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EFFECT OF RIPENING STAGE ON ORGANIC ACID PROFILES AND ANTINUTRIENT CONTENTS OF THREE SPECIES OF WILD EDIBLE MUSHROOM *RUSSULA SSP.*

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Abstract: *The present study was aimed at investigating the antinutrient and organic acid contents of three wild edible mushrooms from Russula genus as a function of their ripening stage. Fresh mushrooms Russula lepida, Russula mustelina and Russula delica were harvested from their natural habit in Brobo area's (7°43'0" N and 4°42'0" W) in center Côte d'Ivoire. The fresh mushrooms were oven dried and ground to obtain the crude flour. Phytate and oxalate contents were investigated using standard colorimetric methods, while organic acid profiles were performed by using HPLC analytical methods. The antinutrient composition showed that the greatest content in phytates (1.60±0.10 mg/100 g) and oxalates (5.17±0.01 mg/ 100 g) were observed respectively, in R. lepida and R. mustelina immature fruiting bodies. These values are much lower than the standard safe limits. As regards organic acid profiles, they revealed the presence of at least fourteen organic acids namely benzoic, oxalic, fumaric, succinic, malic, tartaric, ascorbic, citric, lactic, adipic, propionic, formic, shikimic and acetic acids. The main organic acids in R. lepida were lactic (49.70 mg/ 100 g DW, for post-mature stage), fumaric (36.00 and 31.22 mg/ 100 g DW, respectively for immature and mature stage), citric (21.60 and 31.60 mg/ 100 g DW, respectively for immature and mature stage) and succinic (21.00 mg/ 100 g DW for post-mature stage) acids. Lactic acid (47.90 mg/ 100 g DW) was the dominating organic acid in R. mustelina at immature stage, whereas citric (30.20 mg/ 100 g DW at post-mature stage) and ascorbic acids (21.80 mg/ 100 g DW at post-mature stage) were major in R. delica. R. lepida showed the higher total organic acid contents (ranging from 113.13 mg/ 100 g in immature stage to 103.31 mg/ 100 g in post-mature stage) whatever the ripening stage. As for R. mustelina, the immature stage was better (100.02 mg/ 100 g DW), while R. delica showed better total organic acid content (83.33 mg/ 100 g DW) at post-mature stage.*

Keywords*: Organic acids, edible mushroom, ripening stage, Russula lepida, Russula mustelina, Russula delica.*

1. Introduction

Mushrooms are the best-known example of macrofungi which are wild edible fungi species with large and visible fruiting bodies [1]. Mushrooms species having a close relationship with the host (usually a tree) are mycorrhizal (symbiotic). Those living on dead organic matter are saprotrophic (saprophytes) while species that live on other species in a non-symbiotic relationship are parasitic species

[2]. The mycorrhizal species play a vital ecological role through the symbiotic relationships that they form with trees. They enable trees to grow in nutrient-poor soils. For example, the trees of the miombo woodland of central and southern Africa and the woodland itself would not exist without their fungal partners [3, 1]. Moreover, mushrooms are excellent bio-degraders since they decompose organic waste which releases the nutrients in celluloses, hemicelluloses and lignin. They therefore help in cleaning the environment and recycling the nutrients [4].

The increased interest in consumption of mushrooms as food is as a result of their nutritional, antioxidant and therapeutic values [5]. According to Boa [1], about 1200 species of fungi are used in eighty-five different countries for their gastronomic value and/or medicinal properties. Indeed, wild edible mushrooms are found to be highly rich in proteins, minerals, vitamins, crude fibre and carbohydrate with low fat and oil content [6, 7, 8]. Dué *et al*. [8] reported that they could be used as valuable substitutes for meat or fish especially in developing countries for malnourished children suffering from kwashiorkor (a protein deficiency condition) and for pregnant women. Chittaragi and Naika [9] argue that mushrooms can provide balancing diet compounds in enough quantities for human nutrition. Due to the low fat and oil content, they are recommended as good source of food supplement for patients with cardiac problems or at risk with lipid induced disorders. Also, several mushroom species have been described to exhibit varied biological and medicinal properties such as antioxydants, antibacterial, antifungal, antiviral, anti-inflammatory, antimutagenic, antitumoral and anti-diabetic activities [10, 11, 12, 13, 14, 15]. Therefore, mushrooms have been categorized as therapeutic foods [16]. The bioactive and taste properties of mushrooms are conferred by compounds such as phenolic derivatives and organic acids [17]. Phenolic compounds and organic acids (especially malic, tartaric, citric, and succinic acids) may have a protective role against various diseases due to their antioxidant activity [18, 19]. Furthermore, organic acids were reported to be responsible of organoleptic characteristics of fruits and vegetables, especially mushrooms [20].

In the center of Côte d'Ivoire, wild edible ectomycorrhizal mushrooms of *Russula* genus are widely collected and consumed in many households or sold for the improvement of icomes and livelihood of farmers [21]. The species *Russula lepida*, *Russula mustelina* and *Russula delica* were previously investigated for their bioactive compounds and antioxidative properties [22]. Considering the importance of organic acids, the present paper focuses on their acid organic profile and antinutrient contents as a function of their ripening stage.

2. Materials and methods

Mushroom collection and preparation of sample

Fresh mushrooms were harvested at different ripening stages (immature, mature and post-mature) from their natural habit in Brobo area's (7°43'0" N and 4°42'0" W) in Côte d'Ivoire. Taxonomic identification was achieved by Dr Souleymane Yorou Nourou (Abome Calavy University of Benin/ Munich University of Germany), as *R. lepida*, *R. mustelina* and *R. delica*. After collection, mushrooms were placed in a cooler with ice to keep their freshness and then

transported to the laboratory for flour preparation. Fresh mushrooms (500 g) were cleaned to distilled water then, sorted and free from any kind of waste, drained, and dried at 45°C in an oven for 48 h. Dried mushrooms were ground using a blender to obtain the crude flours.

Antinutritional factors analysis

The titration method as described by Day and Underwood [23] was followed. 1 g of sample was weighed into 100 mL conical flask. 75 mL 3 MH_2SO_4 was added and stirred for 1 h with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25 mL of the filtrate was then taken and titrated while hot against 0.05 M $KMnO_4$ solution until a faint pink colour persisted for at least 30 s. The oxalate content was then calculated by taking 1 mL of 0.05 m $KMnO_4$ as equivalent to 2.2 mg oxalate [24].

Phytate was extracted according to the procedure described by Mohammed *et al*. [25]. 1.0 g Sample was extracted with 3% tri-chloro acetic acid (TCA) at 37°C for 45 min. with simple shaking followed by centrifugation and extractation by using anion exchange column. The extracted phytate (0.2 mL) was mixed with 4.6 ml of distilled water and 0.2 mL of chromogenic solution and the tubes were heated in a water bath at 95°C for 30 min, and then were allowed to cool. The developed color was read at 830 nm against blank. Standard phytate solution was prepared by dissolving sodium phytate in distilled water to prepare different phytate concentrations as described above in the tested samples. The amount of phytate in the tested samples was expressed as mg phytate/100 g sample.

Extraction of Organic Acids

The organic acids of each dried sample of mushroom were extracted according Hasib *et al*. [26] method by grinding (Waring Blendor, Polychimie, Abidjan, Côte d'Ivoire) in distilled water (1:10, w/v) and clarified by centrifuging at 4000 rpm for 30 minutes. The supernatant was first filtered through Whatmann 4 paper, then through 0.45 µm filter (Millipore; Sartorius AG, Goëttingen, Germany) and stored at −20˚C prior further use.

HPLC Analysis of Organic Acids

The separation of the organic acids was carried out as previously reported [22] by using a system consisting of an analytical HPLC unit (Shimadzu Corporation, Japan) in conjunction with a column heating device set at 35˚C with the aid of an oven Meta Therm TM (Interchrom, France), with an ions exclusion column ICSep ICE ORH-801 (40 cm × 5 µm, In terchom, France). The system was also coupled to a pump (Shimadzu LC-6A Liquid Chromatograph), a UV detector (Shimadzu SPD-6A UV Spectrophotometric Detector) and an integrator (Shimadzu Chromatopac CR 6A). Elution was carried out isocratically with sulphuric acid 0.04 N, at a solvent flow rate of de 0.6 mL/min and detection was performed at 210 nm. Organic acids in mushroom extracts were identified by comparing the retention times and spectral data obtained from standards under the same conditions. Quantitation was performed by comparing the peak areas with those of the respective external standards.

Statistical analysis

All chemical analyses and assays were performed in triplicate, unless otherwise indicated. Results were expressed as mean values ± standard deviation (SD).

Analysis of variance (ANOVA) followed by Duncan's test was performed to test for differences between means by employing XLstat 2019 statistical software. Significance of differences was defined at the 5% level ($P<0.05$). Principal component analysis (PCA) was also used in order to discover relationships between independent variables (mushroom species and organic acids).

3. Results and discussion

Antinutritional factors

The phytate and oxalate contents as a function of ripening stage of the three wild edible mushroom *Russula* species are presented in table 1. These results showed that the measured antinutritional parameters decreased significantly during ripening of the different mushroom species. We observed the highest content of phytate (1.60±0.10 mg/100 g) in *R. lepida*, especially in immature fruiting bodies. Gaur *et al*. [27] reported low values of phytate ranging from 0.11 to 0.19 mg/ 100 g for six selected edible mushrooms in India. However, the phytate contents of the present study were much lower than those found for some cultivated and wild edible mushrooms collected from Ethiopia, ranged from 31.3 to 242.8 mg/ 100 g DW [28]. Furthermore, the phytate contents of studied *Russula* species are over13 times lower than the standard safe limit of 22.10 mg/ 100 g [29]. This suggest that whatever the ripening stage, the three studied mushroom species could be consumed without risk of toxicity associated with phytate concentration. As regards oxalate, the highest content (5.17±0.01 mg/ 100 g) was estimated in *R. mustelina* immature fruiting bodies. Oxalate contents obtained in this study were higher than that found for the Oyster mushroom *Pleurotus ostreatus* (0.41 mg/ 100 g) [30], but quite lower than the tolerable limit of 105 mg/100 g recommended by World Health Organization. This suggests the safe for consumption of these mushrooms at any ripening stage. Moreover, it's noteworthy that food processes such as boiling or cooking, fermentation and milling could reduce drastically or remove antinutrient elements [31].

Table 1.

Antinutritional factors content as a function of ripening stage of three *Russula* species

Parameters	Stage	Species		
		R. lepida	***R. mustelina***	***R. delica***
Phytate (mg/100g)	**I**	0.60 ± 0.20^{aA}	1.07 ± 0.02^{aB}	1.60 ± 0.10^{bC}
	M	0.40 ± 0.30^{aA}	0.56 ± 0.22^{bA}	1.46 ± 0.25^{abB}
	PM	0.23 ± 0.23^{aA}	0.53 ± 0.15^{bA}	1.20 ± 0.10^{aB}
Oxalate (mg/100g)	**I**	2.83 ± 0.02^{cA}	5.17 ± 0.01^{cC}	4.34 ± 0.03^{cB}
	M	2.10 ± 0.01^{bA}	4.13 ± 0.02^{bC}	3.26 ± 0.02^{bB}
	PM	0.77 ± 0.02^{aA}	3.03 ± 0.01^{aB}	3.08 ± 0.01^{aC}

Values are mean ± standard deviation of three measurements (n = 3). I: immature; M: mature; PM: post-mature. ^{a,b,c}Identical script indicate no significant difference between mean values in line. ^{A,B,C}Identical script indicate no significant difference between mean values in column.

Organic acids composition

Organic acids are categorized as important food components for the formation of taste and flavour, and determination of the quality and safety of food and food products [32]. They generate important effects on food such as sensorial, antioxidant and acidifying properties [33]. The HPLC-UV analysis (figure not shown) allowed the identification of at least fourteen organic acids namely benzoic, oxalic, fumaric, succinic, malic, tartaric, ascorbic, citric, lactic, adipic, propionic, formic, shikimic and acetic acids. Most of these organic acids has already been found in edible mushrooms [34, 35].

Results summarized in **table 2** indicated that the three mushroom species were characterized by a high variation in their organic acid content. Moreover, these organic acid contents were strictly dependent on ripening stage. For the three ripening stages, *R. lepida* showed the highest total organic acid contents (ranging from 103.31 to 113.13 mg/ 100 g DW), mainly consisting of lactic (49.70 mg/ 100 g DW, for post-mature stage), fumaric (36.00 and 31.22 mg/ 100 g DW, respectively for immature and mature stage), citric (21.60 and 31.60 mg/ 100 g DW, respectively for immature and mature stage) and succinic (21.00 mg/ 100 g DW for post-mature stage) acids. Lactic acid is the product of glycolysis under anaerobic condition. In food, it's characterized as a natural flavour enhancer, mild and lingering [36]. Fumaric and citric acids are well known for their antimicrobial and antioxidant properties making them important agents in the prevention of mushroom browning [37]. *R. mustelina* presented total acid content of 100.02 mg/ 100 g DW at immature stage, with dominating lactic acid (47.90 mg/ 100 g DW), whereas *R. delica* had a total acid content of 83.33 mg/ 100 g DW at post-mature stage. The major acids in *R. delica* were citric acid (30.20 mg/ 100 g DW at post-mature stage) followed by ascorbic acid (21.80 mg/ 100 g DW at post-mature stage). Total organic acid contents found in this study were higher than those found for *Fustulina hepatica* [38].

Table 2.

Organic acids content as a function of ripening stage of three *Russula* species

Organic Acids	Retention time (min)	Stage	Species		
			R. lepida	***R. mustelina***	***R. delica***
Butyric acid	2.53	**I**	nd	nd	0.06 ± 0.01^{aA}
		M	nd	0.05 ± 0.01^{aA}	nd
		PM	nd	nd	nd
Benzoic acid	5.62	**I**	4.00 ± 0.02^{bB}	nd	0.11 ± 0.01^{aA}
		M	3.63 ± 0.03^{aA}	nd	nd
		PM	nd	1.26 ± 0.02^{aA}	3.84 ± 0.01^{bB}
Oxalic acid	9.66	**I**	0.62 ± 0.03^{bC}	0.04 ± 0.01^{aA}	0.61 ± 0.01^{bA}
		M	0.52 ± 0.03^{bB}	0.41 ± 0.01^{aC}	0.61 ± 0.01^{cA}
		PM	0.06 ± 0.01^{aA}	0.21 ± 0.02^{bB}	1.14 ± 0.01^{cB}
Fumaric acid	10.48	**I**	36.00 ± 0.04^{bB}	2.62 ± 0.02^{aB}	nd
		M	31.20 ± 0.02^{cA}	2.14 ± 0.01^{aA}	6.47 ± 0.02^{bB}
		PM	nd	3.10 ± 0.02^{aC}	3.14 ± 0.01^{aA}

Succinic acid	11.25	**I**	5.31±0.02^{bB}	17.70±0.03^{aC}	nd
		M	4.58±0.02^{bA}	0.76±0.02^{aA}	nd
		PM	21.00±0.03^{cC}	1.88±0.3^{aB}	8.16±0.01^{bA}
Malic acid	12.95	**I**	3.27±0.01^{bB}	nd	0.50±0.01^{aA}
		M	0.43±0.02^{aA}	2.49±0.02^{bA}	5.19±0.06^{cB}
		PM	nd	2.85±0.02^{aB}	9.82±0.03^{bC}
Tartaric acid	15.54	**I**	12.10±0.02^{bB}	10.10±0.03^{cC}	3.76±0.02^{aA}
		M	11.40±0.03^{bB}	1.29±0.02^{aA}	6.96±0.01^{bB}
		PM	9.27±0.02^{bA}	9.45±0.01^{bB}	13.10±0.01^{aC}
Ascorbic acid	16.31	**I**	7.87±0.02^{bB}	3.48±0.02^{aB}	nd
		M	8.43±0.02^{bC}	0.48±0.35^{aA}	nd
		PM	4.33±0.02^{aA}	nd	21.80±0.05^{bA}
Citric acid	17.7	**I**	29.60±0.03^{bB}	5.90±0.01^{aC}	30.20±0.01^{bC}
		M	31.60±0.02^{cC}	1.50±0.02^{aA}	2.46±0.03^{bB}
		PM	7.94±0.02^{cA}	2.90±0.00^{bB}	2.16±0.02^{aA}
Lactic acid	20.75	**I**	6.03±0.03^{bA}	47.90±0.01^{cC}	0.46±0.02^{aA}
		M	5.98±0.02^{bA}	5.01±0.01^{aA}	11.40±0.01^{cC}
		PM	49.70±0.02^{cB}	12.10±0.01^{bB}	7.52±0.03^{aB}
Adipic acid	22.42	**I**	0.79±0.02^{cC}	0.52±0.03^{bA}	0.02±0.00^{aA}
		M	0.54±0.02^{aA}	1.13±0.02^{bB}	2.22±0.02^{cB}
		PM	0.60±0.01^{aB}	1.10±0.01^{bB}	nd
Propionic acid	24.15	**I**	3.39±0.02^{cB}	1.53±0.03^{bA}	0.94±0.02^{aB}
		M	4.14±0.02^{cC}	0.41±0.01^{aB}	0.63±0.02^{bA}
		PM	2.00±0.02^{bA}	0.29±0.01^{aC}	5.45±0.02^{cC}
Formic acid	28.04	**I**	1.42±0.03^{bC}	0.24±0.02^{aA}	2.52±0.02^{cC}
		M	0.99±0.02^{cB}	0.21±0.01^{aA}	0.59±0.02^{bB}
		PM	0.26±0.02^{aA}	0.51±0.02^{bB}	0.52±0.02^{bA}
Shikimic acid	34.22	**I**	2.73±0.02^{bA}	7.24±0.02^{cB}	0.02±0.00^{aA}
		M	3.78±0.01^{cB}	1.59±0.01^{aA}	1.65±0.02^{bB}
		PM	5.60±0.02^{bC}	1.85±0.01^{aA}	6.68±0.02^{cC}
Acetic acid	36.46	**I**	nd	2.75±0.01^{aC}	nd
		M	nd	0.49±0.01^{aA}	1.43±0.04^{bA}
		PM	2.55±0.02^{bA}	0.88±0.02^{aB}	nd
Total		**I**	113.13±0.03	100.02±0.02	39.2±0.02
		M	107.22±0.02	17.96±0.01	39.61±0.02
		PM	103.31±0.02	38.38±0.03	83.33±0.02

Values are mean ± standard deviation of three measurements (n = 3). I: immature; M: mature; PM: post-mature. ^{a,b,c}Identical script indicate no significant difference between mean values in line. ^{A,B,C}Identical script indicate no significant difference between mean values in column.

The principal component analysis (PCA) based on the organic acid contents obtained for the three mushroom species as a function of ripening stage is presented in Figure 1. In terms of information, the principal components 1 and 2 contained more than 80 % (81.62) of the total variance explained with eigenvalues for the first two components greater than unity (respectively, 4.226 and 2.922) (Table 3). This value of the total variability is relatively significant. Also, the Kaiser-Meyer-Olkin (KMO) criterion is 0.761 (Table 4) suggesting that sampling is acceptable for the validity of PCA test in this study.

Table 3.

Eigen values of the main components

	F1	**F2**
Eigenvalues	4.226	2.922
Variability (%)	52.262	29.220
Cumulated Percentage	52.262	81.482

Table 4.

Sampling accuracy	
RT	0.149
L1	0.770
L2	0.791
L3	0.802
M1	0.657
M2	0.656
M3	0.957
D1	0.855
D2	0.715
D3	0.769
KMO	**0.761**

The PCA analysis (Figure 1) makes it possible to distinguish the mushroom species in two large groups. One formed by *R. delica* and *R. lepida* and the other represented by *R. mustelina*. The first one better expresses the axis of the first component (52.41% of the total variance explained) and the second the axis of the second component (29.21% of the total variance explained). The first group is characterized by the relationship *R. delica* and *R. lepida* - fumaric, citric, shikimic, malic, tartaric, ascorbic, succinic acids. For *R. mustelina* (which constitutes the second group), PCA analysis revealed a close relation with lactic, succinic and tartaric acids.

The ripening stages were used as additional variables for the PCA analysis (Figure 1). Results suggest a difference in the influence of the ripening stages on the organic acid contents, contributing in different ways to the constitution the study axes.

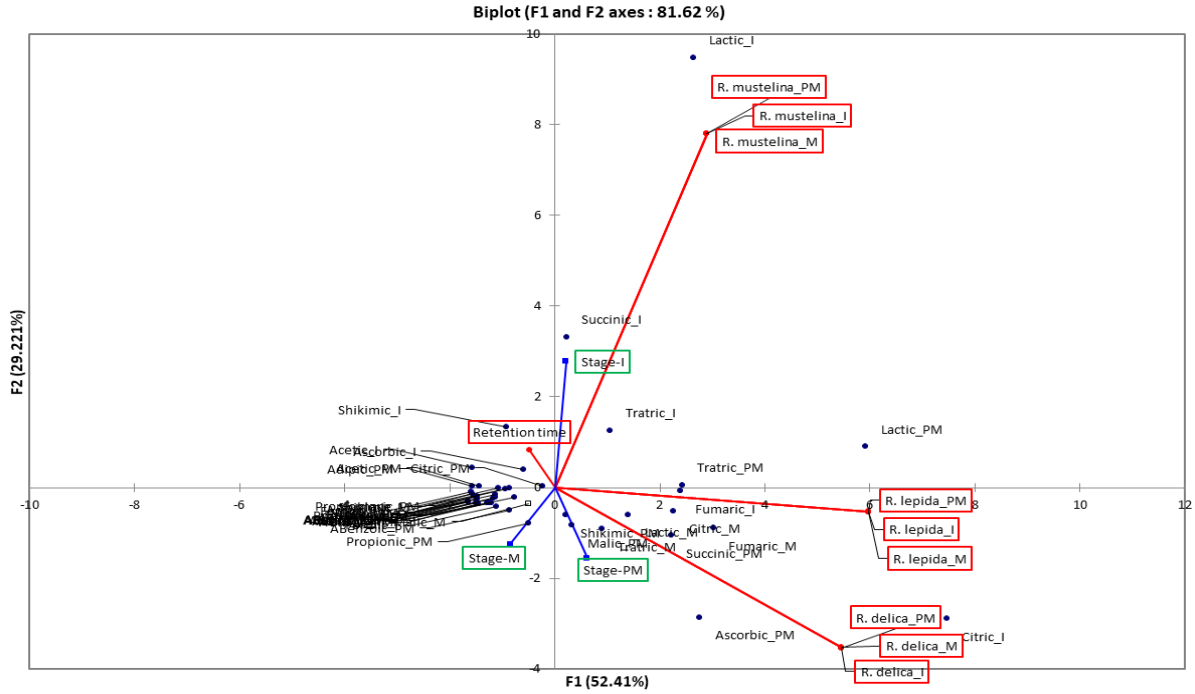

Fig. 1. PCA analysis for organic acids content as a function of ripening stage of three *Russula* species

4. Conclusion

Based on the present study, it appears that *Russula* species from Côte d'Ivoire contained a wide range of organic acids (fourteen). These organic acids are unevenly distributed (with widely varying proportions) according to the species and the ripening stages. *R. lepida* shows the higher total organic acid contents for all ripening stages mainly consisting of lactic, fumaric, citric and succinic. For *R. delica*, the major organic acids were citric and ascorbic acids found at post-mature stage, while lactic acid was the dominating acid at the immature stage in *R. mustelina*. The PCA analysis suggests a relationship between *R. delica* and *R. lepida* which would forme a group, whereas *R. mustelina* constitutes another group.

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