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Effects of maturity stage on the antioxidant components levels and antioxidant properties of three edible mushroom species of genus *Russula* from Côte d'Ivoire

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ABSTRACT

This study aimed to assess the antioxidant capacities of three species of edible ectomycorrhizal mushrooms of the genus *Russula* from Côte d'Ivoire as a function of their maturity stage through changes in the contents of the antioxidant components and properties of their extracts. The contents of total phenolic compounds, flavonoids, tannins and carotenoids were assessed by colorimetric assays whereas ascorbic acid content was estimated by titration. Individual phenolic compounds were analyzed by HPLC for their identification. Three biochemical assays (DPPH scavenging, ABTS scavenging and Ferric reducing antioxidant power) were used to estimate the antioxidant properties. The highest amounts of all the antioxidant components and the majority of individual phenolic compounds analyzed were obtained at the mature stages in the extracts of the three mushrooms. The maximum contents of total phenolic compounds were also obtained in the mature fruiting bodies and were respectively 729.16±0.32, 623.11±0.04 and 832.78±0.01 mg/100g dry matter for *Russula lepida*, *R. mustelina* and *R. delica.* These results were confirmed by the values of the effective concentrations EC_{50}, the lowest of which were obtained at the mature stages and were respectively 0.11±0.03, 0.19±0.09 and 0.09±0.01 mg/ml in terms of DPPH scavenging for *R. lepida*, *R. mustelina* and *R. delica*. This suggests that in terms of the need for natural antioxidants, the mature stage is the appropriate stage for harvesting these mushrooms.

Keywords: Ectomycorrhizal mushrooms, *Russula*, maturity stages, antioxidant components, antioxidants properties

For many years, edible mushrooms have been regarded as real sources of natural antioxidants (phenolic compounds, flavonoids, carotenoids, ascorbic acid and so on) like fruits and vegetables. Several reports have established the correlation between the levels of phenolic compounds with antioxidant activities in the edible mushrooms (Yen and Hung 2000; Cheung *et al*., 2003; Mau *et al*., 2004; Barros *et al*., 2007; Turkoglu *et al*., 2007). For fruits and vegetables, many findings have been published on the effects of the maturity stage on the antioxidant composition and antioxidant properties such as tomatoes (Hdider *et al*., 2013; Dûma *et al*., 2018; Tilahun *et al*., 2018), papaya (Addai *et al*., 2013), mango (Joshi *et al*., 2017) and many other fruits and vegetables (Fawole and Opara 2013; Xie *et al*., 2016; Wulandari *et al*., 2017; Oszmiañski *et al*., 2018). All of these studies have shown that the maturity stage significantly affects the antioxidant potential of fruits and vegetables through the antioxidant components levels and antioxidant activities.

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In edible mushrooms, very few investigations have undertaken this aspect into account. However, some authors have analyzed the influence of maturity stage on the antioxidant component composition and antioxidant properties of some wild edible, commercial, medicinal mushrooms (Barros *et al*., 2007; Tsai *et al*., 2008; Soares *et al*., 2009; Ren *et al*., 2020). These few reports have also indicated the influence of maturity stage on the antioxidant components and the antioxidant properties of edible mushrooms.

In Africa, south of the Sahara and particularly in Côte d'Ivoire, edible mushrooms are picked during the rainy seasons and consumed locally. These are also marketed in the surrounding villages and towns (Anno *et al*., 2016). Especially, in the central part of this country, edible ectomycorrhizal mushrooms living in symbiosis with trees of Caesalpiniaceae family are the object of picking and great consumption in the rainy season (Kouassi 2016). Generally, the picking of these mushrooms is carried out without taking into account the stage of maturity (immature; mature and post-mature); all being picked and put together for later consumption. Previous works revealed that the chemical and nutritional properties were strongly impacted by maturity stage of picking of edible ectomycorrhizal mushrooms of the genus *Russula* from central part of Côte d'Ivoire (Gbotognon *et al*., 2019a; 2019b). According to Barros *et al*. 2007), consumption of mushrooms could contribute to minimize risks of chronic diseases such as cancer through their high contents of natural antioxidants. Nevertheless, it is important to know the ideal maturity stage to pick wild mushrooms regarding antioxidant components contents.

Thus, the aim of this work was to evaluate the effects of the stage of harvest maturity on the contents of total phenolic compounds, flavonoids, tannins, individual phenolic compounds, total carotenoids, â-carotene, lycopene and the antioxidant properties of three mushrooms *Russula* spp from central Côte d'Ivoire. This could help to identify the appropriate harvest stage for these wild mushrooms in terms of levels of antioxidant components and antioxidant properties.

MATERIALS AND METHODS

Reagents and Chemicals

Organic acid standards (citric acid, oxalic acid, ascorbic acid, succinic acid, malic acid, fumaric acid, shikminic acid, tartaric acid, benzoic acid, propionic acid and butyric acid), 2,2'-azino acid -bis (3-ethylbenzothiazoline-6-sulphonic (ABTS), trolox and Folin-Ciocalteu were procured from Sigma-Aldrich (Steinheim, Germany). The standards of phenolic compounds (gallic acid, caffeic acid, vanillic acid, veratric acid, ferulic acid, coumaric acid, quercetin, catechin and rutin) and acetonitrile were products of Merck (Darmstadt, Germany). Aluminum chloride and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was procured from Sigma Chemical Co (St, Louis, MO, USA). Iron (III) chloride was purchased from Merk (KGaA, Darmstadt, Germany) while methanol was obtained from Prolabo (Paris, France) All other chemicals were of analytical grade.

Sample collection

Mushrooms of the genus *Russula* (*R. mustelina*, *R. delica* and *R. lepida*) were picked at different maturity stages from the open forests of the central part of Côte d'Ivoire, in the administrative region of Gbèkè, where they lived in ectomycorrhizal symbiosis with trees of the Caesalpiniaceae family. Maturity stage of each mushroom was identified, as (i) immature stage with closed cap and immature spores, (ii) mature stage with opened cap and immature spores and (iii) post-mature stage with wilting cap and mature spores (Fig. 1). After picking, the mushrooms were immediately transferred to the laboratory and cleaned.

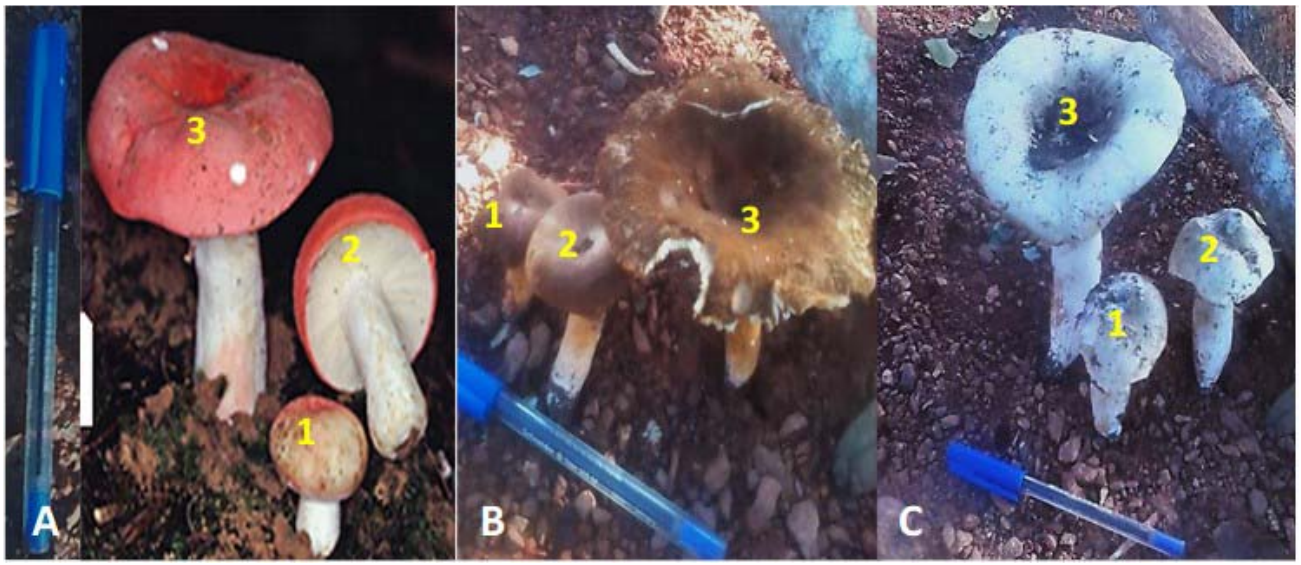

Fig. 1. Mushrooms *Russula* spp (A: *R. lepida*; B: *R. mustelina*; C: *R. delica*) at different stages of maturity: (1) Immature stage (closed cap); (2) Mature stage (opened cap); (3) Post-mature stage (wilting cap)

Extraction of phenolic compounds

Mushrooms of the three samples corresponding to the three *Russula* species were dried at 25°C for ten days until constant weight, according to method of Ribeiro *et al*. (2007) slightly modified by Kouassi *et al*. (2016). Then, each sample was ground into a fine-dried powder (mill IKA, Germany/Deutschland). A sample (10 g) of each fine-dried mushroom powder was extracted by stirring with 50 ml of methanol 80% (v/v) at 25°C for 24 hours and filtered through Whatman paper no 4. The residue was then extracted with two additional 50 ml portions of methanol. The combined methanolic extracts were evaporated at 35°C (rotary evaporator HEILDOLPH Laborata 4003 Control, Schwabach, Germany) until 25 ml, and stored at -20 °C prior to further use.

Determination of total phenolic compounds contents

Total phenolic compounds content was estimated according the Folin-Ciocalteu method (Singleton *et al*., 1999). A volume of 1 ml of methanolic extract of each sample was added to 1 ml of Folin-Ciocalteu's solution in a test tube. After 3 minutes, 1 ml of 20% sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The mixture was allowed to stand at room temperature in a dark environment for 30 min. Absorbance was measured against the reagent blank at 725 nm. Gallic acid was used for the calibration curve with a concentration range of 50-1000 µg/ml. Results were expressed as mg gallic acid equivalent (GAE)/100 g DW (Dry Weight). All experiments were performed in triplicate.

Determination of flavonoids contents

Total flavonoids content was determined according method used by Meda *et al*. (2005), but slightly modified. A volume of 0.5 ml of methanolic extract of each mushroom sample was diluted with 0.5 ml of distilled water. Then, 0.5 ml of aluminium chloride 10 % (W/V) and the same volume of sodium acetate 1M were added. Finally, 2 ml of distilled water was added and absorption reading at 415 nm was carried out after 30 min against a blank sample consisting of a 4 ml methanolic extract without aluminium chloride. Quercetin was used for the calibration curve with a concentration range of 0-100 µg/ml. Results were expressed as mg of quercetin equivalent (QE)/100g DW. All experiments were performed in triplicate.

Determination of tannins contents

Tannins content was determined using the method described by Bainbridge *et al*. (1996). A volume of 1 ml of each methanolic extract was mixed with 5 ml of reaction solution [vanillin 0.1mg/ml in sulphuric acid 70 % (V/V)]. The mixture was allowed to stand at room temperature in a dark environment for 20 min. The absorbance was measured at 500 nm against a blank (without extract). Tannic acid was used for the calibration curve with a concentration range of 0-100 µg/ml. The results were expressed as mg of tannic acid equivalents (TAE)/100 g DW. All experiments were performed in triplicate.

Extraction of carotenoids

Total carotenoids of mushroom samples were extracted according to the method described by Rodriguez-Amaya and Kimura (2004). A mass of 2 g of fine powder of each sample of mushroom previously described for the preparation of the methanolic extract was diluted in 50 ml of acetone and the mixture was filtered. The residue was completely decolorized by repeating the operation by adding 2 times 50 ml of acetone. The three filtrates obtained were combined and taken into a 500 ml separating funnel. Then, 100 ml of petroleum ether was added, and allowed to stand for phase separation. The ethereal phase (containing the carotenoids) was collected and placed in a 200 ml beaker for the subsequent determination of total carotenoids, β-carotene and lycopene.

Determination of total carotenoids contents

The total carotenoids contents of the mushroom samples were estimated by the method used by de Carvalho *et al*. (2012). Absorbance (A) of each ethereal extract of total carotenoids was read with a colorimeter at 450 nm against petroleum ether. The total carotenoids content expressed in mg / 100g of dry matter is calculated according to the following formula:

$$\text{Carotenoid contents (mg/100g DW)} = \frac{AxV}{2592xM_e} \times 1000$$

Where, A = Absorbance; V (ml) = Total ethereal extract volume; M_e (g) = sample mass; 2592= Carotenoids Extinction Coefficient in petroleum ether

Determination of β-carotene and lycopene contents

β-carotene and lycopene contents were determined according to the method of Nagata and Yamashita (1992). Fine powder from each mushroom sample (100 mg) was vigorously stirred with 10 ml of acetone-hexane (4: 6) for 1 min and filtered through Whatman no. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. β-carotene and lycopene contents were calculated according to the following equations:

Lycopene content (mg /100 ml) = $-0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$

β-carotene content (mg / 100 ml) = $0.216A_{663}$ 6 $0.304A_{505} + 0.452A_{453}$

Determination of ascorbic acid content

Ascorbic acid from each mushroom samples was extracted according to method described by Boonkasem *et al*. (2015). Each mushroom powder (10 g) was extracted with 20 ml of 3% (W/V) metaphosphoric acid solution, and then placed under magnetic stirring for 30 min. The extract was centrifuged at 4000 rpm for 20 min. The supernatant collected was used for the determination of ascorbic acid according to method described by Pongracz *et al*. (1971), using 2,6-dichlorophenol (DCPIP). One (1) ml of the supernatant was titrated with 2,6-dichlorophenol indophenol (0.5g/l). Appearance of a persistent champagne pink color, lasting 15 seconds, indicated the end of the titration. One (1) ml of a standard solution of pure ascorbic acid (1 mg/ml) was also titrated with 2,6-dichlorophenol indophenol under

the same conditions. Ascorbic acid content of each mushroom sample was calculated using the following equation:

$$\text{Ascorbic content (mg/100g)} = \frac{(C_{DCPIP} \times V_{eq}) \times 5 \times 100}{m_e}$$

Where, V_{eq} = 2,6-dichlorophenol indophenol volume used for titration of 1 ml of supernatant from each samples; m_e=fine powder mass (g) of each mushroom samples; C_{DCPIP}((g/L) = DCPIP concentration

Identification of individual phenolic compounds by HPLC

The methanolic extracts of each mushrooms samples previously prepared (50 ml) were diluted in 100 ml with distilled water and 20 µl of it was analyzed using an analytical HPLC unit (HPLC (Shimadzu Corporation, Japan) equipped with a binary pump (LC-6A) coupled to a UV-VIS detector (SPD-6A). Phenolic compounds were separated on a column ICSep ICE ORH-801 (length 25 cm) at a temperature set at 30°C. The mobile phase consisted of 50 mM $NaH_4H_2PO_4$ to pH 2.6 (eluent A), a solution of acetonitrile/$NaH_4H_2PO_4$ (80:20, v/v) (eluent B) and 200 mM acid *o*-phosphoric pH 1.5 (eluent C). The operating time was 70 min with a flow rate of 1 ml/min. Phenolic compounds in methanolic extract of mushroom samples were identified through comparison of their retention times and UV-visible spectra with those obtained by injection of the standard solution under the same conditions.

Antioxidant properties

In the present study, the antioxidant activities of mushroom sample extracts were evaluated in terms of DPPH radical scavenging, ABTS cation radical ($ABTS^+$) scavenging and ferric ion reducing antioxidant power (FRAP).

DPPH radical scavenging activity

The free radical scavenging activities of each mushroom sample extracts were measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Hatano *et al*., 1988). Briefly, 2.5 ml of each extract was added to 1 ml of DPPH solution (3 mM), vigorously shaken and maintained for 24 h at room temperature in the dark. Methanol was used instead of mushroom extract as a control. Then the absorbance was measured at 515 nm. The mushroom samples extracts capability to scavenge the DPPH radical was calculated using the following equation:

DPPH inhibition (%) = $[(A_0 - A_1/A_0) \times 100]$, where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample.

The mushroom sample extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of inhibition percentage of DPPH against extract concentration. Trolox was used as standard.

ABTS cation radical scavenging activity

This method had expressed the ABTS cation radical scavenging capacity of our mushroom samples extracts estimated using discoloration test of these ABTS cations (Re *et al*., 1999), which is based on the reduction of $ABTS^+$ radicals by mushrooms antioxidant extracts tested. ABTS was dissolved in distilled water to a 7 mM concentration. ABTS radical cation ($ABTS^+$) was produced by reacting ABTS solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 16 h before use. For the study, the ABTS solution was diluted in ethanol to an absorbance of 0.700 ± 0.02 at 734 nm. After the addition of 30 µl of methanolic extracts of mushroom samples at various concentrations to 3 ml of ABTS solution, the absorbance reading was taken at 30°C 10 min after initial mixing. A control was carried out under the same conditions with methanol

instead of the methanolic extracts of mushroom samples. The mushroom samples extracts capability to scavenge ABTS radical cation ($ABTS^+$) was calculated using the following equation:

ABTS inhibition (%) = $[(A_0 - A_1/A_0) \times 100]$, where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample

The mushroom sample extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of inhibition percentage of $ABTS^+$ against extract concentration. Trolox was used as standard

Ferric reducing antioxidant power (FRAP)

The reducing powers of the mushroom sample extracts were determined according to the method described by Ferreira *et al*. (2007). In a test tube containing 0.1 ml of sample extract prepared at different concentrations (0.5 to 3 mg / ml), are added with 2.0 ml of phosphate buffer (0.2 M, pH 6.6), followed by 2 ml of 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$] (w/v). The whole was incubated at 50 °C in a water bath for 20 min, and then cooled. A volume of 2 ml of 10 % (w/v) trichloroacetic acid (TCA) was then added and the mixture was centrifuged at 3000 rpm for 10 min. Finally, 2 ml of the supernatant was mixed with 2 ml of distilled water and 0.4 ml of ferric chloride [$FeCl_3$] (1g/l). A blank without sample extract was prepared under the same conditions. Absorbance was measured at 700 nm against blank. The increase in absorbance indicated higher reducing power. The extract concentration of samples giving an absorbance of 0.5, i.e. the value of the EC_{50} was estimated from the graph of the absorbance at 700 nm versus the concentration of extract. Trolox prepared as above at different concentrations was used as a standard.

Statistical analysis

All chemical analyses and assays were carried out in triplicate. 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 Statistica 7.1 software. Significance of differences was defined at the 5% level ($p < 0.05$).

RESULTS AND DISCUSSION

The variations in contents of different antioxidant components depending on maturity stage of three *Russula* spp are shown in Table 1. For each of the extracts of the three species of *Russula*, the total phenolic compounds were the major antioxidant components while carotenoids and ascorbic acid were low in quantities. These results were in accordance with those reported by other authors (Barros *et al*., 2007; Robaszkiewicz *et al*., 2010). On the whole, the contents of total phenolic compounds, flavonoids, tannins, total carotenoids, β-carotene, lycopene and ascorbic acid all showed variations with a peak at the mature stage for the three mushroom species. Values of peaks for total phenolic compounds were respectively 729.16±0.32; 623.11±0.04 and 832.78±0.01 mg/100g DW for *R. lepida*, *R. mustelina* and *R. delica*. With respect to total carotenoids, peaks were estimated respectively to 86.92±1.73; 54.44±0.53 and 51.78±2.60 mg/100g DW for *R. lepida*, *R. mustelina* and *R. delica.* The increase in the levels of all the antioxidant components analyzed from immature stage to mature stage with immature spores in the three mushroom species could be explained by the accumulation of these components during maturation. Indeed, it is well known that during the maturation of mushrooms, there is a biosynthesis of variety of secondary metabolites (Turkoglu *et al*., 2007; Soares *et al*., 2009). These results were contrary to those obtained by Saha *et al*. (2012) for the content of phenolic compounds in wild mushrooms *Pleurotus djamor* at different stages of maturity. He reported the highest amount of phenolic compounds in the juvenile stage of fruiting bodies. On the other hand, from the mature stage to the post-mature stage with mature spores, the different antioxidant

Table 1. Antioxidant components contents (mean ± SD) of *Russula* spp at different stages of maturity

Antioxidant components	Maturity Stage	Mushrooms species		
		R. lepida	***R. mustelina***	***R. delica***
Total Phenolic compounds (mg/100 g)	Immature	560.92±0.02[b]	545.45±0.01[b]	551.78±0.02[b]
	Mature	729.16±0.32[c]	623.11±0.04[c]	832.78±0.01[c]
	Post-Mature	389.74±0.04[a]	343.55±0.10[a]	383.26±0.01[a]
Flavonoids (mg/100 g)	Immature	073.14±0.00[b]	119.43±0.02[b]	126.65±0.01[b]
	Mature	087.75±0.01[c]	129.14±0.10[c]	138.64±0.10[c]
	Post-Mature	069.25±0.09[a]	113.44±0.05[a]	116.93±0.02[a]
Tannins(mg/100 g)	Immature	119.62±0.10[b]	119.75±0.05[b]	148.58±0.42[b]
	Mature	134.58±0.01[c]	150.73±0.02[c]	168.86±0.02[c]
	Post-Mature	116.29±0.00[a]	100.07±0.02[a]	151.40±0.36[a]
Total carotenoids (mg/100 g)	Immature	79.29±2.04[b]	52.11±2.21[b]	32.78±0.81[a]
	Mature	86.92±1.73[c]	54.44±0.53[c]	51,78±2.60[c]
	Post-Mature	78.74±1.21[a]	34.55±1.25[a]	33.26±1.68[b]
β-Carotene (mg/100 g)	Immature	13,14±0.98[a]	11.43±1.43[a]	16.65±0,54[b]
	Mature	27.74±0.75[c]	19.14±0.96[c]	18.64±0.48[c]
	Post-Mature	26.25±1.68[b]	13.45±1.13[b]	15.93±0.82[a]
Lycopene(mg/100 g)	Immature	0.96±0.09[b]	1.17±0.20[b]	4.89±0,28[a]
	Mature	1.45±0.12[c]	1.55±0.17[c]	6.88±0.17[c]
	Post-Mature	0.62±0.13[a]	1.00±0.10[a]	5.14±0.62[b]
Ascorbic acid (mg/100 g)	Immature	56.44±1.60[a]	29.43±1,73[a]	62.65±0.86[a]
	Mature	63.14±0. 97[c]	67,14±0,84[c]	87.64±2.48[c]
	Post-Mature	61.93 ±1.78[b]	63,45±1,02[b]	83.42±2.10[b]

In each column different letters mean significant differences ($p < 0.05$)

components have undergone a significant decrease. This could be attributed to the fact that these antioxidant components were involved in the neutralization of reactive oxygen species and free radicals generated by aging fungal cells of the mushrooms. Similar results have were reported by Barros *et al*. (2007) from mature stage with immature spores to post-mature stage with mature spores during development of the wild mushrooms *Lactarius piperatus*.

Fig. 2 shows the chromatographic profiles of some individual phenolic compounds from extracts of *R. lepida*, *R. mustelina* and *R. delica* at different stages of maturity. It was overall noted that the extracts of the mushrooms contained phenolic acids (gallic acid, vanillic acid, caffeic acid, veratric acid, ferulic acid and coumaric acid) and flavonoids (quercetin, catechin and rutin). These results were in agreement with other reports concerning the identification of individual phenolic compounds in edible mushrooms extracts (Palacios *et al*., 2011; Yahia *et al*., 2017; Cayan *et al*., 2020). There were changes in detection of individual phenolic compounds depending on the maturity stage of different species analysed. Regarding *R. lepida*, these changes concerned caffeic acid, veratric acid and quercetin (Fig. 2a). The caffeic acid not detected at the immature stage was well present at the mature and post-mature stages. The veratric acid detected in the first two stages, disappeared in the post-mature stage while the quercetin was only detected at the mature stage. As for *R. mustelina*, the changes also concerned caffeic acid and veratric acid, but also vanillic acid (Fig. 2b). Vanillic acid was only detected at the mature stage while caffeic acid and veratric acid were absent at the immature stage but detected at the Mature and post mature stages. Finally,

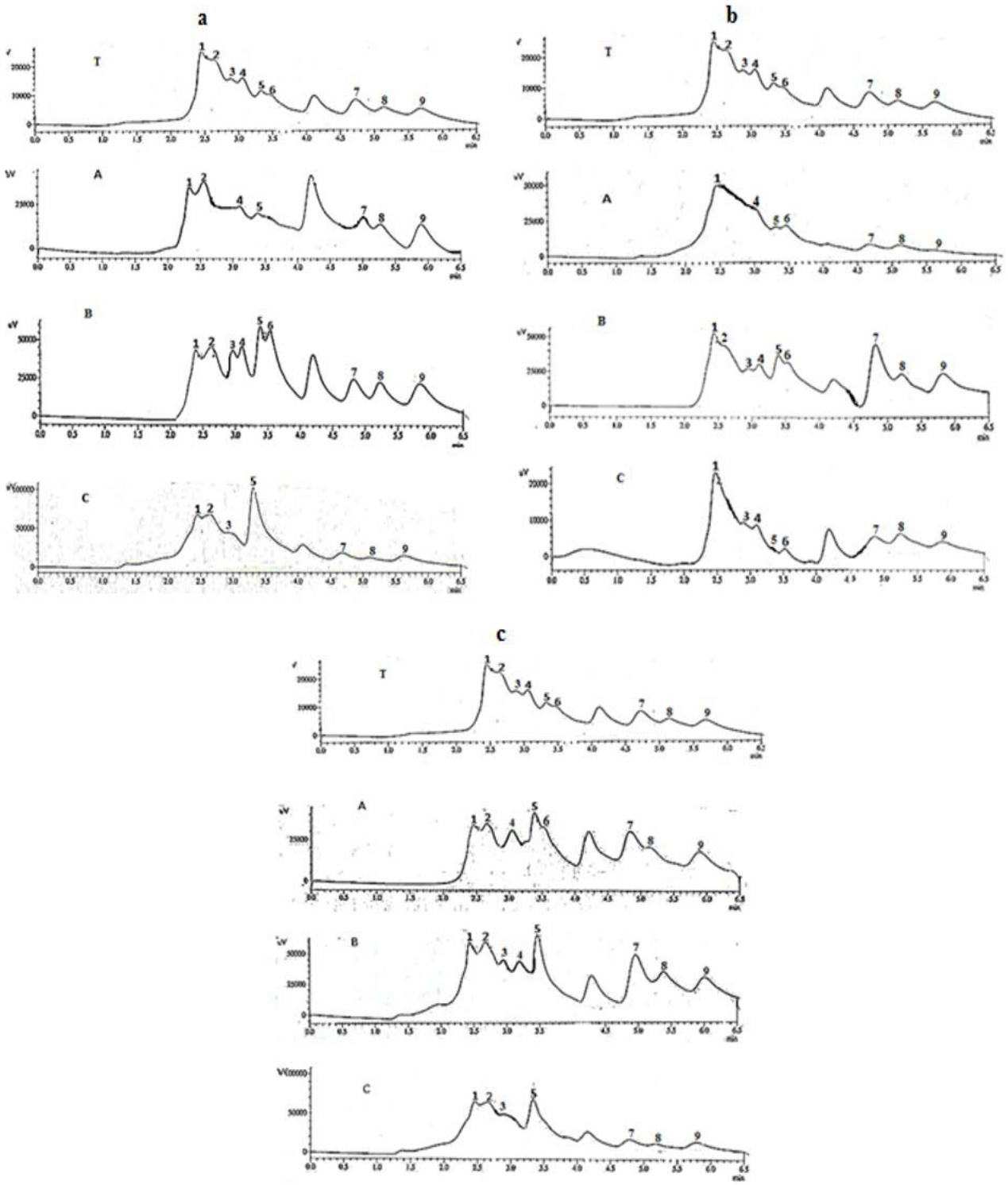

Fig. 2. HPLC-profiles of phenolic compounds of methanolic extracts of three species of *Russula* from Côte d'Ivoire (a: *R. lepida*; b: *R. mustelina*; c: *R. delica*) harvested at three different stages of maturity (T: standards; A: Immature stage; B: Mature stage; C: Post-mature stage). Detection at 210 nm: (1) Gallic acid; (2) Vanillic acid (3) Caffeic acid (4) Veratric acid; (5) Ferulic acid; (6) Quercetin; (7) Catechin; (8) Rutin; (9) Coumaric acid

concerning *R. delica*, the changes concerned caffeic acid and veratric acid and also quercetin (Fig. 2c). Caffeic acid was absent at the immature stage but detected at the other two stages as in the case of the other two mushroom species whereas veratric acid detected at the immature and mature stages, and absent at the post-mature stage. Quercetin was not detected in extracts from all three maturity stages in *R. delica*. Furthermore, in terms of quantity, almost all of the individual phenolic compounds peaked at the mature stages in the extracts of the three mushroom species (data not shown) like total phenolic compounds. This could be attributed to the fact that the phenolic acids and flavonoids individually constitute powerful antioxidants which must have been used in the neutralization of reactive oxygen species and free radicals generated during the aging of fungal cells.

Indeed, phenolic acids such as gallic acid, ferulic acid 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; Shen *et al*., 2019). Likewise, catechin and rutin constitute flavonoids well documented for their antioxidant and anti-free radical properties (Pekkarinen *et al*., 1999; Azevedo *et al*., 2013). On the other hand, all these significant changes in caffeic acid, veratric acid, vanilic acid or even quercetin observed during the growth of the three mushrooms could be due to structural instabilities of some of these phenolic acids (Friedman and Jürgens 2000) or due to possible interconversion between some of them or due to de novo synthesis via shikimic acid (Katsuragi *et al*., 2010) during metabolic phenomena when mushrooms ripen.

Fig. 3 shows the antioxidant activities via DPPH radical scavenging of the extracts from three species of *Russula* harvested at three maturity stages. From the analysis of Figures 3a, 3b and 3c, we could conclude that the percentage inhibition of DPPH increased with the concentration of the extract. The percentages of inhibition obtained with the extracts of the mature stages of the three mushroom species were 75 % at 0.7 mg/ml, 65 % at 0.5 mg/ml and 75 % at 0.4 mg/mg for *R. lepida*, *R. mustelina* and *R. delica*, respectively. These values, however, were still lower than those of the standard which was the trolox (around 85 % at 0.3 mg/ml). Barros *et al*. (2007) reported a good percentage inhibition of DPPH at

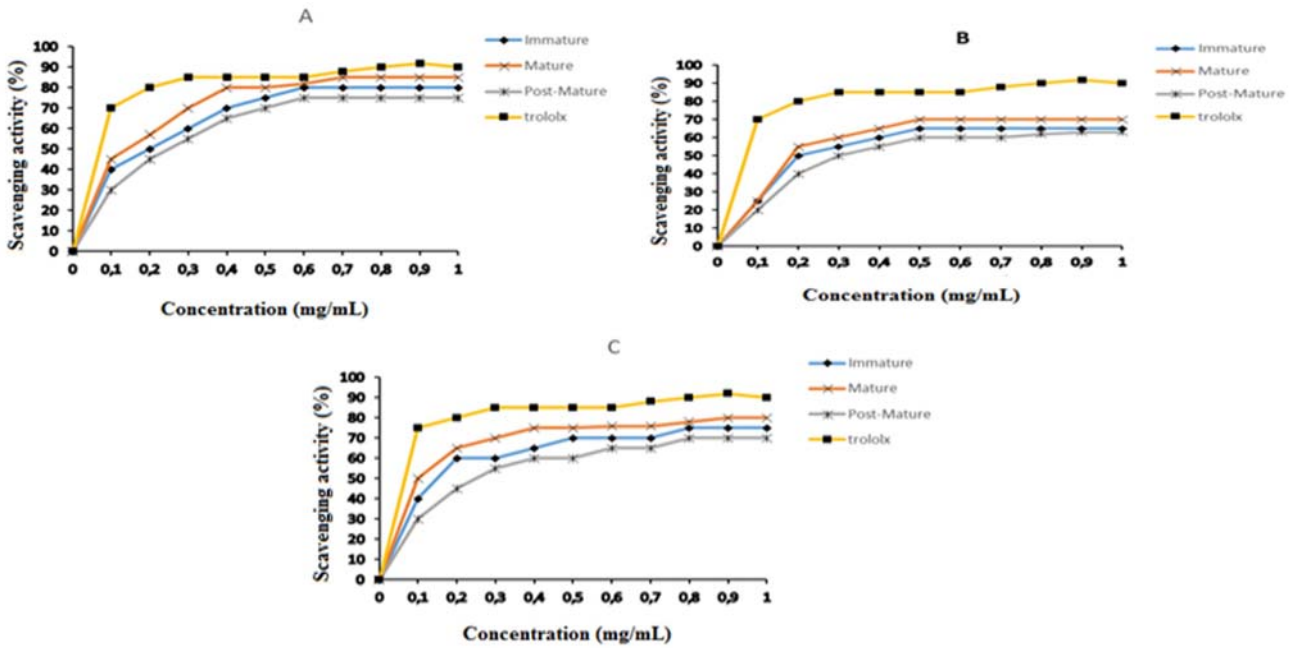

Fig. 3. Antioxidants activities measured by DPPH radical scavenging of méthanolic extracts from three mushroom species of *Russula* (A: *R. lepida*; B; *R. mustelina*; *R. delica*) harvested at different stages of maturity

stage II which corresponds to the mature stage with immature spores for extract from mushroom *Lactarius piperatus*.

In contrast, Soares *et al*. (2009) observed similar percentages of inhibition at all extract concentrations for the mushroom *Agaricus brasiliensis* harvested at two stages of maturity: young and mature. In terms of mechanism, in the DPPH radical scavenging assay, the antioxidant compounds act by donating hydrogen atoms to the DPPH radical molecules. These antioxidant compounds are called anti-radical antioxidants. When these antioxidant components are phenolic compounds, the phenolic radicals obtained are stable due to the conjugated double bonds (Bondet *et al*., 1997). Some authors have shown that the anti-free radical activities estimated by the DPPH scavenging assay are correlated with phytochemical constituents including phenolic compounds and certain pigments (Shalaby *et al*., 2013). This could mean that at the mature stage of development of the three mushroom species *R. lepida*, *R. mustelina* and *R. delica*, the anti-radical antioxidants would have a higher concentration than the other two stages.

As for antioxidants activities via ABTS cation radical scavenging, the results are indicated in Fig. 4. The percentage inhibition of ABTS cation radical increased also with the concentration of the extract. The values obtained were better at the mature stage for the extracts of the three mushrooms. The inhibition of the ABTS cation radical by extracts of *R. lepida* (Fig. 4a) indicate that the extract of the mature stage caused a percentage inhibition of 80% at 1 mg/ml concentration while *R. mustelina*, (Fig. 4b) shows that the ABTS cation radical inhibition of 65% by the extract of the mature stage at 1 mg/ml against 90% inhibition in the standard (trolox) The extract of the mature stage of *R. delica* produced around 80 % of cation ABTS radical inhibition at 1 mg/ml against 90% for the trolox at the same concentration (Fig. 4c). All these levels of inhibition of the ABTS cation radical were better than that reported by other authors for the aqueous extract of the mushroom *Lentinus elodes*, which was 51.5±3.96 % at 5 mg/ml (Shah and Modi 2015).

In the ABTS cation radical scavenging, anti-radical antioxidants which reduce the free radical are also hydrogen donors and according to Re *et al*. (1999), this assay is applicable to lipophilic and hydrophilic antioxidants, including flavonoids, phenolic acids and carotenoids. Previous works has shown that the inhibition of the ABTS cation radical is correlated

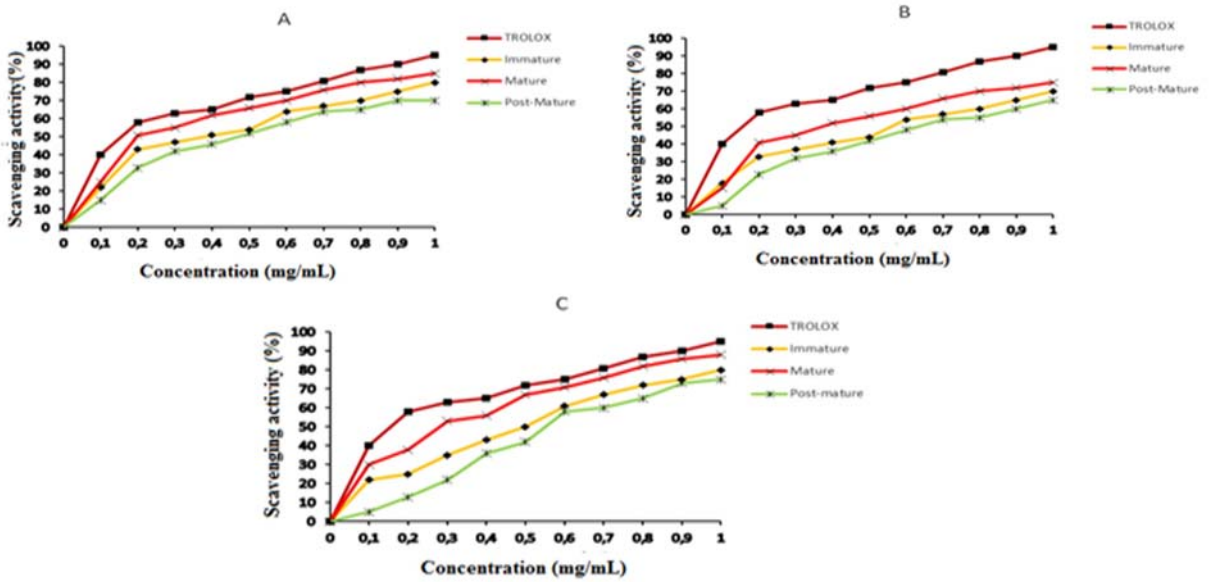

Fig. 4. Antioxidants activities measured by cation ABTS radical scavenging of méthanolic extracts from three mushrooms *Russula* spp. (A: *R. lepida*; B; *R. mustelina*; *R. delica*) harvested at different stages of maturity

with the content of phenolic compounds in mushrooms (Mircea *et al*., 2015; Islam, *et al*., 2016). The results of this work are consistent with this finding since the highest levels of phenolic compounds were effectively observed at the mature stages of the three mushroom species.

Antioxidant activities via ferric reducing power (FRAP) assay exhibited the same trends in terms of reduction of ferric iron (resulting in increased absorbance) that inhibitions of the DPPH radical and cation ABTS radical, i.e. the percentage of reduction increased with the concentration of the extract and the better percentages of reduction were obtained at the mature stages for the three mushroom species (Fig. 5). The extracts of *R. lepida* (Fig. 5a), *R. mustelina* (Fig. 5b) *and R. delica* (Fig. 5c) all exhibited the same reducing power of 0.85 at 1 mg / ml against 0.90 at the same concentration for trolox. Reducing power value obtained for the extracts of the three species of *Russula* was appreciable and comparable to those of many edible mushrooms (Mau *et al*., 2002; Elmastas *et al*., 2006*;* Barros *et al*., 2007; Tsai *et al*., 2008; Soares *et al*., 2009). According to some authors (Prior *et al*., 2005; Karadag *et al*., 2009), the mechanism of reducing power assay is based on the transfer of electrons rather than the transfer of hydrogen atoms.

Table 2 shows the different values of EC_{50}. The value of the effective concentration EC_{50} is inversely correlated with that of the antioxidant activity, that is, the lower the EC_{50}, the higher the antioxidant activity. Values lower than 10 mg/ml are indicative of the effective antioxidant activity ((Lee *et al*., 2007; Soares *et al*., 2009; Sudha *et al.,* 2012). In this work, all the extracts exhibited EC_{50} lower than 10 mg/ml, which indicates their effectiveness. However, the lowest EC_{50} values were observed at mature stages for all three assays and for all three *Russula* mushroom species which is in agreement with the antioxidant activities obtained for the three assays. About the DPPH scavenging assay, the lowest EC_{50} values of extracts of *R. lepida*, *R. mustelina* and *R. delica* obtained at the mature stages were higher than that reported by Barros *et al*. (2007) at the same stage for the mushroom *Lactarius piperatus* (5.19±0.03 mg/L). However, these values were lower than the lowest value obtained for the mushroom *Agaricus bisporus* at stage 3 corresponding to the opened cap stage (0.41 mg/ml) (Tsai *et al*., 2008).

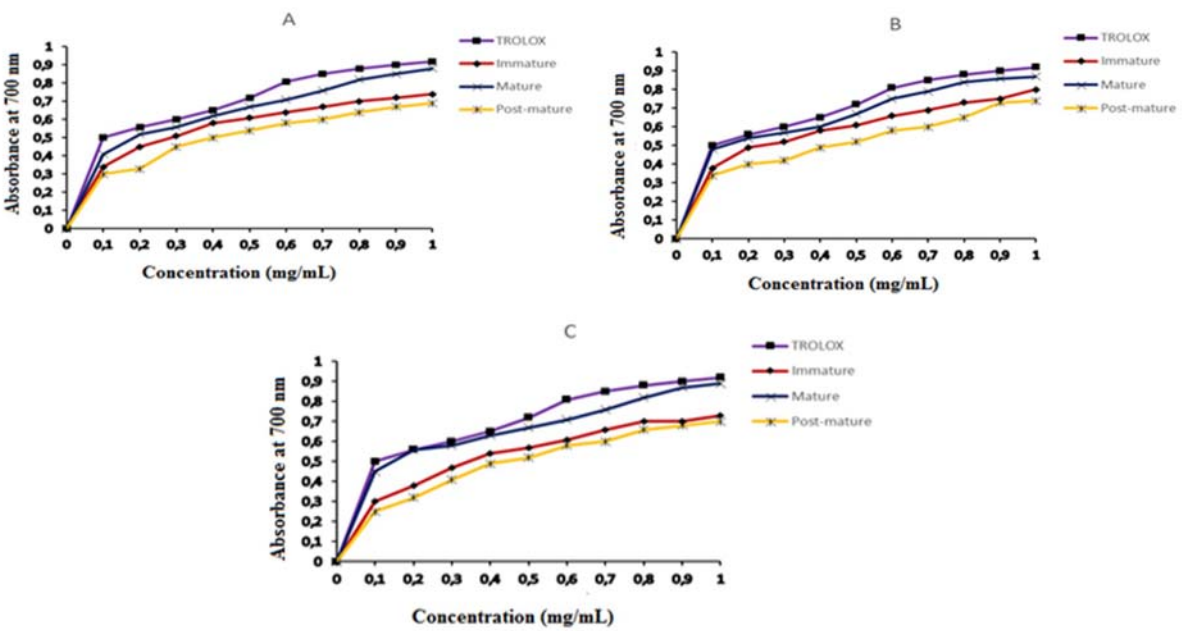

Fig. 5. Antioxidant activities measured by ferric reducing power (FRAP) assay of méthanolic extracts from three mushrooms *Russula* spp. (A: *R. lepida*; B; *R. mustelina*; *R. delica*) harvested at different stages of maturity

Table 2. EC_{50} values obtained for the antioxidants activities of three *Russula* spp. at different stages of maturity

Species	Maturity stage	EC_{50} (mg/mL)		
		DPPH	ABTS	FRAP
R. lepida	Immature	0.31±0.01[b]	0.52±0.01[b]	0.29±0.07[b]
	Mature	0.11±0.03[a]	0.31±0.05[a]	0.18±0.01[a]
	Post-Mature	0.26±0.01[c]	0.61±0.03[c]	0.44±0.03[c]
R. mustelina	Immature	0.22±0.02[b]	0.60±0.03[b]	0.31±0,02[b]
	Mature	0.19±0.09[a]	0.29±0.02[a]	0.16±0.07[a]
	Post-Mature	0.30±0,05[c]	0.69±0.05[c]	0.51±0.04[c]
R. delica	Immature	0.15±0.01[b]	0.43±0,02[b]	0.35±0.01[b]
	Mature	0.09±0.01[a]	0.33±0.01[a]	0.15±0.01[a]
	Post-Mature	0.24±0.01[c]	0.50±0.02[c]	0.50±0.02[c]

In each column different letters mean significant differences ($p < 0.05$)

With regard to ABTS cation radical scavenging assay, the lowest EC_{50} values obtained at mature stages of the three *Russula* were lower than that reported for mushrooms *Russula virescens* (0.44 mg/mL) (Hasnat *et al*., 2014) and *Pleurotus eous* (17.0±0.39 mg/ml) ((Sudha *et al.,* 2012). As for reducing power, the lowest EC_{50} values also obtained at mature stages of the mushrooms, although much higher than that of the mature stage of *Lactarius piperatus* reported by Barros *et al*. (2007) (2.29±0.02 mg/L), were however lower than those of extracts from many mushrooms reported in the literature (Barros *et al*., 2008; Tsai *et al*., 2008, Sudha *et al*., 2012; Hasnat *et al*., 2014). In sum, the three tests showed all the extracts were effective in terms of antioxidant properties. However, the mature stage with immature spores was the best stage in terms of the antioxidant properties of the methanolic extracts of the three mushroom species, as evidenced by the values of EC_{50}.

CONCLUSION

On completion of this study, it was essentially noted that the main antioxidant components of extracts of the mushrooms *R. lepida*, *R. mustelina* and *R. delica* such as total phenolic compounds, flavonoids, tannins, carotenoids (â-carotene and lycopene) and ascorbic acid had undergone variations in terms of overall contents with peaks at the mature stage with immature spores during growth of these mushrooms. The same observation was pointed for individual phenolic compounds known to be powerful antioxidants such as gallic acid, ferulic acid, catechin, rutin and coumaric acid. These results were in agreement with the changes in the antioxidant properties of the extracts of these mushrooms during growth and this was demonstrated by the effective concentration values EC_{50} found for the assays of DPPH radical scavenging, ABTS cation radical scavenging and ferric reducing antioxidant power. Consequently, the mushrooms *R. lepida*, *R. mustelina* and *R. lepida* intended for human consumption should be collected at the mature stage with immature spores so that consumers can make the most of the natural antioxidants to protect them against oxidative damage which cause chronic diseases as the cancer.

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REFERENCES

1. Addai, Z. R., A. Abdullah and S. Abd-Mutalib. 2013. Influence of ripening stages on antioxidant properties of papaya fruit (*Carica papaya* L.). *AIP Conference Proceedings* **1571**: 696-701. https://doi.org/10.1063/1.4858736

2. Anno, H. F. A., E.J.P. Kouadio, K.H. Konan, A.E. Dué, and L.P. Kouamé. 2016. Two widely consumed wild mushrooms from central Côte d'Ivoire: their proximate analysis, mineral composition and amino acids profile. *Annals Food Science and Technology* **17(2):** 139-149.

3. Azevedo, M. I., A.F. Pereira, R.B. Nogueira, F.E. Rolim, G.A. Briton, and D.V.T. Wong. 2013. The antioxidant effects of the flavonoids rutin and quercetin inhibit oxaliplatin-induced chronic painful peripheral neuropathy. *Molecular pain* **9:** 53. DOI: 10.1186/1744-8069-9-53

4. Bainbridge, Z., K. Tomlins, and A. Westby. 1996. Analysis of condensed tannins using acidified vanillin. *J Sci Food Agric* **29:** 77-79.

5. Barros, L., P. Baptista, and I.C.F.R. Ferreira. 2007. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food Chem Toxicol* **45(9):** 1731-1737. DOI: 10.1016/j.fct.2007.03.006

6. Barros, L., T. Cruz, P. Baptista, L.M. Estevinho, and I.C.F.R. Ferreira. 2008. Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem Toxicol* **46(8):** 2742-2747. DOI: 10.1016/j.fct.2008.04.030

7. Bondet, V., W. Brand-Williams, and C. Berset. 1997. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. LWT - *Food Sci Technol* **30:** 609-615. https://doi.org/10.1006/fstl.1997.0240

8. Boonkasem, P., P. Sricharoen, S. Techawongstein, and S. Chanthai. 2015. Determination of ascorbic acid and total phenolics related to the antioxidant activity of some local tomato (*Solanum lycopersicum*) varieties. *Der Pharma Chemica* **7(4) :** 66-70.

9. Cayan F., E. Deveci, G. Tel Cayan, and M.E. Duru. 2020. Identification and quantification of phenolic acid compounds of twenty six mushrooms by HPLC–DAD. *J Food Meas Charact* **14:** 1690-1698. https://doi.org/10.1007/s11694-020-00417-0

10. Cheung, L. M., P.C.K. Cheung, and V.C.F. Ooi. 2003. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem* **81:** 249-255. https://doi.org/10.1016/S0308-8146(02)00419-3

11. de Carvalho, L.M.J., P.B. Gomes, R. Godoy, R.., A.C.R.A. Vieira and S.R.R. Ramos. 2012. Total carotenoid content, á-carotene and â-carotene, of landrace pumpkins (*Cucurbita moschata Duch*): a preliminary study. *Food Res Int* **47(2):** 337-340. https://doi.org/10.1016/j.foodres.2011.07.040

12. Dûma, M., I. Alsiòa, L. Dubova and I. Erdberga. 2018. Bioactive compounds in tomatoes at different stages of maturity. *Proc Latv Acad Sci Section B. Natural, Exact, and Applied Sciences* **72 (2):** 85-90. DOI: https://doi.org/10.2478/prolas-2018-0014

13. Elmastas, M., O. Isildak, I. Turkekul and N. Temur. 2007. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *J Food Compost Anal* **20(3):** 337-345. DOI: 10.1016/j.jfca.2006.07.003

14. Fawole, O.A. and U.L. Opara. 2013. Effects of maturity status on biochemical content, polyphenol composition and antioxidant capacity of pomegranate fruit arils (cv.'Bhagwa'). *S Afr J Bot* **85:** 23-31. https://doi.org/10.1016/j.sajb.2012.11.010

15. Ferreira, I.C.F.R., P. Baptista, M. Vilas-Boas and L. Barros. 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. *Food Chem* **100(4)**: 1511-1516. https://doi.org/10.1016/j.foodchem.2005.11.043

16. Friedman, M. and H.S. Jürgens. 2000. Effect of pH on the stability of plant phenolic compounds. *J Agric Food Chem* **48(6)**: 2101-2110. DOI: 10.1021/jf990489j

17. Gbotognon, O.J., D.M. Koffi, K.H. Konan and E.J.P. Kouadio. 2019a. Effect of ripening stage on organic acid profiles and antinutrient contents of three species of wild edible mushroom *Russula* spp. *Food and Environment Safety* **18(3)**: 191-200.

18. Gbotognon, O.J., K.A. Kouassi, K.H. Konan and E.J.P. Kouadio. 2019b. Proximate composition and nutritional value of three edible mushrooms ectomycorrhizal (*Russula mustelina, Russula Delica and Russula Lepida*) from Côte d'Ivoire according to the maturity stages. *World Journal of Advanced Research and Reviews* **02(03):** 021-030. **DOI:** 10.30574/wjarr.2019.2.3.0040.

19. Hasnat, M.A., M. Pervin, T. Debnath and B.O. Lim. 2014. DNA protection, total phenolics and antioxidant potential of the mushroom *Russula virescens*. *J Food Biochem*: **38(1)**: 6-17. DOI: 10.1111/jfbc.12019

20. Hatano, T., H. Kagawa, T. Yasuhara and T.T. Okuda. 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging affects. *Chem Pharm Bull* **36(6)**: 2090-2097. DOI: 10.1248/cpb.36.2090

21. Hdider, C., R. Ilahy, I. Tlili, M.S. Lenucci and G. Dalessandro. 2013. Effect of the stage of maturity on the antioxidant content and antioxidant activity of high-pigment tomato cultivars grown in Italy. *Food* **7:** 1-7.

22. Islam, T., X. Yu and B. Xu. 2016. Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China. LWT- *Food Sci Technol* **72**: 423-431. DOI: 10.1016/j.lwt.2016.05.005

23. Joshi, H., A. Kuna, M.N. Lakshmi, M. Shreedhar and A.K. Kumar. 2017. Effect of stage of maturity, ripening and storage on antioxidant content and activity of *Mangifera indica* L. var. Manjira. *Int J Food Sci Nutr* **2(3)**: 01-09.

24. Karadag, A., B. Ozcelik and S. Saner. 2009. Review of methods to determine antioxidant capacities. *Food Anal Methods* **2**: 41-60. DOI:10.1007/s12161-008-9067-7

25. Katsuragi, H., K. Shimoda, N. Kubota, N. Nakajima and H. Hamada. 2010. Biotransformation of cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid by plant cell cultures of *Eucalyptus perriniana*. *Biosci Biotechnol Biochem* **74**: 1920-1924. DOI: 10.1271/bbb.100335

26. Kim, Y. J. 2007. Antimelanogenic and antioxidant properties of gallic acid. *Biol Pharm Bull* **30(6):** 1052-1055. DOI: 10.1248/bpb.30.1052

27. Kouassi, K.A. 2016. *Propriétés biochimiques, nutritionnelles et potentialités bioactives de six champignons ectomycorhiziens comestibles du centre de la Côte d'Ivoire*. Thèse N° 323 de l'Université

28. Kouassi, K.A. E.J.P. Kouadio, K.M. Djè, A.E. Dué and L.P. Kouamé. 2016. Edible Ectomycorrhizal Mushrooms *Russula* spp. of Côte d'Ivoire: Total Phenolic Content, HPLC-Profiles of Phenolic Compounds and Organic Acids, Antioxidant Activities. *J Agric Chem Environ* **5**: 73-84. DOI 10.4236/jacen.2016.52008

29. Lee, Y.L., M.T. Yen and J.L. Mau. 2007. Antioxidant properties of various extracts from *Hypsizigus marmoreus*. *Food Chem* **104**, 1-9. https://doi.org/10.1016/j.foodchem.2006.10.063

30. Mau, J. L, C.N. Chang, S.J. Huang and C.C. Chen. 2004. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chem* **87**: 111-118. https://doi.org/10.1016/j.foodchem.2003.10.026

31. Mau, J.L., H.C. Lin and S.F. Song. 2002. Antioxidant properties of several specialty

mushrooms. *Int Food Res J* **35(6)**: 519-526. https://doi.org/10.1016/S0963-9969(01)00150-8

32. Meda, A., C.E. Lamien, M. Romito, J. Millogo and O.G. Nacoulma. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem* **91(3)**: 571-577. https://doi.org/10.1016/j.foodchem.2004.10.006

33. Mircea, C., O. Cioanca, C. Iancu, G. Tataringa and M. Hancianu. 2015. *In vitro* antioxidant activity of some extracts obtained from *Agaricus bisporus* brown, *Pleurotus ostreatus* and *Fomes fomentarius*. *Farmacia* **63(6)**: 927-933.

34. Nagata, M. and I. Yamashita. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon Shokuhin Kogyo Gakkaish* **39(10)**: 925-928. DOI: https://doi.org/10.3136/nskkk1962.39.925

35. Oszmiañski, J., S. Lachowicz, J. Gorzelany and N. Mat³ok. 2018. The effect of different maturity stages on phytochemical composition and antioxidant capacity of cranberry cultivars. *Eur Food Res Technol* **244**: 705-719. DOI: 10.1007/s00217-017-2994-z

36. Palacios, I., M. Lozano, C.D. Moro, M. Arrigo, M.A. Rostagno, J.A. Martínez, A. García-Lafuente, E. Guillamón and A. Villares. 2011. Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chem* **128 (3)**: 674-678. https://doi.org/10.1016/j.foodchem.2011.03.085

37. Pekkarinen, S. S., I.M. Heinonen and A.I. Hopia. 1999. Flavonoids, quercetin, myricetin, kaemferol and (+) catechin and antioxidants in methyl linoleate. *J Sci Food Agric* **79(4)**: 499-506.

38. Pongracz, G., H. Weiser and D. Matzinger. 1971. Tocopherols- Antioxydant. *Fat Science and Technology* **97:** 90-104.

39. Prior, R. L, X. Wu and K. Schaich. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* **53(10)**: 4290-4302. https://doi.org/10.1021/jf0502698

40. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* **26(9-10)**: 1231-1237. DOI: 10.1016/s0891-5849(98)00315-3

41. Ren, X., J. Wang, L. Huang, K. Cheng, M. Zhang and H. Yang. 2020. Comparative Studies on bioactive compounds, ganoderic acid biosynthesis, and antioxidant activity of pileus and stipes of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (Agaricomycetes) fruiting body at different growth stages. *Int J Med Mush* **22(2)**: 133-144. DOI: 10.1615/IntJMedMushrooms.2020033683

42. Ribeiro, B., P. Valentao, P. Baptista, R.M. Seabra and P.B. Andrade. 2007. Phenolic compounds, organic acids profiles and antioxidative properties of beefsteak fungus (*Fistulina hepatica). Food Chem Toxicol* **45(10)**: 1805-1813. https://doi.org/10.1016/j.fct.2007.03.015

43. Robaszkiewicz, A., G. Bartosz, M. Lawrynowicz and M. Soszynski. 2010. The role of polyphenols, â-carotene, and lycopene in the antioxidative action of the extracts of dried, edible mushrooms. *J Nutr Metab* ID 173274: 9. DOI: 10.1155/2010/173274

44. Rodriguez-Amaya, D. B. and M. Kimura. 2004. *Harvest Plus Handbook for Carotenoid Analysis*. International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT), Washington DC and Cali

45. Saha, A. K., S. Acharya and A. Roy. 2012. Antioxidant level of wild edible mushroom:

Pleurotus djamor, Boedijn. *Journal of Agricultural Technology* **8(4)**: 1343-1351.

46. Shah, P. and H.A. Modi. 2015. Comparative Study of DPPH, ABTS and FRAP Assays for Determination of Antioxidant Activity. *Int J Res Appl Sci Eng Technol* **3(6):** 636-641.

47. Shalaby, E.A. and S.M.M. Shanab. 2013. Antioxidant compounds, assays of determination and mode of action. *Afr J Pharm Pharmacol* **7(10**): 528-539.DOI: 10.5897/ AJPP2013. 3474

48. Shen, Y., Y. Guan, X. Song, J. He, Z. Xie, Y. Zhang and D. Tang. 2019. Polyphenols extract from lotus seedpod (*Nelumbo nucifera Gaertn*.): Phenolic compositions, antioxidant, and antiproliferative activities. *Food Science & Nutrition* **7(9)**: 3062-3070. DOI: 10.1002/ fsn3.1165

49. Singleton, V. L, R. Orthofer and R.M. Lamuela-Raventos. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology* **299:** 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

50. Soares, A.A., C.G.M. Souza, F.M. Daniel, G.P. Ferrari, S.M.G. Costa and R.M. Peralta. 2009. Antioxidant activity and phenolic content of *Agaricus brasiliensis* (*Agaricus blazei Murrill*) in two stages of maturity. *Food Chem* **112(4)**: 775-781. http://dx.doi.org/10.1016/ j.foodchem.2008.05.117

51. Srinivasan, M., A.R. Sudheer and V.P. Menon. 2007. Ferulic acid: therapeutic potential through its antioxidant property. *J Clin Biochem Nutr* **40**: 92-100. DOI: 10.3164/jcbn.40.92

52. Sudha. G., V. Sabapathy, B.I.S. Rajan and L. Ponnusamy. 2012. Antioxidant activity of various extracts from an edible mushroom *Pleurotus eous*. *Food Sci Biotechnol* **21(3)**: 661-668. DOI: 10.1007/s10068-012-0086-1

53. Tilahun, S., D.S. Park, S. Tifsehit, R.C. Han and C.S. Jeong. 2019. Maturity stages affect nutritional quality and storability of tomato cultivars. CyTA - *Journal of Food* **17(1)**: 87-95. https://doi.org/10.1080/19476337.2018. 1554705

54. Tsai, S.Y., T.P. Wu, S.J. Huang and J.L. Mau. 2008. Antioxidant properties of ethanolic extracts from culinary-medicinal button mushroom *Agaricus bisporus* (J. Lange) Imbach (Agaricomycetideae) harvested at different stages of maturity. *Int J Med Mush* **10(2)**: 127-137. DOI: 10.1615/intjmedmushr. v10.i2.30

55. Turkoglu, A., M.E. Duru, N. Mercan, I. Kivrak and K. Gezer. 2007. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chem* **101(1)**: 267-273. https://doi.org/10.1016/j.foodchem. 2006.01.025

56. Wulandari, P., B.S. Daryono and Supriyadi. 2017. The effect of ripening stages on the antioxidant potential of melon (*Cucumis melo* L.) cultivar Hikapel. *AIP Conference Proceedings* **1854 (1):** 020-039

57. Xie, G., J. Wang, X. Xu, R. Wang, X. Zhou and Z. Liu. 2016. Effect of different ripening stages on bioactive compounds and antioxidant capacity of wild *Rosa laevigata Michx*. *Food Sci Technol* **36(3)**: 396-400. http://dx.doi.org/ 10.1590/1678-457X.00715

58. Yahia, E.M., F. Gutiérrez-Orozco and M.A. Moreno-Pérez. 2017. Identification of phenolic compounds by liquid chromatography-mass spectrometry in seventeen species of wild mushrooms in Central Mexico and determination of their antioxidant activity and bioactive compounds. *Food Chem* **226**: 14-22. DOI: 10.1016/j.foodchem.2017.01.044

59. Yen, G. C. and C.Y. Hung. 2000. Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian tsao (*Mesona procumbens* Hemsl.). *Food Res Int* **33**: 487-492. DOI:10.1016/S0963-9969(00)00073-9