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Change in Iodine Contents during Ripening of two *Capsicum annuum* L. Cultivars Fruits at Brazzaville, CONGO

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ABSTRACT----To identity iodine, assess its concentration and analyse the variation of this concentration following the ripeness stages, its titrating from Capsicum annuum L. dried fruit extract by sodium thiosulfate and starch solution test were achieved. Small and big Capsicum annuum cultivars were planted according to completely randomised design onto eight rows with eight seedlings whose four was useful. One-factor Anova incorporating the means comparison according to Student-Newman-Keuls' test at 5% level was applied. Likewise, Pearson's linear correlation at 1‰ probability was used. Results shown that small and big Capsicum annuum L. cultivars contained iodine. People should consum Capsicum annuum L. fruits to avoid goiter. Other animal and plant foods also contain iodine. Iodine concentration discriminated the fruit ripeness stages in small Capsicum annuum L. cultivar. At the taste pre-ripeness stage (PMG), iodine concentration was higher than the ripe one (MG). Consequently, the consumption of its fruits is recommended at taste pre-ripeness stage to optimise the ingesting of iodine. In the same way, iodine concentration of small Capsicum annuum L. cultivar at taste pre-ripeness stage was significantly higher than the big one. At such phenological stage it will be advised people to eat small Capsicum annuum L. fruit than the big one when fruit changes. Duration of "sowing-flowering", "sowing-fruit set" and "sowing-fructification" in small Capsicum annuum L. cultivar was shorter than in the big one. Such shorter duration of small Capsicum annuum L. will allow the early obtention of its fruits than those from the big one and thus, their early selling.

Keywords----goiter, mental deficiency, congenital malformations, completely randomised design, groundness, sowing, fruit set

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1. INTRODUCTION

Capsicum annuum L. is dicotyledon plant of the Solanaceae (Guyot, 1992). Asia, America and Africa yield 88.30% of the world yielding. In the World, China is the largest pepper producer with yearly average yielding of 125.000.000 tons (Anonyme, 2006). In Republic of Congo, no information is available about the yielding as well as people dependently living of *Capsicum annuum* L.

In Brazzaville several cultivars of *Capsicum annuum* L. are cultivated and sold. Little works have been achieved on the morphological characterisation of *Capsicum* spp L. Among characteristics described, no information is available on the presence and quantity of iodine in pepper fruit. Indeed, iodine is micronutrient which plays an important role in human body. It allows the good functioning of thyroid gland of which the deficit triggers the goiter, mental deficiency, congenital malformations (Delange, 1998; Delange, 2000), among others.

The identification, assessment and variation of the content of iodine in *Capsicum annuum* L. fruit according to ripening stage in the two cultivars are poorly known. Indeed, scientific community did not pay attention to the above mentioned constraints. The solving of these constraints might help to find solutions to some pathologies such as goiter, mental deficiency, congenital malformations among others.

We hypothesise that *Capsicum annuum* L. fruit contains iodine and its content varies as a function of ripeness stage as well as cultivar.

The objective of this work was to identify, assess and analyse the variation of iodine content in *Capsicum annuum* L. fruits from two cultivars.

2. METHODOLOGY

Experimental Site, Climate, Soil Characteristics, Plant Material and Experimental Conditions

Trial was conducted at experiment field of Faculty of Sciences and Techniques based on Bacongo quarter, situated at 15°15'17.3'' West longitude, 4°17'1.7'' North latitude and 291 metres above sea. Experimentation spread out from March to September 2018. Climate is the lower Congo type characterised by two main seasons. It is about rainy season from October to May and dry season from June to September (Samba-Kimbata, 2001). Soil has particle structure and its texture is sand clay.

Plant materials consisted of two cultivars locally called "big *Capsicum annuum* L." and "small *Capsicum annuum* L." Fruit of "big *Capsicum annuum*" is big while the small one is effectively small. Their ripe fruits were first purchased at local market. From these, seeds were extracted, dried at laboratory then sowed in nursery. After the germination, seedlings obtained were transplanted in the experimental field according to the planned experimental design agreed.

A one-complete factor scheme in a completely randomised design was used. Factor "cultivar" with eight seedlings was laid out onto each row. In all, sixty four seedlings, accounting for sixty four treatments, were planted onto the eight rows. Experimental unit was accounted for by four seedlings, thus four useful plants, out of eight. This corresponded to sixteen useful seedlings per cultivar. They were used for measurements. Here, treatment was defined as one variant of factor "cultivar". All of treatments were carried out in quadruplicate.

Experimental area was 25 m^2. At the nursery, 65 days after, seedlings regenerated from seeds were transplanted in the field. Gap between two seedlings onto the same row were 0.5 metre whereas the one between two rows was 1 metre. Plantation density was 2.56 seedlings / m^2.

Planted seedlings were watered with watering can of 10 litres every day in the morning and evening. Weeds were controlled every week.

Variables Measurement and Titrating of Iodine

Growth variables and parameters obtained from iodine titrating were measured and calculated, respectively. Diameter at collar, height of seedling and number of branches were growth variables. Measurements were achieved every two weeks after the transplanting in the field. These ended at the beginning of fructification. Volumetric titrating according to Sandell-Kolthoff (1937) was applied. It consists, first, in drying, in oven at 50°C for 48 hours, the harvested fruits at physiological ripeness, veraison, taste, pre-ripeness and ripeness as reported Mbama *et al* (2018). Then these dried fruits were ground. These groundness were first mixed with distilled water then with 1 ml of sulfuric acid (98.08 g/mole). A volume of 0.5 ml of potassium iodide (1%) was added to the solution prepared. Titrating was done with sodium thiosulfate (0.012 mole per litre). At equivalence, tested yellowish solution becomes colourless. The latter was tested with starch solution to evidence the presence of iodine characterised by blue coloration. Volume of sodium thiosulfate (Veq) was recorded. From this, concentration of iodine (Ci) was deducted from solutions of each groundness of cultivar fruits at different ripeness stages. Content in iodine in four phenological stages such as taste ripeness (MG), physiological ripeness (MP), veraison (V) and taste pre-ripeness (PMG) was assessed.

Statistical Analysis

Xlstat and SPSS softwares, versions 2007 and 22.0 were used, respectively. Pearson's linear correlation at 5% probability was used. Nonetheless, the normality of distribution of the measured variables was verified. General Linear Model of Anova freeing the investigator of respect of the distribution normality constraint at 1% level was applied.Regarding Anova, the following model, corresponding to completely randomised design was used: $Y = \mu + T + \varepsilon$. Where, Y is the response variable; μ is the general mean; T, accounts for the treatment; ε, the error ε_{ij} is supposed to be normally distributed with null mean and variance σ2, that is to say, $\varepsilon_{ij} \sim N(0, \sigma 2)$ (Lakhal–chaieb, 2015).

3. RESULTS

Relevance of Descriptors

Variables named the volume of sodium thiosulfate and iodine concentration were very highly correlated (r = 1*****; p-value <0.00001; table 1). Coefficient of correlation of the fitted curve (R^2) was 100%. Consequently, the choice was focused on the iodine concentration. The latter was used for the rest of work.

Table 1. Linear relationship between sodium thiosulfate volume and iodine concentration.

Dependent variable	Criterion	Veq*	Ci*
Veq	Pearson's correlation	1	1.000*****
	p-value		<0.00001
	N*	32	32
Ci	Pearson's correlation	1.000*****	1
	p-value	<0.00001	
	N*	32	32

Legend.
Veq* : Volume at equivalence of sodium thiosulfate. **Ci*:** Concentration of iodine. **N* :** Size of treatments. Value accompanied by three asterisks reveals that the correlation is very highly significant, namely at 1‰ 00 likelihood.

Effect of ripeness stage on the expression iodine concentration in fruit of each of two cultivars

Iodine concentration was discriminating enough to structure the variants of factor "Ripeness stage" into distinct sub-sets. Sure enough, the variation of "Ripeness stage" induced the variation of iodine concentration. This one can be structured into distinct groups (p-value = 0.035; table 2).

Two sub-sets were evidenced. First, accounted for taste ripeness (MG), was characterised by low iodine concentration (Mean = 0.007 mole per litre). Second, known as taste pre-ripeness (PMG), was marked by high iodine concentration (Mean = 0.0110 mole per litre). Coefficient of variation stretched out from 9.09 to 14.29% (Table 3).

Moreover, two classes were identified. Firstly, composed of big *Capsicum annuum* L. cultivar, was singular in low iodine concentration (Mean : 0.0075 mole per litre). Secondly, consisted of small *Capsicum annuum* L., was distinguished by high iodine concentration (Mean : 0.011 mole per litre). This corresponded to real gap of 0.0035 mole per litre (Table 4). Magnitude of variation was 13.09 and 15.53%, respectively (Table 4).

Table 2. Assessment of factor "Ripeness stage" on the expression of iodine concentration by means of analysis of variance.

Source	SS*		df*		MS*		F*		p-value*	
Cultivar	Small	Big	Small	Big	Small	Big	Small	Big	Small	Big
Corrected model	0.00005	0.00003	3	3	0.00002	0.00001	4.000	1.645	0.035	0.231
Intercept	0.00100	0.00100	1	1	0.00100	0.00100	264.000	213.774	0.000	0.000
Ripeness stage	0.00005	0.00003	3	3	0.00002	0.00001	4.000	1.645	0.035	0.231
Error	0.00005	0.00007	12	12	0.00000	0.00001				
Total	0.00100	0.00100	16	16	0.00010					
Corrected total	0.000099	0.000098	15	15						

Legend.
SS* : Sum of squares. **df* :** Degree of freedom. **MS* :**Mean square. **F* :** Fisher-Snedecor's test statistics. **p-value :** Critical level.

Table 3. Classification of means of the iodine concentration as a function of ripeness stage from two cultivars.

Small *Capsicum annuum*			**Big *Capsicum annuum***		
Ripeness stage*	**Mean Ci***	**CV (%)***	**Ripeness stage**	**Mean Ci***	**CV (%)***
MG	0.0070a	14.29	**MP**	0.0075a	10.00
MP	0.0080ab	12.50	**PMG**	0.0075a	12.50
V	0.0080ab	12.50	**MG**	0.0098a	12.50
PMG	0.0110b	9.09	**V**	0.0105a	9.09

Legend.
Ripeness stage*. MG : Taste ripeness. **MP :** Physiological ripeness.**V:** Veraison. **PMG:** Taste pre-ripeness. **Ci* :** Concentration of iodine. **CV (%)* :** Coefficient of variation in percentage. Numbers followed by the same letter in column are not significantly different according to Student-Newman-Keuls' test at 5% likelihood.

Table 4. Variation of iodine concentration in fruits of the big and small *Capsicum annuum* cultivars at taste pre-ripeness stage.

Cultivar	Mean*	CV %
Big	0.008a	13.09
Small	1.875b	15.53

Legend.
Mean*: Values followed by the same letter in column are not significantly different after the Student's two-samples t testat 1‰ likelihood.

Phenological stages in the two *Capsicum annuum* cultivars

In the two cultivars, the seed emergence of the seedlings majority and transplanting in the field had comparable durations. These were 7 and 65 days, for interval between sowing and seed emergence as well as from sowing to transplantation in the field, respectively. In constrast, when we consider the duration of flowering, fruit set and fructification, values of big *Capsicum annuum* were above of those from small *Capsicum annuum*. Indeed, big *Capsicum annuum* recorded 88, 103and 128 daysafter sowing for flowering, fruit set and fructificationas against 76, 96 and 115 days concerning small *Capsicum annuum* cultivar, respectively (Table 4).

Table 4. Duration of phenological stages of seedlings from two tested *Capsicum annuum* cultivars.

Phenological stage	Duration (day after sowing)	
	Small *Capsicum annuum*	Big *Capsicum annuum*
Sowing	-	-
Seed emergence	7	7
Transplantating	65	65
Flowering	76	88
Fruit set	96	103
Fructification	115	128

4. DISCUSSION

Iodine was identified in fruit of big and small *Capsicum annuum* L. cultivars. Its concentration was assessed and analysed. To our knowledge no information is available concerning titrating of iodine in *Capsicum annuum* L. cultivars fruit. Nevertheless, Von Fellenberg (1930), Dunn *et al.*, (1993), Anonyme (https://fr.wikipedia.org/wiki/Teneur_en_iode_des_aliments, accessed on 2 nd September 2019) reported works on milk, urine and some foods. Our works shown that, first, iodine exists in fruits of the two *Capsicum annuum* L. cultivars. Likewise, small *Capsicum annuum* L. cultivar displayed higher iodine concentration at taste pre-ripeness stage.

Iodine is present in *Capsicum annuum* L. fruits of the two cultivars (Table 2). Indeed, titrating of iodine from *Capsicum annuum* L. dried fruit extract by sodium thiosulfate and starch solution test were positive. In other plant and animal materials, the presence of iodine was also revealed Anonyme (https://fr.wikipedia.org/wiki/Teneur_en_iode_des_aliments, accessed on 2 nd September 2019). From this, we advise people to eat fruits of *Capsicum annuum* L. to ingest iodine and, thus, to prevent goiter.

Variable "iodine concentration" was the most relevant (Table 1). Sure enough, sodium thiosulfate is only reagent which allowed to titrate iodine in *Capsicum annuum* L fruits. Thus, that is the concentration of iodine which is the most important variable. Issali *et al*., (2008) used the Principal Component Analysis to identify relevant variables. Here, we identified iodine concentration as the most relevant variable than sodium thiosulfate volume by means of Pearson's linear correlation. In the future, we will use iodine concentration to describe populations of plant or animal species.

In small *Capsicum annuum* L. cultivar, iodine concentration discriminated the fruit ripeness stages. At the taste pre-ripenessstage (PMG), iodine concentration was higher than the ripe one (MG). Probably, concentration in secondary metabolites would be more important in pre-ripeness stage than the ripe one. In tomato and pepper, iodine content is 0.10 µg/100g and 0.12 µg/100g, respectively (Haldimann *et al*., 2005; Leufroy *et al*., Rose *et al*., 2001). Therefore, the consumption of small *Capsicum annuum* L. fruit is recommended at taste pre-ripeness stage to optimise the ingesting of iodine and thus, to avoid goiter.

In the same way, statistically, iodine concentration of small *Capsicum annuum* L. cultivar at taste pre-ripeness stage was upper than the big one. This might find an explanation through the iodine metabolism which would be more active in small *Capsicum annuum* L. cultivar than in the big one. Iodine content increased from physiological maturity to taste maturity then again decreased. Thus, at taste pre-ripeness stage it will be preferable to eat small *Capsicum annuum* L. fruit than the big one when fruit changes.

The duration "sowing-seed emergence" and "sowing-transplantation" was comparable for the two cultivars. In contrast, duration of "sowing-flowering", "sowing-fruit set" and "sowing-fructification" differed in the two cultivars. The latter could find an explanation through the difference in genetic constitution, and thus the product of genes activity would be different. The duration "sowing-transplantation" and "sowing-fructification" is 40-45 days after sowing and 105-130 days after sowing respectively (Fondio et al, 2009). The cultivation of small *Capsicum annuum* L. allows the early obtention of the fruits and thus, their early selling.

5. CONCLUSION

We hypothesised that *Capsicum annuum* L. fruit contains iodine and its concentration varies as a function of ripeness stage as well as cultivar. According to our obtained results, the two *Capsicum annuum* L. cultivars contain indeed iodine. This one varies after the phenological stages in small *Capsicum annuum* fruits. Nonetheless, no difference was noted on these two cultivars.

Iodine is present in *Capsicum annuum* L. fruits of the two cultivars. It would be desirable that people to eat fruits of *Capsicum annuum* to ingest iodine.

Iodine concentration was the most relevant variable. In the future, we will use iodine concentration to describe populations of plant or animal species.

In small *Capsicum annuum* L. cultivar, iodine concentration discriminated the fruit ripeness stages. At the taste pre-ripeness stage (PMG), iodine concentration was higher than the ripe one (MG).The consumption of small *Capsicum annuum* L. fruit is recommended at taste pre-ripeness stage to optimise the ingesting of iodine. In the same way, iodine concentration of small *Capsicum annuum* L. cultivar at taste pre-ripeness stage was higher than the big one. Therefore, at taste pre-ripeness stage it will be preferable to eat small *Capsicum annuum* L. fruit than the big one when fruit changes. Iodine content increased from phyiological maturity to taste maturity then again decreased.

Duration of "sowing-flowering", "sowing-fruit set" and "sowing-fructification" differed in the two cultivars. The cultivation of small *Capsicum annuum* L. allows the early obtention of the fruits and thus, their early selling.

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