Original article

Changes in the chemical composition of the fruits of *Grewia coriacea* Mast. during development and ripening

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Changes in the chemical composition of the fruits of *Grewia coriacea* Mast. during development and ripening.

Abstract — **Introduction**. *Grewia coriacea* is a widespread plant species that occurs spontaneously in tropical forests of Central Africa. It is one of the most important commercial spontaneous fruit crops in the Republic of Congo (Brazzaville), where it is harvested, sold and consumed on a rather large scale. However, similar to many other spontaneous products of the tropical rain forest in Africa, *G. coriacea* remains poorly studied and its nutritional potential is unknown. Because the fruit of *G. coriacea* is an important food for indigenous people in a large portion of the Congo River Basin, we studied the evolution of some compounds of this fruit starting from its fruit setting until its maturation. **Materials and methods**. The contents of various pigments (chlorophyll a and b, and carotenoids), and concentrations of soluble sugars, ascorbic acid and soluble proteins of the fruit pulp of *G. coriacea* were analyzed in the laboratory and they were followed during maturation. **Results and discussion**. The curves of evolution of the studied parameters followed some anticipated trends: increasing, decreasing or relative stability. Increasing applied to the carotenoids (3,0 $mg \cdot g^{-1}$ at the beginning of the fruit development up to 8.2 $mg \cdot g^{-1}$ at the end of its maturation), soluble sugars (9,9 $\mu g \cdot g^{-1}$ up to 75,4 $\mu g \cdot g^{-1}$), ascorbic acid (25 $mg \cdot 100\ g^{-1}$ up to 230 $mg \cdot 100\ g^{-1}$) and soluble proteins (7 $\mu g \cdot g^{-1}$ up to 40 $\mu g \cdot g^{-1}$). In contrast, the two chlorophyll pigments (chlorophyll a and b) showed an overall decreasing trend. Finally, the concentration of soluble proteins remained rather constant in the later stages, despite an initial increase in the early stage of development. **Conclusion**. To our knowledge, these results are the first data published concerning the temporal changes in physico-chemical parameters of *G. coriacea* fruits in tropical Africa. The study shows that the studied fruits have high nutritional potentials, and that their ability to accumulate rather high levels of ascorbic acid could make them a potential source of vitamin C.

Congo / *Grewia coriacea* / fruits / developmental stages / maturation / chemical composition / chlorophylls / carotenoids / sugars / ascorbic acid / proteins

Évolution de la composition chimique des fruits de *Grewia coriacea* Mast. au cours de leur développement et de leur maturation.

Résumé — **Introduction**. *Grewia coriacea* est une espèce végétale répandue qui pousse naturellement dans les forêts tropicales d'Afrique centrale. C'est l'un des fruits de cueillette le plus commercialisé au Congo (Brazzaville) où il est récolté, vendu, et consommé sur une assez large échelle. Cependant, comme beaucoup d'autres productions spontanées de la forêt tropicale africaine, l'espèce *G. coriacea* reste mal étudiée et ses potentialités alimentaires sont inconnues. Comme le fruit de *G. coriacea* est une importante ressource alimentaire pour les populations d'une grande partie du bassin fluvial du Congo, nous avons étudié l'évolution de certains composés de ce fruit à partir de sa nouaison jusqu'à sa maturation. **Matériel et méthodes**. Les teneurs en différents pigments (chlorophylle a et b, caroténoïdes) et les concentrations en sucres solubles, acide ascorbique et protéines solubles de la pulpe de fruits de *G. coriacea* ont été analysées en laboratoire et suivies au fur et à mesure de la maturation. **Résultats et discussion**. Les courbes d'évolution des paramètres étudiés ont suivi certaines tendances prévisibles : augmentation, diminution ou relative stabilité. L'augmentation s'est appliquée aux carotenoïdes (3,0 $mg \cdot g^{-1}$ en début de développement du fruit à 8.2 $mg \cdot g^{-1}$ en fin de sa maturation), sucres solubles (9,9 $\mu g \cdot g^{-1}$ à 75,4 $\mu g \cdot g^{-1}$), acide ascorbiques (25 $mg \cdot 100\ g^{-1}$ à 230 $mg \cdot 100\ g^{-1}$) et protéines solubles (7 $\mu g \cdot g^{-1}$ à 40 $\mu g \cdot g^{-1}$) . En revanche, les deux pigments chlorophylliens (chlorophylles a et b) ont eu globalement tendance à décroître. En fin de maturation du fruit, la concentration en protéines solubles est restée plutôt stable en dépit de l'augmentation observée au début du développement du fruit. **Conclusion**. À notre connaissance, ces résultats seraient les premières données publiées concernant l'évolution, en fonction du temps, des paramètres physicochimiques des fruits de *G. coriacea* en Afrique tropicale. L'étude montre que les fruits étudiés ont des potentiels nutritionnels élevés et que leur capacité d'accumuler des niveaux relativement élevés d'acide ascorbique pourrait en faire une source potentielle de vitamine C.

Congo / *Grewia coriacea* / fruits / stade de développement / maturation / composition chimique / chlorophylle / caroténoïde / sucres / acide ascorbique / protéine

1. Introduction

Grewia coriacea Mast. is a widespread species that occurs spontaneously in tropical forests in the Central African region. It is a Tiliaceae, a family with approximately 725 known species belonging to 40 genera. In the Republic of Congo-Brazzaville, 29 species belonging to 10 different genera have been identified so far.

Plants belonging to this family can be small trees or shrubs ranging from (4 to 25) m in height and (12 to 40) cm in diameter. In Central Africa, several parts of the plants are used for different purposes. For instance, *Grewia*'s wood is used in the manufacture of wooden plates, spoons and forks. The barks are used by indigenous people to produce liquid extracts used as medicine for intestinal gut diseases and syphilis [1]. Fruits of *G. coriacea* are grouped in bunches, ovoid drupes of (2.5 to 4.0) cm long and about (1.8 to 4) cm wide [2]. They are light-green in early development stages and turn black when fully mature and are harvested for consumption and/or production of local fruit juices. *G. coriacea* is one of the most important commercial spontaneous fruit crops in Central Africa, particularly in the Congo (Brazzaville), where it is harvested, sold and consumed on a rather large scale. It is believed to be a good source of ascorbic acid, pectin, sugar, proteins and several minerals [1].

Changes in the chemical composition of comestible fruits have been extensively studied in plants such as *Dacryodes edulis* [3], guava [4], *Acerola* sp. [5], *Mespilus germanica* Dutch. [6], *Diospyros lotus* L. [7], *Cyphomandra lutacea* (Cav.) and *Sendton* sp. [8]. Overall, it is now well accepted that such changes are caused by a series of concerted biochemical and physiological processes, and understanding these changes should help improve the nutritional contribution of fruits in human diets [7]. Unfortunately, most of the spontaneous products of the tropical rain forest in Central Africa, including *G. coriacea,* remain poorly studied. Our paper reports on our preliminary effort aimed at identifying and characterizing, biologically and chemically, comestible spontaneous products of the tropical forest in Congo-Brazzaville, with an emphasis on their nutritional facts. The fruits of *G. coriacea* have three major parts, which are the skin, the pulp and a very small kernel. Our study focused on the pulp chemistry because it is the edible portion of the fruit and it represents on average 80% of its weight.

2. Materials and methods

2.1. Plant material

Ten trees growing spontaneously in a natural forest were selected randomly. From the selected trees, fruits were picked manually at different stages of development, from fruit setting until fruit maturation, in 2005, and, for a given development stage, twenty fruits (two fruits collected for each of the ten selected trees) were weighed individually; the average weight per harvest was calculated. The analysis focused on the pulp, which is the edible part of the fruit. The fruits were peeled using a scalpel, the skin discarded and the pulp collected after extruding the kernel. Pulp obtained from fruits belonging to the same stage of development was mixed and ground to produce homogenous mixtures from which aliquot samples were then taken for different analyses, which were conducted in duplicate; the mean values are reported.

2.2. Extraction and determination of soluble protein

Soluble proteins were extracted from the samples using a method adapted from that described previously by Attibayéba and Paulet [9] and Khales and Baaziz [10]. One g of the pulp materials was mixed and homogenized with 5 mL of 20 mM tris-HCl buffer (pH 7.5) containing 50 mM of KCl, 5 mM of $MgCl_2$, 10 mg of ascorbic acid, and 25 mg of insoluble polyvinylpyrrolidone (PVP). After centrifugation for 15 min at 5000 rpm, the supernatant was removed for the determination of the concentration of soluble proteins by the method of Bradford [11], and using Bovine Serum Albumin (BSA) as standard.

2.3. Soluble sugar determination

For the determination of soluble sugar, 5 g of pulp materials from each development stage were boiled in 50 mL of distilled water for 15 min and then homogenized in a mortar. The obtained mixture was then filtered and the residual material in the mortar collected through sequential rinsing with warm distilled water and filtered as well. The obtained liquid fractions were combined and used for analysis. Five mL of 5% potassium ferrocyanide and 5 mL of 20% $ZnSO_4$ were added to 25 mL of the obtained extract and allowed to react for 15 min. This solution was then centrifuged at 5000 rpm for 15 min and the supernatant collected and used for the determination of soluble sugars, which was determined with the anthrone-H_2SO_4 reagent as described by Fairbairn [12]; glucose was used as standard.

2.4. Ascorbic acid determination

Ascorbic acid was determined by the method of El Bulk *et al.* [4] with 2.5 g of pulp materials dissolved into 20 mL of 1% oxalic acid, then homogenized for 2 min. The mixture was next blended and brought to a final volume of 25 mL with 1% oxalic acid followed by centrifugation at 5000 rpm. After this centrifugation step, 5 mL of the supernatant were titrated with standard sodium thiosulfate for the determination of ascorbic acid concentrations.

2.5. Chlorophyll and carotenoid determination

For each development stage, two g of fruit pulp were homogenized in a mortar with 10 mL of 95% ethanol and a pinch of calcium carbonate and clean white sand. The homogenate was vacuum-filtered through a Whatman No. 1 filter paper and the residue rinsed three times with 95% ethanol. The filtrates were combined and adjusted to 50 mL with ethanol. Three mL were taken from this solution for the measurement of absorbance on a spectrophotometer at 662 nm (OD_{662}), 664 nm (OD_{664}) and 440 nm (OD_{440}), respectively [13]. The corresponding concentrations ($mg \cdot g^{-1}$) were calculated using the following equations:

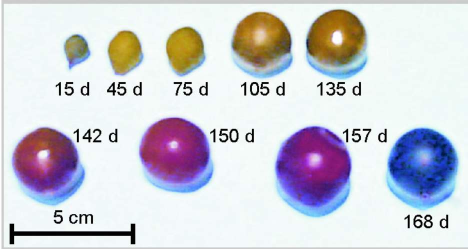

Figure 1.
Fruits of *Grewia coriacea* at different stages of development and ripening. The fruits change color at about day 135 and become ready for harvesting and consumption at about day 157. (Color picture on www.fruits-journal.org.)

$Conc_{chlorophyll\ a} = [9.78 \times (OD_{662})] - [0.99 \times (OD_{664})]$,

$Conc_{chlorophyll\ b} = [21.43 \times (OD_{644})] - [4.65 \times (OD_{644})]$,

$Conc_{carotenoids} = [4.7 \times (OD_{440})] - [1.38 \times (OD_{662})] + [5.48 \times (OD_{644})]$.

3. Results and discussion

Our observations indicated that the fruits (*figure 1*) seem to grow very rapidly during an initial phase that lasts about (45 to 75) d. During this phase, the fruits increase in volume. At this point, they are light green in color, the content is liquid and the kernel is absent. The kernel appears only when the fruits have reached the stage of 105 d after fruit setting (*figure 1*). At this stage, the weight of the fruits is near what would be the maximum average weight measured of 10.4 g. Once the fruits have reached physiological maturity (at about 135 d), they start to change color from green to purple, and then black. The fruit color increases in intensity, with black-purple to black and ready for picking, at 150–157 d after fruit setting, (*figure 1*). The full change in color from light green to black takes about (5 to 6) months.

A growth curve corresponding to the change in the average weight of the fruits versus time had a sigmoidal trend (*figure 2*). These observations concur with data reported earlier on changes in the chemical composition of several other fruits [5, 8, 14–16]. In addition, trends of measured temporal changes in concentrations of pigments (chlorophyll a and b, and the carotenoids) are quite similar to what is commonly

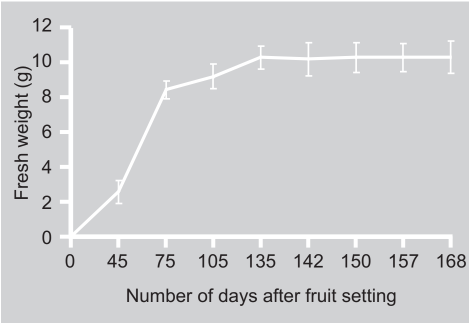

Figure 2.
Temporal evolution of fresh weight of the fruits of *Grewia coriacea* during different stages of development and ripening. Each point represents the average weight of 20 fruits and the vertical bars correspond to standard deviation.

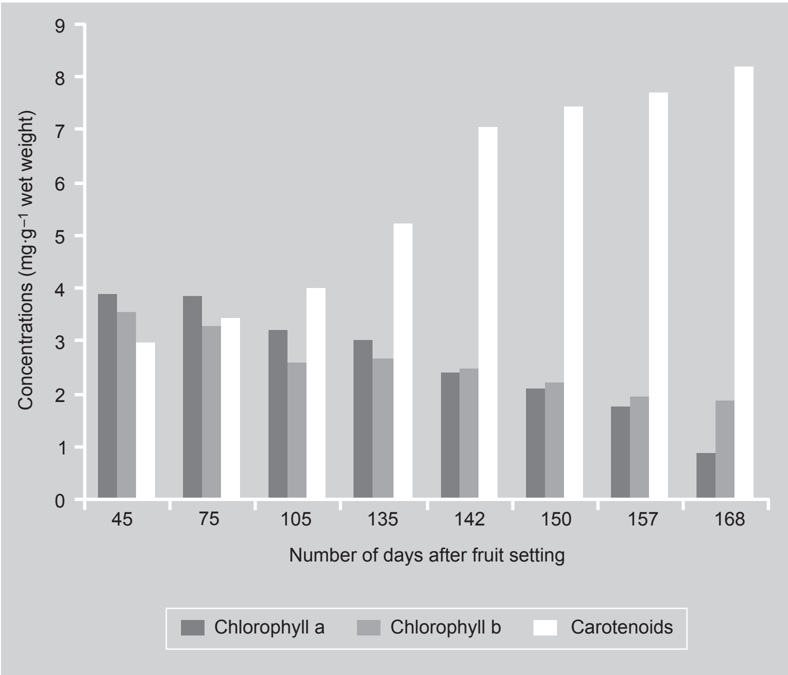

Figure 3.
Trends of chlorophyll and carotenoïds in the pulp of *Grewia coriacea* fruits during development and ripening.

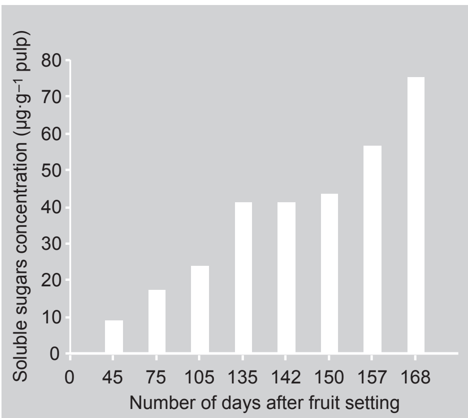

Figure 4.
Soluble sugar concentrations at various stages of development and ripening of the fruits of *Grewia coriacea*.

reported for most fruits (*figure 3*): the decreasing chlorophyll a and chlorophyll b overlap with concentrations of the carotenoids, which increased from 3.0 $mg \cdot g^{-1}$ at the 45-d stage up to 8.2 $mg \cdot g^{-1}$ at the 168-d stage. Measured levels of carotenoids at the stages of (135, 142, 150, 157 and 168) d after fruit setting were significantly higher than those of chlorophyll a and b. These results suggest that pigment change associated with the ripening of the fruits proceed largely via a common mechanism and obey a general law determined by a fruit physiological stage. These results are in agreement with those reported by Solovenchenko *et al.* [17], where chlorophyll content alone has been suggested as a suitable internal marker of fruit ripeness. In general, changes in both chlorophyll and carotenoid contents should be used to follow the ripening process, as reported in studies on apple fruits on and off the tree [17].

It is worth noting that, in addition to changes in the concentration of pigments, there are changes in the concentrations of sugars, ascorbic acid and soluble proteins; according to Wang *et al.* [18], the concentrations of several other chemical compounds can be used as indicators of fruit development and ripening processes.

Soluble sugar content increased gradually during the early stages of development and significantly at the last stages of ripening (*figure 4*). It was 9.9 $\mu g \cdot g^{-1}$ at the stage of 45 d after fruit setting and, when the fruits were 168 d old, they accumulated up to 75.4 $\mu g \cdot g^{-1}$ of soluble sugar in the pulp. Also, levels of ascorbic acid increased slowly in the first (45 to 135) d after fruit setting (25 $mg \cdot 100\ g^{-1}$ at 45 d), before accumulating at a much higher rate during ripening between (142 and 168) d after fruit setting (230 $mg \cdot 100\ g^{-1}$ at 168 d, *figure 5*). These trends are similar to those reported in the literature by Mowlah and Itoo [19], El Bulk *et al.* [4], and Glew *et al.* [7]. Finally, the concentrations of ascorbic acid and soluble sugars in the pulp of *G. coriacea* reached values similar to those reported for a good number of well-studied fruits found in the literature [4, 7, 8, 19, 20].

The concentration of soluble protein content was quite low in the immature stages of 45–75 d after fruit setting (7 µg·g^{-1} at 45 d) and increased to remain constant (40 µg·g^{-1}) at later stages (105–168 d after fruit setting). The results showed no significant variations at ripe stages of fruit (*figure 6*). This observation is in contradiction with trends reported by De Assis *et al.* [5], suggesting that temporal changes in chemical composition of fruits should not be generalized, and should be studied on a case-by-case basis.

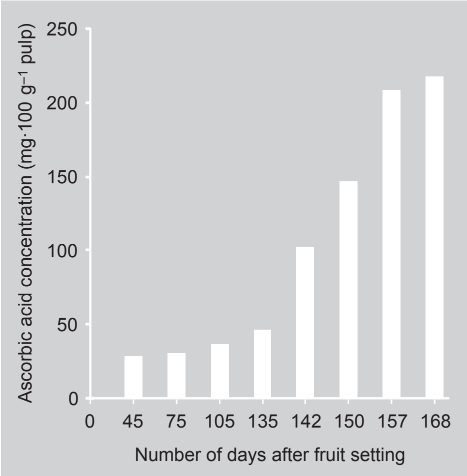

Figure 5.
Ascorbic acid concentrations at various stages of development and ripening of the fruits of *Grewia coriacea*.

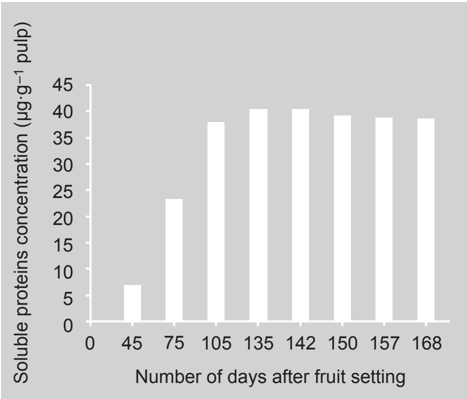

Figure 6.
Soluble protein concentrations at various stages of development and ripening of the fruits of *Grewia coriacea*.

4. Conclusion

The preliminary work that we achieved represents, to our knowledge, the first report on temporal changes in the chemical composition of the fruits of *G. coriacea* Mast. during development and ripening. Our study demonstrates that chemical compounds associated with nutritional benefits to humans (*e.g.*, sugars [7], protein and ascorbic acid) reach their maximum values near 145 d after fruit setting. It is worth noting that the high ascorbic acid content measured in these fruits opens the door for consideration of this spontaneous and comestible fruit as a raw source of vitamin C. Further studies will be needed to evaluate the commercial benefits of using these fruits to produce vitamin C concentrates.

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Evolución de la composición química de los frutos de *Grewia coriacea* Mast. en el transcurso de su desarrollo y de su maduración.

Resumen — **Introducción**. *Grewia coriacea* es una especie vegetal expandida que crece naturalmente en los bosques tropicales del África central. Se trata de uno de los frutos de cosecha más comercializados en el Congo (Brazzaville) en donde se cosecha, se vende, y se consume a una escala relativamente grande. No obstante, como ocurre con otras muchas producciones espontáneas del bosque tropical africano, la especie *G. coriacea* permanece mal estudiada y se desconocen sus potencialidades alimentarias. Dado a que el fruto de *G. coriacea* es un recurso alimentario importante para las poblaciones de una gran parte de la cuenca fluvial del Congo, estudiamos la evolución de ciertos componentes de este fruto a partir de su fructificación hasta su maduración. **Material y métodos**. Se analizaron en laboratorio los contenidos de diferentes pigmentos (clorofila a y b, carotenoides), así como las concentraciones de azúcares solubles, ácido ascórbico y proteínas solubles de la pulpa de frutos de *G. coriacea,* posteriormente fueron sometidos a seguimiento durante la maduración. **Resultados y discusión**. Las curvas de evolución de los parámetros estudiados siguieron ciertas tendencias previsibles: aumento, disminución, o estabilidad relativa. El aumento se aplicó a los carotenoides (3,0 mg·g^{-1} al principio de desarrollo del fruto a 8.2 mg·g^{-1} al final de su maduración), azúcares solubles (9,9 µg·g^{-1} a 75,4 µg·g^{-1}), ácido ascórbico (25 mg·100 g^{-1} à 230 mg·100 g^{-1}) y proteínas solubles (7 µg·g^{-1} a 40 µg·g^{-1}). Por el contrario, ambos pigmentos clorofilianos (clorofilas a y b) tuvieron generalmente tendencia a decrecer. Al final de maduración del fruto, la concentración de proteínas solubles se mantuvo más bien estable a pesar del aumento observado al principio del desarrollo del fruto. **Conclusión**. De acuerdo con nuestros conocimientos, estos resultados serían los primeros datos publicados referente a la evolución, en función del tiempo, de los parámetros físico-químicos de los frutos de *G. coriacea* en el África tropical. El estudio muestra que los frutos estudiados poseen potenciales nutricionales elevados y que su capacidad para acumular niveles relativamente elevados de ácido ascórbico podría ser una fuente potencial de vitamina C.

Congo / *Grewia coriacea* / frutas / etapas de desarrollo / maduración / composición quimica / clorofilas / carotinoides / azucares / ácido ascórbico / proteínas