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Some phytochemicals and Antioxidant activity of the *Capsicum annuum* type « Ahalimancou », pepper cultivated in the East of Côte d'Ivoire

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Abstract

This study was carried out to assess the levels of certain phytochemicals and the antioxidant activity of "Ahalimancou" type pepper (*Capsicum annuum*) consumed in the east of Côte d'Ivoire.The pepper were harvested at the green, yellow, orange and red color stages of ripening. Then, the contents of total phenols, carotenoids, capsaicinoids and antioxidant activity were determined in the extract of the peppers. These parameters were determined respectively by spectrometry, HPLC and by the DPPH test. The results of the analysis of *Capsicum annuum* peppers of the "Ahalimancou" type showed that the yield of the extraction of phytochemicals evolves until the peppers ripen, with a higher value in red fruits. In addition, pepper fruits total phenol contents increase from 487.93 ± 9.52 mg GAE / 100 g DM (the yellow stage) to 617.20 ± 7.02 mg GAE / 100 g DM (orange stage). Also, carotenoids contents evolved from 5.06 ± 0.23 mg / 100g DM (green stage) to 55.90 ± 3.56 mg / 100 g DM (red stage). Likewise, the capsaicinoid contents increase with the pepper's fruits ripening. The capsaicin content increased from 792.29 ± 2.42 mg / 100 (green fruit) to 1175.63 ± 2.01 mg / 100 g (red fruit), that of dihydrocapsaicin increased from 218.70 ± 5 .65 (green stage) to 423.60 ± 4.63 mg / 100 g DM (red stage) and then, total capsaicinoids varied from 1167.10 ± 3.06 mg / 100g DM (green stage) to 1599.20 mg / 100 g DM (red stage). In the case of antioxidant activity, from 12 µg / mL of orange and red pepper extracts, more than 80 % inhibition of the DPPH radical is observed. These results underline the importance of Capsicum annuum "Ahalimancou" peppers in the contribution of human health.

Keywords: Pepper, *Capsicum annuum*, capsaicinoids, total phenols, carotenoids, antioxidant activity

INTRODUCTION

The pepper (*Capsicum annuum*) is a vegetable plant which fruits, widely used in the world, are very important for human feeding because of its bioactive compounds richness (Caporaso *et al*., 2013). In Côte d'Ivoire, the marketing of pepper fruits is a fully-fledged activity for rural and even urban populations, by offering opportunities to improve their incomes (Fondio *et al*., 2015). In addition, pepper remains a very important agricultural crop, not only because of its economic importance but, especially for its nutritional and medicinal value. Indeed, pepper is an important source of phytochemicals or secondary metabolites (Maji *et al*., 2016). All of these phytochemicals induced high antioxidant capacity in pepper fruits because it represents an excellent source of carotenoids (Alam *et al*., 2018). According to Fiedor *et al*. (2014), carotenoid compounds are most efficiently reacting with peroxyl radicals that formed under oxidative conditions and they play a crucial role in the protection of the cellular membrane and lipoprotein against oxidative damage. Pepper is also an excellent source of capsaicinoids which are found to exert multiple pharmacological and physiological effects, including the activities of analgesia, anticancer, anti-inflammation, antioxidant, and anti-obesity (Zhang *et al*., 2013). Capsaicin and other members of the capsaicinoids group produce a large number of physiological and pharmacological effects on the gastrointestinal tract, the cardiovascular and respiratory system as well as the sensory and thermoregulation systems (li *et al.*, 2019). Capsaicin has been used as an analgesic against arthritis pain and inflammation (Deal *et al.*, 1991). According to Moore *et al.* (2003), it shows anticancer effect and for Szolcsanyi (2004), it is active against neurogenic inflammation (burning and stinging of hands, mouth, and eyes).

Phenols are compounds that have the ability to destroy radicals because they contain hydroxyl groups. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals; hence, they play an important role in antioxidant activity (De Gaulejac *et al.*, 1999). Notwithstanding the proven nutritional and medicinal potential of *Capsicum annuum* peppers, "Ahalimancou" type remains a problem in its large-scale use. In fact, there is no scientific work to promote the above variety in Côte d'Ivoire. Even the studies by Kouassi *et al.*, (2012) on the knowledge and use of the type of pepper (*Capsicum*) cultivated in Côte d'Ivoire did not mention the "Ahalimancou" type. In addition, no scientific work deals with the phytochemical composition and antioxidant activity of the "Ahalimancou" type. Because of this, the ignorance of *Capsicum annuum* pepper type "Ahalimancou" is used locally. As a result, this type of pepper is on the verge of extinction in Côte d'Ivoire.

This study is the first to highlight certain phytochemicals and the antioxidant activity of *Capsicum annum* peppers, type "Ahalimancou" harvested at different stages of ripening consumed in eastern Côte d'Ivoire.

MATERIAL AND METHODS

2.1. Material

2.1.1. Biological material

The fruits (in green, yellow, orange and red colored) of pepper (*Capsicum annuum*) type "Ahalimancou" were used in this study. These fruits were harvested from an experimental plot at the Nangui Abrogoua University (Abidjan, Côte d'Ivoire).

2.1.2. Chemical products

The analysis reagents used in this study were obtained from different manufacturers. Those purchased from Polychimie (Côte d'Ivoire) are: 95% methanol, acetone, petroleum ether, Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyle (DPPH) and sodium carbonate (Na2CO3). The standards namely gallic acid, quercetin, ascorbic acid and β-carotene are from Sigma Aldrich (Germany).

2.2. Methods

2.2.1 Extraction of phytochemicals

For 2 hours, 5 g of fresh pepper fruits were macerated into 200 ml of 80 % methanol, with magnetic stirring. The operation was repeated 3 times in succession until the plant matrix was exhausted. After filtration on Whatman filter paper (outline: grade 1V; diameter: 270 mm), the macerates were combined and concentrated in a rotary evaporator (Rotavapor R-300, BUCHI, Switzerland) at 40 ° C to provide the crude extract methanol.
The extraction yield (Y) was determined by the following mathematical formula:

$$Y\ (\%) = \frac{Weight\ of\ extract}{Weight\ of\ pepper\ fruits} \times 100$$

2.2.3 Determination of total phenols

The method of **Singleton et al. (1999)** using the Folin-Ciocalteu reagent allowed the determination of total phenols. To 1 mL of methanolic fresh fruit extract contained in a test tube was added 1 mL of Folin-Ciocalteu reagent. The tube was left to stand for 3 min in the dark and then 1 ml of 20% (w / v) sodium carbonate solution was added to it. The contents of the tube were then made up to 10 mL with distilled water and the tube was placed in the dark for 30 min. The optical density (OD) reading was taken at 725 nm against a blank, using a UV-Visible spectrophotometer (Jenway 7315 Spectrophotometer, SHIMADZU, Japan). Under the same conditions as the test, a standard range was established from a stock solution of gallic acid (1 mg / mL) and made it possible to determine the quantity of phenols in the samples. The results are expressed in mg of gallic acid equivalent (GAE) per gram of dry matter.

2.2.4 Determination of carotenoids

The carotenoids content were measured according to the method described by Rodriguez-Amaya (2001). A quantity of 2 g of sample was ground using a grinder (MC-BL 6731J, Japan). Twice in succession, the ground material obtained was diluted in 50 ml of acetone and then filtered. The filtrates obtained were introduced into a 500 ml separating funnel and 100 ml of petroleum ether were added to it. After light stirring, the mixture is left to stand for phase separation. The ethereal phase (containing the carotenoids) is recovered in another separating funnel and the absorbance of the ethereal phase is read using a spectrophotometer at 450 nm against petroleum ether.

$$Caroteno\ddot{i}ds\ content\ (mg/100g) = \frac{A_{450}.V.10^{-3}}{2592w_s}$$

V: volume (mL) of the extracted phase containing the carotenoids.
w_S: weight (g) of the sample.
2592: carotenoids mass extinction coefficient in petroleum ether.

2.2.2. Capsaicinoid extraction and quantification by HPLC

The capsaicinoids were extracted according to the modified method of Maillard et al. (1997). A volume of 10 mL of methanol was added to 0.5 g of pepper powder. The suspension was stirred for 2 min and centrifuged at 3000 rpm for 10 min in a centrifuge (ALC International, Milan, Italy). The extraction was repeated 10 times on the solid residues. The methanolic solution was filtered and evaporated in an oven at 40 ° C. The concentrate was dissolved in 3 mL of methanol and analyzed by HPLC.

For HPLC analysis, the method of Fogliano et al. (1999) was followed. The solution obtained was analyzed by a Shimadzu mod HPLC system. LC- 10ADVP equipped with a binary pump coupled to a UV-VIS Diode detector. Chromatographic separation of capsaicinoids was carried out on a 4u Fusion - RP 80 reverse phase synergic column mm 150 mm x 4.6 mm (Phenomenex, Castel Maggiore, BO, Italy). The identification of the capsaicinoids extracted was carried out by comparing the retention times and the UV spectra of the pure compounds. The chromatograms were recorded at 279 nm. The limits of detection (LDD) and quantification (LDQ) were obtained from the standard deviations of the responses using the following equations: LDD = 3.3 x σ / P; LOQ = 10 x σ / P; where σ = standard deviation of the response, P = slope of the calibration curve.

2.2.5 Determination of antioxidant activity by trapping Radical DPPH •

The anti-free radical activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test according to the method of **Kabran *et al*. (2012)**. This test is based on the discoloration of the DPPH solution with an active extract. Vitamin C and quercetin were chosen as positive controls. The crude methanolic extracts of the dried fruits were used for this test. A range of seven concentrations (200, 100, 50, 25, 12.50, 6.25 and 3.125 µg / mL) of the extracts and of each reference was prepared with methanol. In a test tube, 2.5 mL of extract was added to 1 mL of DPPH solution (3 mM in methanol). The mixture was incubated in the dark for 30 min followed by reading the absorbance in a spectrophotometer at 517 nm against the blank. The percentage inhibition of DPPH is calculated according to the following formula:

$$AA\ (\%) = \frac{(Ab - At) \times 100}{DOb}$$

Ab: absorbance of the blank sample (1 mL of DPPH + 2.5 mL of methanol)
At: absorbance of the test sample (1 mL of DPPH + 2.5 mL of methanolic extract).
AA: Antioxidant Activity

2.2.6 Statistical analysis

All the measurements were carried out in triplicate. The results obtained at the end of the experiments were first processed using Microsoft Excel 2007. The means of the parameters (total phenol, carotenoid and capsaicinoid contents) measured at different levels of the ripening stage were compared by one-way analysis of variance (ANOVA 1) using Statistica version 7.1 software. The statistical significance for the results was determined by Tukey's multiple comparisons test. $P < 0.05$

3. RESULTS

3.1 Extraction yield of bioactive compounds

Phytochemicals are extracted from the pepper at each stage of ripening and the extraction yield is calculated (Figure 1). The extraction yield increased significantly ($p <0.05$) from the green to the red stage of ripening. It is between 22.114 % (green stage) and 30.282 % (red stage).

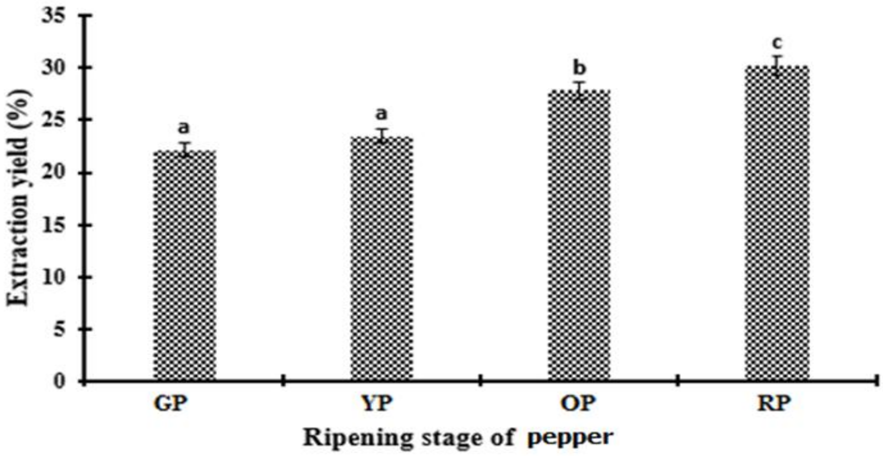

Figure 1: Evolution of the extraction yield of phytochemicals from the chili peppers *Capsicum annuum* type "Ahalimancou" during ripening. *The bars with a common letter indicate that extraction yields are not significantly different at p = 0.05. GP: Green Pepper; YP: Yellow pepper, OP: Orange pepper and RP: Red pepper*

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3.2. Total phenol content in chili peppers *Capsicum annuum* type "Ahalimancou" at each stage of ripening

The total phenol (TP) contents of the fruits of the pepper (*Capsicum annuum*) type "Ahalimancou" are determined at each stage of their ripening (Figure 2). The TP content increases significantly (p <0.05) from the yellow to the orange stage, with values between 452.81 ± 5.42 mgGAE / 100 g and 617.20 ± 7.02 mg GAE / 100 g DM. However, from the green to yellow stage and from the orange to red stage, the contents of total phenols did not differ significantly (p> 0.05). The total phenol contents of the first two stages of ripening are respectively 452.81 ± 5.42 mgGAE / 100 g and 487.93 ± 9.52 mgGAE / 100 g DM and that of the last two stages of ripening have respective values. of 617.20 ± 7.02 mgGAE / 100 g DM and 597.33 ± 8.12 mgGAE / 100 g DM.

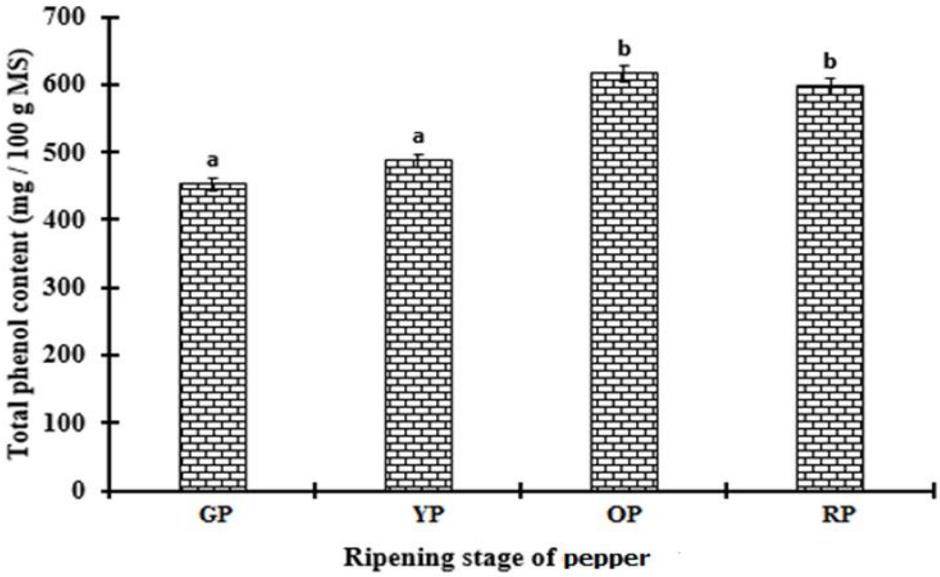

Figure 2: Evolution of the total phenol content of the chili peppers *Capsicum annuum* type "Ahalimancou" in ripening. *The bars with different letters indicate a significant difference (at 5 % threshold) in the total phenol contents between the ripening stages of the pepper.GP: Green Pepper; YP: Yellow pepper, OP: Orange pepper and RP: Red pepper*

3.3. Carotenoids content

The figure 3 shows the evolution of the carotenoid content of pepper fruits (*Capsicum annuum*) type "Ahalimancou" during ripening. The carotenoid content increases significantly (p <5%) from the green stage to the red stage. Indeed, the value of 5.06 ± 0.23 mg / 100g DM of carotenoids observed in the green stage of the pepper reaches 10.13 ± 0.65 mg / 100g DM in the yellow stage, 44.00 ± 1.25 mg / 100g DM in the orange stage and 55.90 ± 3.56 mg / 100g DM in the red stage of ripening.

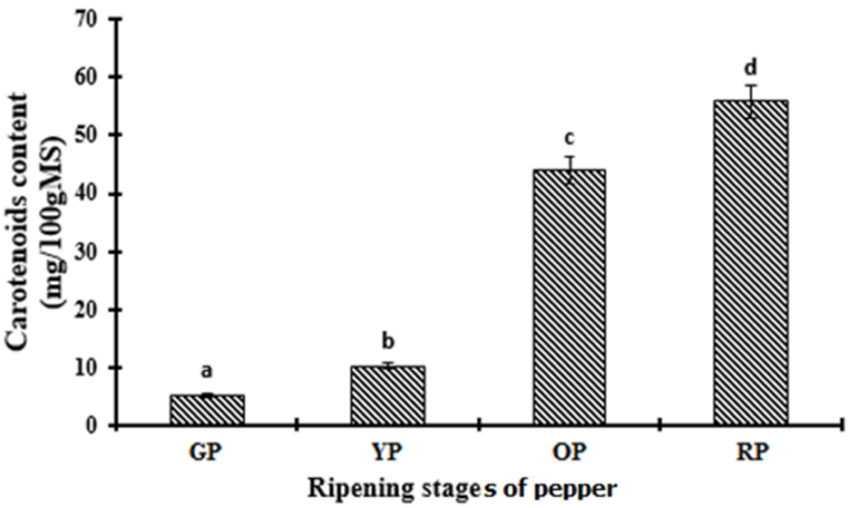

Figure 3: Evolution of the total carotenoid content of the chili peppers *Capsicum annuum* type "Ahalimancou" during ripening.*The bars marked with non-identical letters indicate a significant difference in the total carotenoid contents between the different stages of ripening of the pepper at p = 0.05. GP: Green Pepper; YP: Yellow pepper, OP: Orange pepper and RP: Red pepper*

3.4. Total capsaicinoids content during ripening

The evolution of the contents of capsaicin, dihydrocapsaicin and total capsaicinoids during the ripening of the chili peppers *Capsicum annuum* type "Ahalimancou"is shown in figure 4. The capsaicin content increases significantly (p <0.05) from the yellow stage, passing from 791.39 ± 2.42 to 1175.63 ± 2.01 mg / 100g DM for the red stage. This variation in the levels of capsaine is of the same order as that of the contents in total capsaicinoids which pass from 1110.10 ± 3.06 mg / 100g DM (green stage) to 1599.20 mg / 100g DM (red stage). Regarding the content of dihydrocapsaicin in pepper, the value of 318.70 ± 5.65 obtained at the green stage is not significantly different (p> 0.05) from that of the yellow stage (342.40 ± 3. 21 mg / 100 g DM). However, at the orange stage of ripening, a significant increase (p <0.05) in the content of dihydrocapsaicin (398.10 ± 2.23 mg / 100 g DM) is observed. This value, which reaches 423.60 ± 4.63 mg / 100 g DM in the red stage, is not significantly different (p> 0.05) from the previous stage.

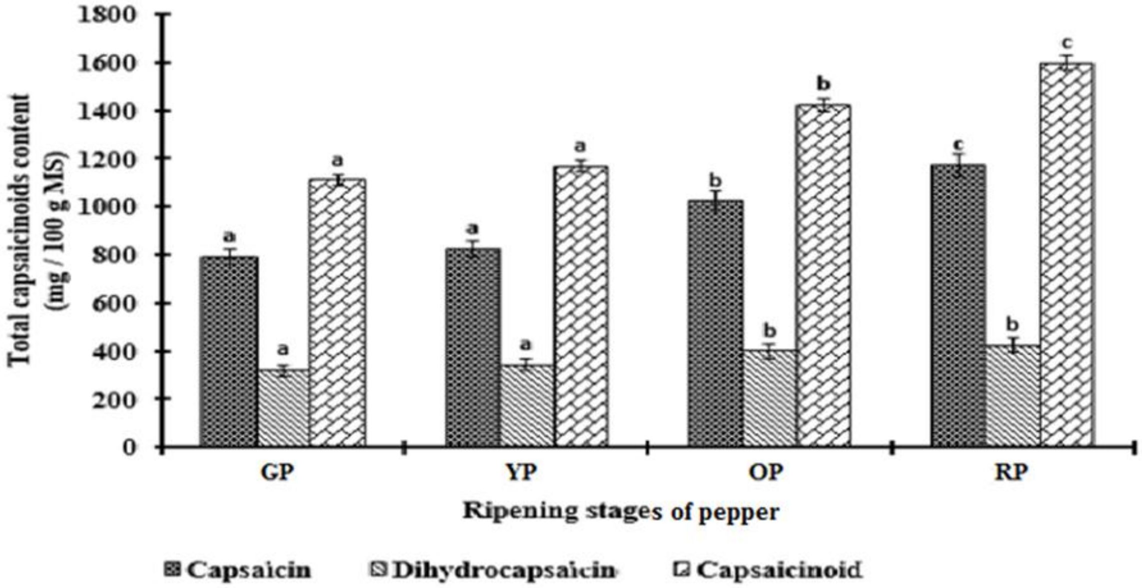

Figure 4: Evolution of the capsaicinoid content of the chili peppers *Capsicum annuum* type "Ahalimancou" during ripening.*The bars with a common cross-group letter indicate that the capsaicin or dihydrocapsaicin or total capsaicinoid contents of the pepper are not significantly different during ripening at p = 0.05. GP: Green Pepper; YP: Yellow pepper, OP: Orange pepper and PR: Red pepper*

3.5. Antioxidant activity of the chili peppers *Capsicum annuum* type "Ahalimancou" at each stage of ripening

The figure 5 shows the antioxidant power of the extracts of the "Ahalimancou" pepper to trap the DPPH radical. In fact, the results show a concentration-dependent effect at the different concentrations of the extracts (from 3.125 µg / ml to 200 µg / ml), resulting in a progressive increase in the DPPH inhibition percentages from 8.04 ± 0. 14 to 65.42 ± 1.92%, from 15.59 ± 0.64 to 64.25 ± 0.36%, from 27.24 ± 0.9 to 86.69 ± 0.64%, and from 38. 40 ± 1.46 to 88.66 ± 0.51% respectively for green, yellow, orange and red pepper fruits. In addition, the values obtained at the concentration of 200 µg / mL for peppers of the orange and red stage are close to those of vitamin C (94.33 ± 2.22%) and quercetin (92.78 ± 1.88%).

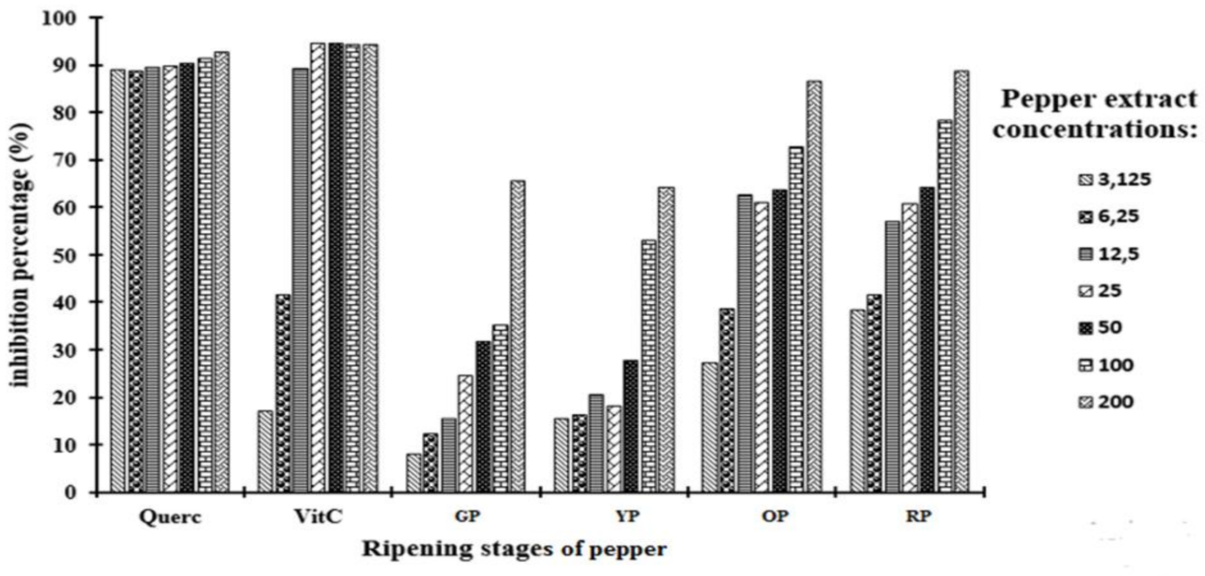

Figure 5: Percentage of inhibition of the DPPH of the chili peppers *Capsicum annuum* type "Ahalimancou". *GP: Green pepper; YP: Yellow pepper; OP: Orange pepper; RP: Red pepper*

4. DISCUSSION

In this study, the yield of the extraction of phytochemicals from the fruits of the "Ahalimancou" pepper increases from the green stage of ripening to the red one. Such an increase of phytochemicals extraction yield would be due to its accumulation as secondary metabolites in plants, in response to biotic and abiotic stresses (Belwal et al., 2019). In addition, Hervert-Hernandez et al. (2010) reported that Capsicum annuum has a wide range of phytochemicals. In this study, phytochemicals such as total phenols, carotenoids and capsaicinoids are present in the extracts of the "Ahalimancou" pepper. These compounds are more abundant in extracts of peppers from the red stage of ripening. In fact, the content of total phenols in pepper fruits is significantly influenced ($p < 0.05$) by the different stages of ripening.

The results obtained corroborate those of Chande et al. (2020) and Hamed et al. (2019). These authors mentioned that, the red pepper fruits displayed in general the highest level of total phenols compared to the others fruits color. In addition, previous reports by Belwal et al. (2019) suggested that fruits and vegetables ripening was due to a significant accumulation of total phenols. The presence of total phenols in the fruits of the "Ahalimancou" pepper would be an advantage in human food because, these substances which cannot be synthesized by the human body participate in the prevention of oxidative stress (Oboh *et al*., 2007). Otherwise. These phytophenols are reputed to be excellent natural antioxidants (Asnin *et al*., 2015) which, beyond preserving the sensory qualities of the pepper, reinforce its nutritional value (Alam *et al*., 2018). In addition, a significant increase in the carotenoid content of pepper fruits from the green to the red stage was observed. Indeed, Cervantes-Paz *et al*. (2014) have shown that carotenoids are responsible for the orange and red coloring of peppers. In addition, Hornero-Mendez *et al*. (2000) signified that the levels of lutein and neoxanthine, high in green fruits, decrease with maturation while the contents of β-carotene, violaxanthin, capsantine and capsorubin (responsible for the red color) increase. The carotenoid contents of the fruits of the chili peppers *Capsicum annuum* type "Ahalimancou" at the various stages of ripening studied are higher than those recorded by Collera-Zúñiga *et al*. (2007) on varieties of red peppers (Capsicum annuum) which vary from 6.76 to 7.52 mg / 100 g DM. Pepper type such as Ahalimancou is an important source of capsaicinoids at all stages of its fruits ripening. According to Reilly *et al*. (2001), the particularity of hot pepper is its high content of the monophenolic compounds capsaicins such as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin. A gradual increase in the capsaicinoid content of green to red fruits has been observed. Such an increase in the capsaicinoid content during ripening has also been reported by Bae *et al*. (2014) in cayenne pepper where the capsaicin content increased from 14.95 mg / 100 g DM (green stage) to 21.17 mg / 100 g DM (red stage). These results also corroborate those of Eissa *et al*. (2007) who observed a variation in the capsaicinoid content of the indigenous peppers analyzed in Mexico. It should be noted that capsaicinoids belong to the family of alkaloids and are classified as secondary metabolites (Ben-Chaim *et al*., 2006). Their effects go far beyond taste and their roles in the plant help us understand how its use can improve human health. Indeed, the studies of Ramos-Torres *et al*. (2016) found that capsaicinoids cause the cell cycle to stop and trigger apoptosis in human prostate carcinomas. They are also used to inhibit the action of Helicobacter pylori, a gastric pathogen (Satyanarayana, 2006; Shruti *et al*., 2010). With regard to the therapeutic applications of capsaicinoids, the Ahalimancou pepper cultivar, cultivated in eastern Côte d'Ivoire, could have various health benefits.

The antioxidant effect of the fruits of the chili peppers *Capsicum annuum* type "Ahalimancou" was revealed at all stages of ripening in this study. For Rojita *et al*. (2011), the presence of total phenols, carotenoids and capsaicinoids in peppers is an indicator of the antioxidant activity that they could contain. Also, it is evaluated that vegetables are rich in total phenolics showed strong antioxidant activity at the same time (Sayın and Alkan, 2015). This antioxidant activity was found to be greater in the fruits of the "Ahalimancou" chjlli pepper at red stage of ripening. Our results are far superior to those of Medina-Juárez *et al*. (2012) who found a strong antioxidant activity in the fruits of red peppers with an inhibition of 44.66 %. For Álvarez-Parrilla *et al*. (2011), the degree of fruit ripening may explain the difference in antioxidant activity.

5 CONCLUSION

The antioxidant activity of the chili peppers *Capsicum annuum* type "Ahalimancou" has been evaluated at each stage of its fruits ripening. It emerges from this study that the phytochemicals which confer to pepper fruits this antioxidant power are more present in the orange and red stages of ripening. Thus, the pepper fruits, harvested at the orange and red stage of ripening, contained the highest total phenol, carotenoid and capsaicinoid amount and showed strong antioxidant activity. So, the cultivated pepper's variety, called locally "Ahalimancou" in the East of Côte d'Ivoire, is therefore an important source of natural antioxidant.

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