Koffi *et al.*, 2022). Indeed, the Ivorian forests are full of numerous edible spontaneous plants whose biochemical and nutritional composition remain unknown and yet, these plants could play a leading role in the fight against malnutrition and various nutritional deficiencies. Unfortunately, a large part of this resource is underexploited, even neglected (Padulosi and Hoeschle-Zeledon, 2004).

In particular, protein crops constitute an interesting source of proteins in addition to cereals for human food, either in the form of seeds or in the form of split products enriched with proteins (Boutin *et al.*, 2006). Among these plants with high nutritional values are legumes which are characterized by both high energy density and high nutritional density.

The main characteristic of legume seeds is their high protein content (20-50%) (Kouakou *et al.*, 2022a; Kouakou *et al.*, 2022b). Saber bean (*Canavalia ensiformis*), Saber pea (*Canavalia gladiata*) and caprit pea (*Canavalia rosea*) are also legumes grown in the Gbèkê region (Côte d'Ivoire) which are little valued but whose nutritional characteristics can be as interesting as those of traditional dried vegetables (Rémond and Walrand, 2017).

The work of Tchumou (2017) showed that the maturity stage influenced the physicochemical, mineral and phytochemical composition content of *Phaseolus lunatus* seeds. The maturity stage of the harvest could affect the nutritional quality of the seeds of the three *Canavalia* species. This work aims to monitor the evolution of phenolic and antioxidant compounds depending on the stage of maturity to better benefit from these legumes.

Material and methods

Biological material

The biological material used to carry out this study consisted of seeds of three species of legumes, *Canavalia gladiata*, *Canavalia rosea* and *Canavalia ensiformis* harvested at five harvest stages numbered from S1 to S5 (Fig. 1).

Determination of the phytochemical composition of seeds of three Canavalia species

Preparation of methanolic extracts of phenolic compounds

The extraction of methanolic extracts of phenolic compounds was carried out according to the modified method of Ribeiro *et al.* (2007). An amount of 10 g of powder was incubated at approximately 25 °C for 24 h in 50 mL of 80% (v/v) methanol with mechanical stirring. The mixtures obtained for each of the samples after incubation were centrifuged separately at 4000 rpm for 5 min with a refrigerated centrifuge (Refrigerated centrifugal TGL-16). The supernatants were collected and the pellets of each sample were extracted twice in succession under the same conditions. The different methanolic extracts obtained were concentrated by evaporation of methanol at 35 °C until obtaining 50 mL of solution, using a rotary evaporator (rotary evaporator HEILDOLPH Laborata 4003 Control, Schwabach, Germany). The different solutions obtained were used for the different analyses.

Determination of total phenolic compound

The contents of total phenolic compounds or polyphenols in the different powder samples were determined according to the method of Singleton *et*

al. (1999) using Folin-ciocalteu's reagent. The contents of total phenolic compounds were expressed in mg gallic acid equivalent (GAE)/100 g dry weight (DW).

Determination of total flavonoids

Total flavonoid contents were evaluated using the method described by Meda *et al.* (2005) using aluminum chloride. The results were expressed as mg quercetin equivalent (QE)/100 g dry weight (DW).

Determination of total tannins

The total tannin contents were determined according to the method used by Bainbridge *et al.* (1996) using vanillin reagent. The results were expressed as mg tannic acid equivalent (mgTAE) / 100 g dry weight (DW).

Separation and identification of phenolic compound

The methanolic extracts of phenolic compound from the different legume seed samples prepared previously were diluted in 100 mL of distilled water. Using an analytical HPLC unit (Shimadzu Corporation, Japan) equipped with a binary pump (LC-6A) coupled to a UV-VIS detector (SPD-6A) each sample was analyzed. The phenolic compounds were separated on an ICSep ICE ORH-801 column (length 25 cm) at a temperature set at 30°C. The phenolic compounds of the methanolic extract of the legume samples were identified by comparison of their retention times with those obtained by the injection of the standard solution comprising the standard phenolic compounds under the same conditions.

Quantification of phenolic compound

The phenolic compounds of the different samples were quantified by estimating their concentrations expressed in mg/kg of dry weight from the average of the peak areas of each of the standard phenolic compounds. Thus, the concentration of a phenolic compound x identified in a sample was calculated using the equation described by AOAC (2012).

Dosage of carotenoids

Total carotenoids from the different legume seed samples were extracted according to the method described by Rodriguez-Amaya and Kimura (2004). These extracts were used for the determination of total carotenoids, anthocyanins and lycopene.

The total carotenoid contents of the seed powder samples of the three legumes collected at different stages of maturity were estimated by the method used by Carvalho *et al.* (2012).

Dosage of anthocyanins

Total anthocyanins were defined according to the differential pH method of Giusti and Wrolstad (2001) thanks to the fact that the structure of anthocyanins undergoes a reversible transformation during a change in pH which is manifested by different absorption spectra.

Determination of lycopene content

The lycopene contents of the seed powders of the three legumes were determined according to the method of Nagata and Yamashita (1992).

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 ($P<0.05$).

Results and discussion

Phenolic compound contents of the seeds of three species of legumes

The phenolic analysis of legume seeds harvested at different stages of maturity shows a significant decrease ($P < 0.05$) in the level of phenolic compounds such as total polyphenols, tannins and flavonoids (Table 1).

The total polyphenol contents of the methanolic extracts of the seeds of the species studied are significantly different at the 5% threshold, and

experienced a progressive decrease during maturation. The values of these contents oscillate between 120.52 and 77.26 mg / 100 g DW; 90.80 and 70.91 mg/100 g DW and between 120.52 and 38.42 mg/100 g DW, respectively for *Canavalia gladiata*, *Canavalia rosea* and *Canavalia ensiformis*.

Table 1. Contents of some polyphenols of three species of legumes harvested at different stages of maturity.

Species	Maturity stage	Total polyphenols (mg/100g DW)	Tannins (mg/100g DW)	Flavonoids (mg/100g DW)
Canavalia gladiata	S1	120,52 ± 0,03 ^{p}	59,32 ± 0,18 ^{n}	12,13 ± 0,03 ^{n}
	S2	104,12 ± 0,02 ^{m}	48,52 ± 0,19 ^{m}	11,03 ± 0,02 ^{k}
	S3	90,31 ± 0,03 ^{h}	43,95 ± 0,180 ^{k}	10,42 ± 0,03 ^{j}
	S4	81,75 ± 0,29 ^{g}	36,30 ± 0,16 ^{h}	2,89 ± 0,29 ^{c}
	S5	77,26 ± 0,29 ^{e}	33,07 ± 0,26 ^{fg}	0,00 ± 0,00 ^{a}
Canavalia rosea	S1	90,80 ± 0,04 ^{i}	40,74 ± 0,18 ^{j}	10,43 ± 0,03 ^{j}
	S2	81,41 ± 0,02 ^{g}	38,81 ± 0,18 ^{i}	9,29 ± 0,02 ^{h}
	S3	80,63 ± 0,03 ^{f}	32,71 ± 0,20 ^{f}	5,13 ± 0,03 ^{e}
	S4	77,29 ± 0,29 ^{e}	26,32 ± 0,23 ^{d}	4,76 ± 0,29 ^{d}
	S5	70,91 ± 0,29 ^{c}	21,16 ± 0,11 ^{a}	0,00 ± 0,00 ^{a}
Canavalia ensiformis	S1	120,52 ± 0,03 ^{p}	33,63 ± 0,18 ^{g}	13,09 ± 0,02 ^{q}
	S2	92,28 ± 0,03 ^{j}	28,85 ± 0,180 ^{e}	12,62 ± 0,03 ^{p}
	S3	73,03 ± 0,02 ^{d}	26,14 ± 0,05 ^{d}	11,63 ± 0,03 ^{m}
	S4	64,07 ± 0,29 ^{b}	24,48 ± 0,10 ^{c}	7,58 ± 0,29 ^{f}
	S5	38,42 ± 0,29 ^{a}	23,73 ± 0,29 ^{b}	2,27 ± 0,29 ^{b}

The means ± standard deviation, assigned different lowercase letters in the same column for each parameter, are significantly different at $P < 0.05$ according to the Duncan test.

As for flavonoids, the results also revealed a significant decrease at the 5% threshold in the methanolic extracts of the seeds of the legumes studied during maturation. However, these values remained low for all seeds of the species studied. The flavonoid contents were the highest, obtained at stage S1, were respectively 12.13 mg / 100 g DW; 10.43 mg / 100 g DW and 13.09 mg / 100 g DW for *Canavalia gladiata*, *Canavalia rosea* and *Canavalia ensiformis*. Regarding the tannin content, it appears overall that these contents also decreased significantly at the threshold of 5% from the first maturity stage defined S1 to the last stage S5. They were included in the intervals of 59.32 to 33.07 mg / 100 g DW; 40.74 to 21.16 mg / 100 g DW and 33.63 to 23.73 mg / 100 g DW respectively for the species *Canavalia gladiata*, *Canavalia rosea* and *Canavalia ensiformis*.

The qualitative and quantitative analysis of phytochemical compounds in legume seeds has always been of great interest to researchers due to their bioactive potential. Regarding total phenolic compounds, flavonoids and gallic tannins, the significant decrease during seed maturation was also observed by some authors during a similar study on legume beans (*Vicia faba* L.) (Benchikh *et al.*, 2014; Boukhanouf *et al.*, 2016) and *Pistachia lentiscus* (Ballistreri *et al.*, 2009). Indeed, the work of the authors indicated that the contents of total polyphenols, tannins and flavonoids were higher at the early stages of ripening and decreased significantly throughout the growth process. Holderbaum *et al.* (2014) hypothesized that legumes synthesize more polyphenolic compounds from the start of the development of their still very fragile (immature) fruits (seeds) in order to protect them against pathogens, predators and abiotic stress.

The depletion of phenolic content during maturation would therefore be caused by the conversion of

soluble phenolic compounds into insoluble phenolic compounds, which are linked to cell wall polysaccharides and/or the oxidation of phenolic compounds by polyphenol oxidases. However, Persic *et al.* (2018) as well as Gbotognon (2021), reported an increase in the polyphenol content, during ripening, of hazelnuts (*Corylus avellana*) and mushrooms of the *Russula* genus respectively. Salamatullah *et al.* (2021) reported that the contents of phenolic compounds in peanut kernels (*Arachis hypogaea* L.) oscillated between decrease and increase during ripening. Quantitatively, the results on the contents of phenolic compounds are slightly lower than those of "voandzou", reported by Yagoub and Abdalla (2007). The total polyphenol contents reported by these authors were between 94 to 217 mg/100 g of DW. However, the contents obtained in the present study were very high compared to the values reported by Mbaiogaou *et al.* (2013) on seeds of other legume species (0.033 to 0.083 g / 100 g DW). These results confirm literature data indicating that berries, fruits and the seed coats of colored seeds and vegetables are rich in polyphenols (Peschel *et al.*, 2006; Mbaiogaou *et al.*, 2013). According to Hegedűsová (2015), variation in polyphenolic content can be attributed to genetic factors, variety, agricultural factors, growing conditions, ecological and climatic factors, maturity level at the time of harvest and places of cultivation and storage after harvest. The difference in total polyphenol content could also be due to the geographical region where the plant grows and even to the method of extraction of these compounds.

Table 2. Phenolic compound contents of seeds of three legume species harvested at different stages of maturity.

Species	Stages	Tanic acid (mg/Kg DW)	Galic acid (mg/Kg DW)	Coumaric acid (mg/Kg DW)	Catechins (mg/Kg DW)	Caffeine (mg/Kg DW)	Sodium cinnamate (mg/Kg DW)	Quercetin (mg/Kg DW)
Canavalia gladiata	S1	0.10 ± 0.01 ^{g}	0.09 ± 0.01 ^{f}	0.10 ± 0.01 ^{hi}	0.36 ± 0.01 ^{i}	0.09 ± 0.01 ^{e}	0.06 ± 0.01 ^{d}	0.07 ± 0.01 ^{efg}
	S2	0.07 ± 0.01 ^{def}	0.06 ±0.01 ^{de}	0.09 ± 0.01 ^{hi}	0.19 ± 0.01 ^{g}	0.07 ± 0.01 ^{de}	0.04 ± 0.01 ^{bcd}	0.05 ± 0.01 ^{cde}
	S3	0.05 ± 0.01 ^{bcd}	0.06 ± 0.01 ^{cd}	0.06 ± 0.01 ^{efg}	0.13 ± 0.01 ^{e}	0.06 ± 0.01 ^{cd}	0.03 ± 0.01 ^{bc}	0.04 ± 0.01 ^{bcd}
	S4	0.04 ± 0.01 ^{abc}	0.04 ±0.01 ^{bc}	0.05 ± 0.01 ^{def}	0.12 ± 0.01 ^{de}	0.04 ± 0.01 ^{abc}	0.03 ± 0.01 ^{bc}	0.03 ± 0.01 ^{bc}
	S5	0.02 ± 0.01 ^{a}	0.03 ± 0.01 ^{bc}	-	0.10 ± 0.01 ^{cd}	0.03 ± 0.01 ^{ab}	0.02 ± 0.01 ^{b}	0.02 ± 0.01 ^{b}
Canavalia rosea	S1	0.09 ± 0.01 ^{fg}	0.06 ± 0.01 ^{de}	0.06 ± 0.01 ^{fg}	0.04 ± 0.01 ^{b}	0.77 ± 0.01 ^{k}	0.77 ± 0.01 ^{j}	0.08 ± 0.01 ^{fg}
	S2	0.08 ± 0.01 ^{efg}	0.04 ± 0.01 ^{bcd}	0.05 ± 0.01 ^{cdef}	0.03 ±0.01 ^{b}	0.50 ± 0.01 ^{j}	0.70 ± 0.01 ^{i}	0.04 ± 0.01 ^{bcd}
	S3	0.06 ± 0.01 ^{cde}	0.03 ± 0.01 ^{bc}	0.02 ± 0.01 ^{bc}	0.03 ± 0.01 ^{b}	0.30 ± 0.01 ^{i}	0.34 ± 0.01 ^{h}	0.02 ± 0.01 ^{b}
	S4	0.04 ± 0.01 ^{abc}	0.02 ± 0.01 ^{b}	0.02 ± 0.01 ^{b}	0.02 ± 0.01 ^{ab}	0.22 ± 0.01 ^{h}	0.18 ± 0.01 ^{g}	-
	S5	0.03 ± 0.01 ^{ab}	-	-	-	0.17 ± 0.01 ^{g}	0.12 ± 0.01 ^{f}	-
Canavalia ensiformis	S1	0.07 ± 0.01 ^{def}	0.09 ± 0.01 ^{f}	0.11 ± 0.01 ^{hi}	0.21 ± 0.01 ^{g}	0.23 ± 0.01 ^{h}	0.04 ± 0.01 ^{bcd}	0.06 ± 0.01 ^{df}
	S2	0.05 ± 0.01 ^{bcd}	0.08 ± 0.01 ^{ef}	0.08 ± 0.01 ^{gh}	0.16 ± 0.01 ^{f}	0.18 ± 0.01 ^{g}	0.02 ± 0.01 ^{b}	0.39 ± 0.01 ^{h}
	S3	0.05 ± 0.01 ^{bcd}	0.06 ± 0.01 ^{de}	0.06 ± 0.01 ^{ef}	0.12 ±0.01 ^{de}	0.14 ± 0.01 ^{f}	-	0.04 ± 0.01 ^{bcd}
	S4	0.04 ± 0.01 ^{abc}	0.04 ± 0.01 ^{bcd}	0.04 ± 0.01 ^{bcde}	0.08 ± 0.01 ^{c}	0.13 ± 0.01 ^{f}	-	0.02 ± 0.01 ^{b}
	S5	0.02 ± 0.01 ^{a}	0.02 ± 0.01 ^{b}	0.03 ± 0.01 ^{bcd}	0.03 ± 0.01 ^{b}	0.06 ± 0.01 ^{cd}	-	-

The means ± standard deviation, assigned different lowercase letters in the same column for each parameter, are significantly different at $P < 0.05$ depending on the test of Duncan; - : not determined.

Identification and quantitative analysis of phenolic compounds

The identification and quantitative analysis of the phenolic compounds present in the methanolic extracts of the seeds of the three species of *Canavalia* collected at different stages of maturity (Table 2) made it possible to note the presence of all the phenolic compounds at all stages of maturity for the species *Canavalia gladiata* except coumaric acid which was only present at stages S1 and S4, then absent at stage S5. On the other hand, the two other *Canavalia* species studied recorded the absence of at

least two phenolic compounds at certain stages of maturity. Indeed, in addition to not having been detected at stage S5 like gallic acid and coumaric acid, quercetin was also absent at stage S4 and S5 of extracts of the *Canavalia rosea* species. In *Canavalia ensiformis* extracts, quercetin was not detected at the S5 stage and cinnamate was only present at the S1 and S2 maturity stages.

Table 3. Contents of total carotenoids, anthocyanins and lycopenes of three species of legumes harvested at different stages of maturity.

Species	Maturity stage	Total carotenoids (mg/100 DW)	Anthocyanins (mg/100 DW)	Lycopènes (mg/100 DW)
Canavalia gladiata (CG)	S1	9.03 ± 0.05 d	1.93 ± 0.05 e	3.12 ± 0.05 f
	S2	12.33 ± 0.05 h	2.29 ± 0.05 g	4.29 ± 0.05 i
	S3	15.30 ± 0.57 k	3.64 ± 0.04 n	5.90 ± 0.04 q
	S4	20.30 ± 0.14 p	5.11 ± 0.01 t	7.16 ± 0.25 t
	S5	15.73 ± 0.43 m	4.07 ± 0.03 q	5.60 ± 0.06 n
Canavalia Rosea (CR)	S1	8.26 ± 0.05 b	1.56 ± 0.05 d	1.97 ± 0.05 c
	S2	10.42 ± 0.05 g	2.07 ± 0.05 f	2.11 ± 0.05 d
	S3	12.05 ± 0.03 h	3.20 ± 0.02 j	4.02 ± 0.03 h
	S4	15.94 ± 0.02 m	5.53 ± 0.02 u	6.13 ± 0.04 r
	S5	13.44 ± 0.04 j	3.34 ± 0.02 k	5.04 ± 0.04 k
Canavalia ensiformis (CE)	S1	9.39 ± 0.05 e	1.07 ± 0.05 b	2.97± 0.05 e
	S2	10.02 ± 0.05 f	2.13 ± 0.05 f	4.04 ± 0.05 h
	S3	12.70 ± 0.01 i	3.42 ± 0.03 m	4.83 ± 0.03 j
	S4	16.41 ± 0.02 n	5.02 ± 0.03 s	6.65 ± 0.02 s
	S5	15.98 ± 0.01 m	3.06 ± 0.01 i	5.41 ± 0.01 m

The means ± standard deviation, assigned different lowercase letters in the same column for each parameter, are significantly different at p< 0.05 depending on the test of Duncan.

The results showed significant variations at the 5% threshold for each phenolic compound from one maturity stage to another at the level of the methanolic extracts of the seeds of the three legume species studied. The different contents of all the phenolic compounds detected evolved in a decreasing manner during the maturation of the legume seeds. Generally, catechin was the most predominant in the species *Canavalia gladiata* with contents which oscillated respectively from 0.10 to 0.36 mg/Kg DW. As for *Canavalia rosea* and *Canavalia ensiformis*, caffeine was the most abundant phenolic compound with contents respectively between 0.17 and 0.77 and between 0.06 and 0.23 mg / Kg DW. For all species, stage S1 was the stage with the highest contents of phenolic compounds and stage S5, that of the lowest.

In this study, seven phenolic compounds were monitored individually during the maturation of the seeds of the three legumes studied. Large significant variations were observed during the maturation of these seeds. Within the species itself, certain compounds were present throughout the maturation of the seed, while others were only present at the beginning of maturation before disappearing during the final stages of seed development. All these variations could be due to structural instabilities of some of these phenolic acids (Friedman and Jürgens, 2000). The phenomena of interconversion between certain phenolic compounds or even certain metabolic syntheses, during the maturation of legumes, could also be responsible for this variability (Katsuragi *et al.*, 2010).

Overall, tanic acid, gallic acid, coumaric acid, catechin, caffeine, sodium cinnamate and quercetin have the maximum of their concentration at the beginning of seed maturation, i.e. at the S1 stage of all legumes. For all seeds, phenolic compound concentrations declined throughout seed growth. This could be explained by the fact that these compounds, being powerful antioxidants, must have been used in the neutralization of reactive oxygen species and free radicals generated during cell development during maturation. Indeed, phenolic acids such as gallic acid,

quercetin and coumaric acid are recognized as key antioxidants in the neutralization of reactive oxygen species in biological systems (Aruoma *et al.*, 1993; Kim, 2007 ; Srinivasan *et al.*, 2007). Also, catechin is a compound with antioxidant and anti-radical properties (Pekkarinen *et al.*, 1999; Azevedo *et al.*, 2013).

Total carotenoids, anthocyanins and lycopenes contents of seeds of the three species of legumes

The contents of total carotenoids, anthocyanins and lycopenes in the seeds of the three legume species depending on the stage of harvest maturity are presented in Table 3. Total carotenoids underwent a significant increase at the threshold of 5% from stage S1 to stage S4 with peaks correspond to 20.30; 15.94 and 16.41 mg/100 g DW respectively for *Canavalia gladiata*, *Canavalia rosea* and *Canavalia ensiformis*. A decrease in total carotenoid levels was observed from stage S4 to stage S5.

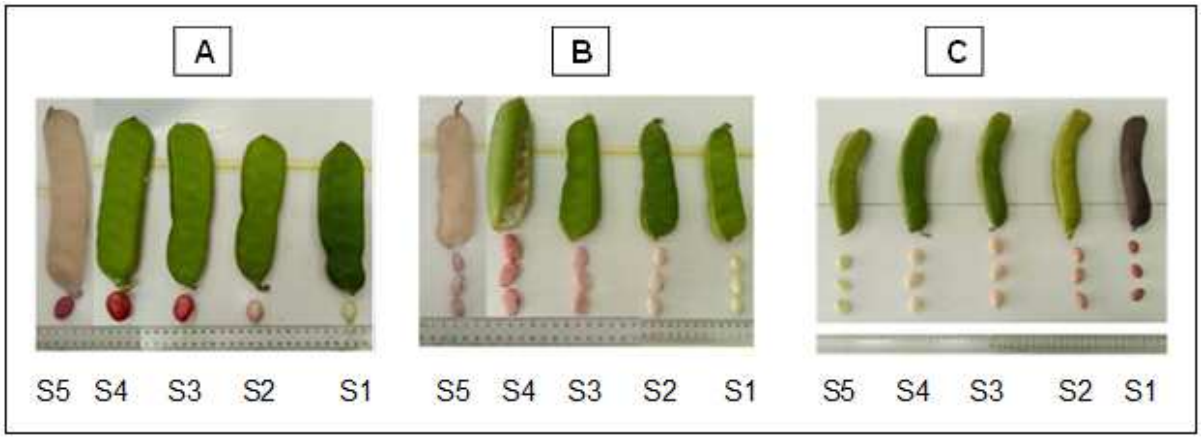

Fig. 1. Presentation of the seeds of the three legumes studied
A = pods and seeds of *Canavalia gladiata*
B = pods and seeds of *Canavalia rosea*
C = pods and seeds of *Canavalia ensiformis*
S1 = harvested 30 days; S2 = harvested 40 days; S3= harvested 50 days;
S4= harvested 60 days and S5= harvested 80 days after fertilization.

Anthocyanins experienced the same evolution as those of total carotenoids. In indeed, for each of the legume species, the anthocyanin content experienced an increase significant increase at the 5% threshold from stage S1 to stage S4. Peaks of 5.11; 5.53 and 5.02 mg/100 g DW were achieved for *Canavalia gladiata*, *Canavalia rosea* and *Canavalia ensiformis* respectively. Also, a decrease in the level of anthocyanins was observed from the S4 stage (Table 3).

The lycopene contents were also estimated at each stage of maturity of harvesting legumes in order to monitor their development during maturation. He was observed the same trends as for carotenoids and anthocyanins for all species of legumes studied. The highest values were obtained at stage S4 of each species and correspond to 7.16 mg / 100 g DW (*Canavalia gladiata*); 6.13 mg / 100 g DW (*Canavalia rosea*) and 6.65 mg / 100 g DW (*Canavalia ensiformis*). At stage S5, a decrease in lycopene levels was also observed (Table 3).

Just like phenolic compounds, the evolution of the contents of total carotenoids, anthocyanins and lycopenes during the maturation of the seeds of the legumes studied was from the lowest contents to the highest. The highest contents were therefore observed at the S4 stages of maturity. The carotenoids which constitute provitamins A are powerful natural antioxidants found in fruits and vegetables (Barros *et al.*, 2008). The peaks of total carotenoids,

anthocyanins and lycopenes obtained at stage S4 could be explained by the fact that the biosynthesis of these pigments intensifies until maturity to compensate for the loss of antioxidant compounds (phenolic compounds and flavonoids) entering the metabolism to be used for the neutralization of reactive oxygen species and free radicals generated.

Conclusion

At the end of this study, it appears that the phytochemical parameters of these legumes were significantly influenced by the maturation process. The results indicated that the contents of phenolic compounds and antioxidants which represent the bioactive compounds of legumes were reduced during ripening. Even if the bioactive elements are found at their maximum at the S1 stage of development; the analyzes showed that the seeds of legumes harvested at the S4 stage of maturity contained very strong antioxidants which could have good antibacterial potential. As a result, they would be equally beneficial in combating the damage caused by free radicals and oxidative stress on the body and the health of populations. Thus, for the consumption of the seeds of these three *Canavalia* species, the choice of the appropriate harvest maturity stage is the S4 stage, for all the species studied.

Conflict of interest

There are no conflicts of interest declared by any of the authors.

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