

## Original Research Article

### Total Polyphenols, Total Flavonoids, Condensed Tannins, and Antioxidant Activity of *Borassus aethiopum* (Arecaceae) mature fruits' Peels, and combined Peel-Pulps, dried at different temperatures.

#### Abstract

To measure their possible beneficial contributions on the rabbits' health, *Borassus aethiopum* mature fruits' peels and peels combined with the pulp were dried at 60, 65, 70 and 75°C. Then, after 5 days of drying in ovens, the products were crushed, sieved, and extracted with distilled water through maceration and decoction for 1 hour. Afterwards, total phenols (TP), total flavonoids (TF), condensed tannins (TC) contents, and antioxidant activity (AAO) were assessed. As a result, the peels presented the highest content of total polyphenols with 0.051 mg GAE/g for the product dried at 70°C. Whereas the lowest polyphenol content was observed in the peel-pulp combined part with 0.040 mg GAE/g, dried at 60°C. However, concerning the TF, the peel with the fruit pulp dried at 75°C and that of the peel dried at 60°C presented the best contents. Indeed, the products dried at different temperature revealed that the peels contain higher flavonoid contents than the peel-pulp combined, respectively for 0.256 mg QE/g and 0.169 mg QE/g. Regarding AOA, they showed a much higher free radical scavenging capacity in the peels than in the peel-pulp parts. The highest antioxidant activity value was gained with the peel dried at 70°C with 3.347 µmol TE/g. While the lowest value was observed with the peel-pulp dried at 70°C for 1.014 µmol TE/g. With condensed tannins, the best outputs were collected through decoction with the peeldried at 60°C for 0.468±0.003 µg ECat/g. With the composed peel-pulp portions, the best extracts were collected at 70°C for 0.100±0.003 µg ECat/g. In conclusion, *Borassus aethiopum* ripe fruits' peels and the peels combined with the pulps can be dried between 65 and 70°C. So, they could be good sources of fibers and phytochemicals for rabbits' diets.

**Keywords:** Antioxidant activities, *Borassus aethiopum*, condensed tannins, rabbits, total polyphenols

#### Introduction

*Borassus aethiopum* is a giant palm tree with a very hard false trunk, fasciculate roots, and long fan-shaped leaves. The plant is almost cosmopolitan, and its different parts are used in many fields such as economic, ecological, food and health (Dieng *et al.*, 2015). Indeed, medicinal plants are a group of plants with great socio-economic importance because they contain active components used in the treatment of various diseases (Sereme *et al.*, 2008). In addition, on the health level, *Borassus aethiopum* mature fruits' extracts were able to neutralize *Staphylococcus aureus* and *Bacillus subtilis* bacteria (Sarkodie *et al.*, 2015). Some works of Tiho *et al.* (2018) and Ali *et al.* (2010) carried out on *Borassus aethiopum* ripe fruits, showed that its mature fruits' pulp is rich in lipids and reducing sugars, which makes its conservation difficult because of fermentation problems. Similarly, its carotenoid and vitamin C contents are very high, indicating it as an important source of vitamins A and C (Ali *et al.*, 2010). Specifically, the pulp is also rich in minerals such as calcium, magnesium, and phosphorus (Ali *et al.*, 2010; Tiho *et al.*, 2018). Since ancient times, in view of preserving foods products, drying is one of adequate preservation methods. Indeed, this process remains a common practice (Cantin *et al.*, 2011). However, drying poor technique mastery leads to the nutritional and therapeutic values losses (Piga *et al.*, 2004). Moreover, Sarkodie *et al.* (2015) and Tiho *et al.* (2017) results showed that *Borassus aethiopum* has significant anti-inflammatory and antioxidant properties. Hence, this work hypothesis was that the drying temperature of the peels and peels combined with their pulp would affect the resulting products' nutritional values. So, the subsequent objectives were to find the best drying temperature that would allow the best extractions and the fruit part that would contain more phenolic compounds including total polyphenols, total flavonoids, total tannins, and condensed tannins.

#### Material and methods

##### *Borassus aethiopum* ripe fruits' collection

The fruits were collected within the graduate school of agronomy at the National Polytechnic Institute Felix Houphouët Boigny in Yamoussoukro, Côte d'Ivoire, and the surrounding areas. Then the fruits were carefully sorted, and the rotten ones were discarded. In fact, the first step was to separate the peel and the pulp from the core. Then, the second step was to peel the fruit with the peel and the pulp together. Afterwards, the two components, including the peel and peel combined with the pulp, were dried in an oven at 60, 65, 70 and 75°C for 5 days, until constant masses were reached (Tiho *et al.*, 2017). Then the dried products were ground. Finally, through a laboratory sieve

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(Iso 3310-1BODY 36 LMESHs-Steel/RF S/N 04003699, Body = 200 mm × 50 mm), large particles were removed. The powders were put in glass bottles and kept in a dry place, protected from light until the analysis (Tiho *et al.*, 2017).

### Samples extraction

The powders were extracted by maceration and decoction, using only distilled water as a solvent. In a 100 mL bottle, 60 mL solvent was added to 1 g of powder. During the extractions, the mixture was put on a magnetic stirrer. A bar magnet was immersed in each mixture consisting of distilled water and the sample (Tiho *et al.*, 2017).

**Comment [pp2]:** The procedure in "SAMPLE PREPARATION" tell that maceration and decoction use 60 mL + 1g powder, but "DECOCTION" is performed by 120 mL + 1 g powder. Why?

### Maceration

Macerations were performed on RSLAB 5NC Multiplot 10 magnetic stirrer, and the plate rotation speed was set at 1100 rotations per minute (rpm). Next, a bar magnet was dipped into each mixed solution. The various Erlenmeyer flasks were closed with aluminum foil to avoid solution evaporation. By increasing the durations recommended by Shewale and Rathod (2018) by 30 min, the extractions lasted 1 hour. Finally, the different solutions were filtered with clean cotton and placed in the refrigerator before the analyses.

### Decoction

A closed-circuit device was made by joining a cooling system and a heating system. It was used to perform the decoction extraction mode. Thus, the mixture was heated on an electric oven stirrer Agimatic-N, from West Germany), set at 100°C. Here, 1g of sample was immersed in 120 mL of distilled water. After observing the first bubble boil, the mixture boiled for 30 minutes, and the heater was turned off. Thereafter, the solution remained on the assembly for an additional 15 minutes to collect the last drops (Tiho *et al.*, 2017). Secondly, the flask was removed from the stirrer-heater, and the whole was left for an additional 15 minutes for cooling. Finally, the solution was filtered.

### Total polyphenols assessment

Yeddes *et al.* (2013) method was lightly modified and used for the determination of total polyphenols. Succinctly, 2.5 mL of the Folin-Ciocalteu's reagent was added to 30 µL of extract. The mixture was then placed in the dark in a room at room temperature for 2 minutes. Then, 2 mL of sodium carbonate solution with a concentration of 75 g.L<sup>-1</sup> was added. Then, the mixture was put for 15 minutes in a water bath at 50°C and cooled with tap water. Absorbance was read at 760 nm on a spectrophotometer with distilled water as a blank. A calibration line was made with gallic acid at different concentrations. The concentration of total polyphenols was expressed in milligrams per liter of gallic acid equivalent extract (mg GEA/mL). This concentration can be converted into mg AGE/g, by multiplying the concentration read on the spectrophotometer by 1/9.06 (Equation 1).

$$F_c \left( mg \frac{AGE}{g} \right) = R_c * \frac{1}{9.06 * 10^3} \quad (\text{Equation 1, for macerations})$$

F<sub>c</sub> stands for Final content.

R<sub>c</sub> stands for Read content on the spectrophotometer.

Concerning the decoction extracts, the maceration conversion factor will be multiplied by 2 due to the volume of solvent which is 120 mL (Equation 2).

$$F_c \left( mg \frac{AGE}{g} \right) = R_c * \frac{1}{18.12 * 10^3} \quad (\text{Equation 2, for decoctions})$$

**Comment [pp3]:** "gallic acid equivalent " should be GEA, AGE, or GAE?? Please use the same word for entire manuscript

### Total flavonoids assessment

The determination of total flavonoids was carried out according to the method of Marinova *et al.* (2005). In a 25 mL flask, 2.5 mL of the extract was added, then 0.75 mL of 5% sodium nitrate (NaNO<sub>2</sub>) was added. In addition, 0.75 mL of Aluminum Chloride (AlCl<sub>3</sub>) was added. Then, the mixture was placed in the dark for 6 minutes. After this time, 5 mL of sodium hydroxide (NaOH, 1N) was added, and the volume was completed with distilled water to 25 mL. Finally, the absorbance was read at 510 nm on a spectrophotometer. A calibration curve has been established. The total flavonoid contents of the extracts were expressed in mg of quercetin equivalent per liter (mg QE/L). This result can be converted to (mg QE/g) with equation 3 for macerations and equation 4 for decoctions.

$$F_c \left( mg \frac{QE}{g} \right) = R_c * \frac{1}{0.6} \quad (\text{Equation 3; for maceration})$$

$$F_c \left( mg \frac{QE}{g} \right) = R_c * \frac{1}{1.2} \quad (\text{Equation 4; for decoction})$$

### Antioxidant activity of extracts

Among many ways to assess antioxidants activities, Awika *et al.* (2003) highlighted the fact that 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) is inexpensive and easy to use, and most importantly, its pH is stable. The method is based on the ability of

**Comment [pp4]:** 1) "ANTIOXIDANT ACTIVITY OF EXTRACT" should be placed after "CONDENSED TANNIN EVALUATION"  
2) you should perform at least 2 different methods for antioxidant activity.

compounds to reduce the radical-cation **ABTS\*+** (2,2-azimobis-3-ethylbenzothiazoline-6-sulfonic acid). The test was carried out according to Teow *et al.* (2007) method. The radical-cation **ABTS\*+** was produced by reaction of 8 mM ABTS (87.7 mg in 20 mL of distilled water) and 3 mM of potassium persulfate (0.0162 g in 20 mL of distilled water) in a 1:1 ratio (v/v). The mixture was then incubated in the dark for 12-16 hours at room temperature. This **ABTS\*+** solution was diluted with methanol to obtain a solution whose absorbance was at 734 nm. Thus, 3.9 mL of this dilute solution of **ABTS\*+** was added to 100  $\mu$ L of the extract. Afterwards, the mixture was put in the dark for 6 minutes. The residual absorbance of the **ABTS\*+** radical was then measured at 734 nm in UV-visible spectrophotometer and should be between 20 and 80% of the absorbance of the blank. The concentrations were expressed in  $\mu$ mol Trolox equivalent per liter of extract ( $\mu$ mol Trolox E./L). The activity of the compounds is expressed in Equivalent Trolox (Trolox E.) which corresponds to the concentration of Trolox. Thus, the higher the E. Trolox value, the more effective the antioxidant. The concentration is converted into ( $\mu$ mol TE/g). The inhibition rate I (%) of **ABTS\*+** was obtained with equation 5.

$$I(\%) = \frac{\text{Abscontrol} - \text{AbsExtract}}{\text{Abscontrol}} * 100 \quad \text{Equation 5}$$

With  $\text{Abs}_{\text{control}}$  is the absorbance of the diluted **ABTS\*+** solution

$\text{Abs}_{\text{Extract}}$  is the absorbance of the **ABTS\* solution + diluted** sample.

The concentration in micromole of Trolox equivalence of the extract **per liter** was obtained with equation 6.

$$F_c \left( \frac{\mu\text{mol TE}}{\text{g}} \right) = R_c * \frac{1}{2.4} \quad (\text{Equation 6, maceration})$$

$$F_c \left( \frac{\mu\text{mol TE}}{\text{g}} \right) = R_c * \frac{1}{4.8} \quad (\text{Equation 7, decoction})$$

#### Condensed tannins evaluation

Condensed tannins were determined by the Vanillin method in an acid medium (Hagerman and Bulter, 1989). This method is based on the ability of vanillin to react with condensed tannin units in the acid presence to produce a colored complex measured at 500 nm. The vanillin reactivity with tannins only involves the polymer first unit. In 50  $\mu$ L of extract, 3 mL of 4% methanolic vanillin were added. Then, 1.5 mL hydrochloric acid (HCl) solution was added to the mixture. Then, the mixture was incubated in the dark for 15 minutes before reading in the spectrophotometer at 500 nm. Catechin was used as a standard and the results were expressed in microgram of catechin equivalent per milliliter ( **$\mu$ g CatE/mL**). This result was converted into microgram of Catechin Equivalent per gram ( **$\mu$ g CatE/g**) with equation 8 et equation 9. Abbassia *et al.* (2018) discovered that, for condensed tannins assessment, distilled water was more efficient than methanol, ethanol, and acetone.

$$\frac{R_c}{F_c} = \frac{\frac{\mu\text{g CatE}}{\text{mL}}}{\frac{\mu\text{g CatE}}{4.55 \text{ mL}}} = \frac{\mu\text{g CatE}}{\text{mL}} * \frac{4.55 \text{ mL}}{Q_m} = \frac{\mu\text{g CatE}}{\text{mL}} * \frac{4.55 \text{ mL}}{\frac{1.9}{60 \text{ mL}} * 50 \mu\text{L}} = \mu\text{g CatE} * \frac{4.55}{\frac{2}{6} * 10^{-3} \text{ g}} = 5.5 \cdot 10^3 \mu\text{g E. Cat} / \text{g}$$

$$F_c \left( \frac{\mu\text{g E. Cat}}{\text{g}} \right) = R_c * \frac{1}{5.5} \quad (\text{Equation 8, macerations})$$

$$F_c \left( \frac{\mu\text{g E. Cat}}{\text{g}} \right) = R_c * \frac{1}{11} \quad (\text{Equation 9, decoctions})$$

#### Statistical analysis

The results were generated in triplicate. Subsequently, for the statistical tests, the results were subjected to a factorial analysis of variance (ANOVA II), using XLSTAT 2014. Least square means were separated according to the Newman-Keuls method in a 95% confidence interval. After examining the one-factor effect on the extracts, the results were given in terms of the interaction between the dried products drying temperatures and the extraction methods.

#### Results and discussion

##### Total polyphenol content

According to Viscoliet *et al.* (2000), polyphenols are a large group of phytochemicals in fruits. Table 1 summarizes total polyphenols' contents according to the products that were the peels (Pe) and the peels plus the pulps (Pe+Pu) dried at 60, 65, 70 and 75°C. Looking at the peels, and the peels combined with the pulps, the extracts significantly differ according to drying temperatures. Herein results show 4 different groups, following of least square means. Particularly, among the peels, the highest extraction was obtained at 70°C, for 0.051 $\pm$ 0.001 mg GAE/g of dried product. From 65°C to 70°C, total phenolic compound extraction increased by 0.005 mg GAE/g, and this gap represented an increase

**Comment [pp5]:** You must correct the radical symbol

**Comment [pp6]:** What is this??

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of 10.87% ( $p < 0.0001$ ). Moreover, from 60°C to 65°C and 65°C to 70°C, total phenols' extractions increased by 0.005 mg GAE/g at each step ( $p < 0.0001$ ).

This increasing output alongside an increasing drying temperature may be because the hard peels became drier, so more friable. Then, it became easier to ground them and free more phytochemicals. Generally, the pulp portion acted as diluting the peels phytochemicals' contents. Because of the high protein content in *Borassus aethiopicum* pulp (Tiho *et al.*, 2018), the polyphenols are sequestered (Rodriguez *et al.*, 2016). So, a high drying temperature destroyed the proteins in pulps, and the polyphenols are released. Indeed, at 60, 65, and 70°C, important protein content in the peels combined with the pulps limited total phenols' extraction. So, the drying temperature is an important factor that influences the products' nutritional values.

**Table 1.** Products total polyphenol contents (mg AGE/g)

Product	$\mu \pm SE$ (mg GAE/g)	Products comparison	p-values
(Pe)70	0.051 $\pm$ 0.001 <sup>a</sup>		
(Pe)65	0.046 $\pm$ 0.001 <sup>b</sup>	(Pe)70 vs (Pe)65	<0.0001
(Pe+Pu)75	0.043 $\pm$ 0.001 <sup>c</sup>	(Pe)65 vs (Pe+Pu)75	0.0008
(Pe)60	0.041 $\pm$ 0.001 <sup>d</sup>	(Pe+Pu)75 vs (Pe)60	0.0203
(Pe)75	0.040 $\pm$ 0.001 <sup>d</sup>	(Pe)60 vs (Pe)75	0.1660
		(Pe+Pu)75 vs (Pe)75	0.0015
(Pe+Pu)60	0.040 $\pm$ 0.001 <sup>d</sup>	(Pe)75 vs (Pe+Pu)60	1
		(Pe+Pu)75 vs (Pe+Pu)60	0.0028
(Pe+Pu)70	0.040 $\pm$ 0.001 <sup>d</sup>	(Pe+Pu)60 vs (Pe+Pu)70	0.5249
		(Pe+Pu)75 vs (Pe+Pu)70	0.0008
(Pe+Pu)65	0.040 $\pm$ 0.001 <sup>d</sup>	(Pe+Pu)70 vs (Pe+Pu)65	0.8985
		(Pe+Pu)75 vs (Pe+Pu)65	0.0008

$\mu$ : Least square mean, SE: standard error, mg GAE/g: mg Gallic Acid Equivalent per g, Pe: Peel, Pu: Pulp, Pe+Pu: Peel + Pulp (together); 60, 65, 70 and 70°C are the drying temperatures

<sup>a, b, c, d</sup>: Least square means bearing different superscripts in the column [ $\mu \pm SE$  (mg EGA/g)] differ significantly ( $p < 0.05$ )

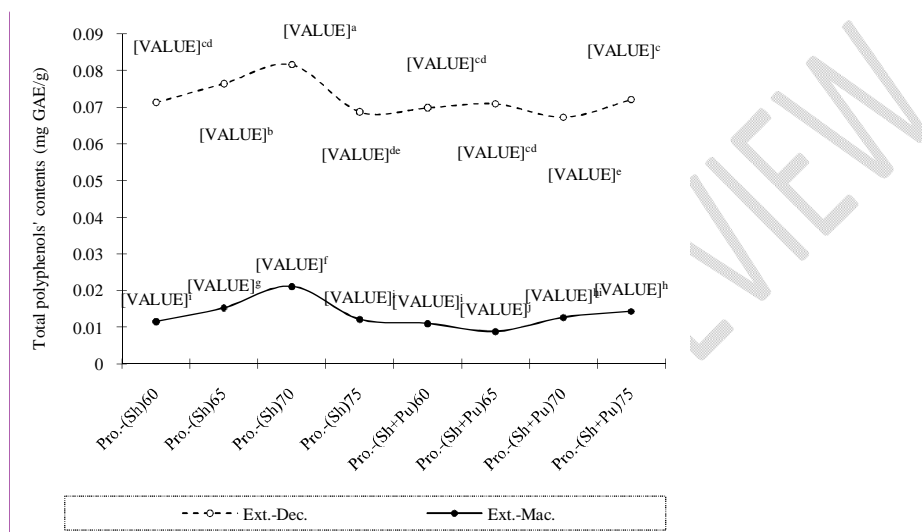
Obviously, *Borassus aethiopicum* peels contain much more polyphenols compared to the peels combined with the pulps. For example, when these parts were dried at 70°C, the peels released 0.051 $\pm$ 0.001 mg GAE/g while the peels combined with the pulps delivered 0.040 $\pm$ 0.001 mg GAE/g. Notably, this 0.011 mg GAE/g, which represented 27.50% increase from the peels combined with the pulps was highly important ( $p < 0.0001$ ). These same tendencies were reported elsewhere. For instance, when Muralidhara *et al.* (2019) examined the phenols content in mango fruits' peels and pulp. In fact, *Mangifera indicavar* Neelum delivered 119.30 and 357.92 mg GAE/100 g, respectively for pulp and peels, thus an increase of 200.02% from pulp to peels (Muralidhara *et al.*, 2019). Similarly, Lenucciet *et al.* (2022) concluded that phenolic compound contents in peels compared to that in pulp can reach 4 folds. Moreover, besides these fruits containing colored pulp covered by peels, Costanzo *et al.* (2022) reported that *Citrus reticulata* peels and pulps differ greatly on polyphenols contents. As an illustration, they extracted 0.9 mg GAE/g of pulp, and 4.2 mg GAE/g of peels, more than 4 folds.

Looking at Slimenet *et al.* (2021) work on prickly, the polyphenol quantities were much greater in the peels than in the pulp. Mainly, the polyphenol content in the peels of spiny *Opuntia ficus indica* was three times that of the pulp. Also, polyphenols content in the spineless skin was 2.21 times higher than that of the pulp. Similar results were observed for *Opuntia stricta*, whose peel polyphenols content was 1.66 times higher than that of pulp (Yeddes *et al.*, 2013). Again, peach fruits (*Prunus*) revealed that the total phenolic compounds amount ranged from 11.1 to 128.5 mg GAE/100 g fresh weight for pulp extracts and from 42.7 to 211.4 mg GAE/100 g for peels extracts (Stojanovic *et al.*, 2016). In apricot varieties, Fan *et al.* (2017) revealed that the peels polyphenol content was 1 to 3 times higher than that of the pulp tissues. According to Viscoliet *et al.* (2000) polyphenols contribute to the organoleptic quality of foods derived from plants (colour, astringency, aroma, bitterness). These molecules play an important role by acting directly on fruits and vegetables nutritional quality, and their impact on consumer health through antioxidant effect, protective effect against the appearance of certain cancers, etc... The beneficial effect of polyphenols on cardiovascular health has been attributed in part to their direct effect on blood vessels, more specifically on the endothelium.

Turning to the extraction mode, total polyphenols extracted relied heavily on the extraction mode, whose were the maceration and the decoction (Figure 1). Namely, for peels extraction from maceration to decoction at 70°C, the polyphenols' extracts increased from

**Comment [pp8]:** QUESTION= Were these TP contents from maceration or decoction, please indicate.

0.0212±0.001 mg GAE/g to 0.0817 mg GAE/g. This important increase was 285.38% yield improvement ( $p < 0.0001$ ). Despite the protein presence in the pulp, the extractions of the portions of peels combined with the pulps were also improved. Indeed, when peels combined with pulps were dried at 65°C, the polyphenols extracts were improved by 706.82%, from 0.0088±0.001 mg GAE/g to 0.071±0.001 mg GAE/g. In fact, the decoction efficacy has been well documented for decades. For example, Reynoso-Camacho *et al.* (2021) mentioned that the decoction of citrus by-products allows the extraction of important phytochemicals. Furthermore, Touré *et al.* (2022) compared decoction and maceration extractions on *Parkia biglobosa* parts, and concluded that from maceration to decoction, the extracts were improved by 49.03%. Without a doubt, decoction was the best extraction method for total polyphenols. Summing up, the best drying temperature was 70°C, and the most valuable product was the peels for total polyphenols' content (Table 1, Figure 1). Indeed, at 70°C, and through the decoction the peels delivered 3.85 times the amount collected through maceration.



**Figure 1.** Total polyphenols' contents (mg GAE/g) according to the extraction mode ( $\sigma = 0.001$ ; Sh: shell (peel); Sh+Pu: shell + pulp (peel+pulp); 60, 65, 70, and 75 (drying temperatures, °C). a, b, c, d, e, f, g, h, i, j, k: Least square means bearing different superscripts differ significantly ( $p < 0.05$ ).

#### Total flavonoids contents

According to Stoclet and Schini-Kerth (2011), flavonoids are the most abundant polyphenolic compounds found in plants. Mainly, these flavonoids contained in fruits, leaves and bark have protective effects against various conditions (Stoclet and Schini-Kerth, 2011). Herein results revealed that the combination of peel and pulp were better source of total flavonoids (Table 2). In fact, the combined peels and pulp delivered 0.302±0.005 mg QE/g versus 0.256±0.005 mg QE/g, and the gap was an increase of 17.97% ( $p < 0.0001$ ). Certainly, high protein, fat and reducing sugar contents inhibit the extraction of phytochemicals (Tiho *et al.*, 2018). Also, a high drying heat destroys protein, fat and reducing sugars contents (Tiho *et al.*, 2018). Drying at 75°C for 5 days led to a severe dry of peels. During drying, the peels rolled up and closed the pulp internally, thus protecting it against severe heat. So, the pulp was well dried and less exposed to the heat. Thus, the well dried internal pulp was a good source of total flavonoid while drying at 75°C. Admittedly, 75°C was not a good drying temperature for the peels, because they allowed only 0.132±0.005 mg QE/g.

**Table 2.** Products total flavonoid contents (mg QE/g)

Products	$\mu \pm SE$ (mg QE/g)	Products comparison	p-values
(Pe+Pu)75	0.302±0.005 <sup>a</sup>		
(Pe)65	0.256±0.005 <sup>b</sup>	(Pe+Pu)75 vs (Pe)65	<0.0001
(Pe)60	0.218±0.005 <sup>c</sup>	(Pe)65 vs (Pe)60	<0.0001
(Pe)70	0.204±0.005 <sup>d</sup>	(Pe)60 vs (Pe)70	0.0465
(Pe+Pu)60	0.169±0.005 <sup>e</sup>	(Pe)70 vs (Pe+Pu)60	<0.0001

**Comment [pp9]:** 1) You should still use Pe instead of Sh.  
2) scale in the x-axis should be in line with GAE/g values and with the name of the samples.

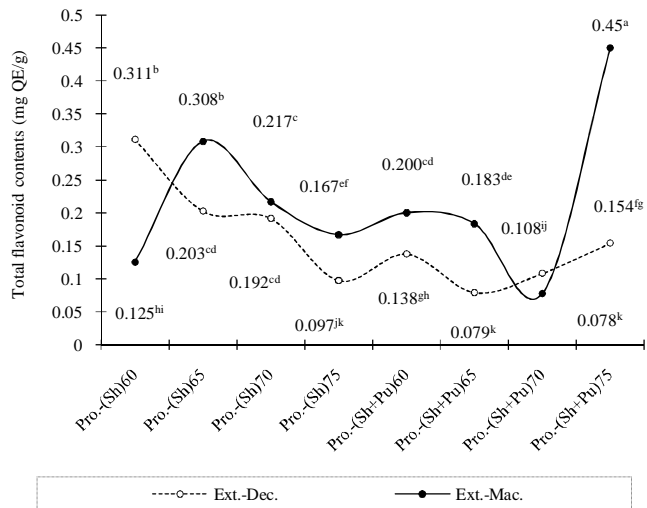
(Pe)75	0.132±0.005 <sup>f</sup>	(Pe+Pu)60 vs (Pe)75	<0.0001
(Pe+Pu)65	0.131±0.005 <sup>f</sup>	(Pe)75 vs (Pe+Pu)65	0.9182
		(Pe+Pu)60 vs (Pe+Pu)65	<0.0001
(Pe+Pu)70	0.093±0.005 <sup>e</sup>	(Pe+Pu)65 vs (Pe)70	<0.0001

μ: Least square mean, SE: standard error, QE/g: Quercetinequivalent, Pe: Peel, Pu: Pulp, Pe+Pu: Peel + Pulp (together)

a, b, c, d, e, f, g: Least square means bearing different superscripts in the column [μ ±SE (mg EQ/g)]

Looking at the peels (Sh, Figure 2), from 65 to 75°C, an important loss was observed from 0.256±0.005 to 0.132±0.005 mg QE/g; resulting in a loss of 48.44%, which was significant (p<0.0001). After the first position, only peels followed with moderate drying temperatures. Especially, 65, 60, and 70°C products delivered 0.256, 0.218, and 0.204±0.005 mg QE/g, respectively (Table 2, 0.0001<p<0.0465). Importantly, each variation of 5°C led to significant differences in extractions (p<0.0001). Yeddes *et al.* (2013) showed that flavonoid contents in peels of *Opuntia ficus indicaw* was higher than in pulps, 4-fold higher for the thornless and 2.30-fold higher for the spiny. In different peach varieties' peel extracts, flavonoid contents ranged from 21.9 to 94.9 mg EQ/100 g, while these amounts decreased in pulp extracts to 5 to 58.9 mg QE/100g (Fan *et al.*, 2017). So, the flavonoid contents determined in the peach peels were 1 to 3 times higher than those of the corresponding pulps (Fan *et al.*, 2017). This concentration is an asset for health, since flavonoids, by their function, protect blood vessels from cholesterol-related damage (Abdou, 2009).

The extraction method had a huge impact on the extracts (Figure 2). When the products were dried at severe heats, the maceration gave the best extracts. Conversely, when the products were obtained at moderate heat, the decoction gave the best extracts. So, the additional heat during the decoction tended to destroy the flavonoids because they are very sensible to the heat (Md-Yusof *et al.*, 2019). In fact, when the extraction time is set, phytochemicals extract tends to increase from low drying temperatures, reach a high extracts level, and then decrease alongside the drying temperature elevation (Md-Yusof *et al.*, 2019). To resume, Md-Yusof *et al.* (2019) found that, when extracting total flavonoids in a medium of 80% of ethanol, the best drying temperature was 55°C. Above and below this reference of 55°C, the extracts decreased. This tendency of reaching a top extract surrounded by low extracts was also observed by Pham *et al.* (2020). When the peels combined with pulps were dried at 75°C, an additional heat by boiling at 100°C during the decoction destroyed the flavonoids. In contrast, when they were extracted during maceration, at room temperature, the results were the best among others. So, peels combined with pulps dried at 75°C delivered 0.450±0.007 mg QE/g through maceration, while this quantity dropped to 0.154±0.007 mg QE/g through the decoction (Figure 2, p<0.0001).



**Figure 2.** Total flavonoids' contents (mg QE/g) according to the extraction mode

σ=0.007; Sh: shell (peel); Sh+Pu: shell + pulp (peel+pulp); 60, 65, 70, and 75 (drying temperatures, °C).

a, b, c, d, e, f, g, h, i, j, k: Least square means bearing same superscripts do not differ significantly (p<0.05).

**Comment [pp10]:** 1) You should still use Pe instead of Sh.  
2) scale in the x-axis should be in line with QE/g values and with the name of the samples.

Taking care only of the peels (Sh), total flavonoids extracted at 60, 65, 70 and 75°C were 0.311, 0.203, 0.192, and 0.0097±0.007 mg QE/g, respectively. So, it may be concluded that, with pure peels, boiling at 100°C during the decoction revealed that alongside an increasing drying temperature, the total flavonoids were destroyed increasingly. But drying at 60°C needed additional boiling during decoction to give an efficient result. Doubtless, drying at 60°C, the decoction improved the total flavonoids extract by 2.48 times, from 0.125 with the maceration to 0.311±0.007 mg QE/g with the decoction.

*Borassus aethiopus* mature fruits' peels are good source of fibers, its fibrous pulp is rich in carotenoids, and it's a good source of reducing sugars too (Tiho *et al.*, 2018; Tiho *et al.*, 2020). According to Gonzalez *et al.* (1971), the rabbit body temperature fluctuates between 39.3 and 40.5°C, while ambient temperature goes from 5 to 35°C. So, its internal digestive fluids could be a good place for maceration at a moderate corporal temperature. Thus, drying the peels or the peels combined with the pulps at 65, 70 and 75°C could allowed good feedstuffs for rabbits. So, flavonoids antibacterial and many other feed advantages could be beneficial to rabbits.

### Condensed tannins content

According to Naumann *et al.* (2017), tannins are divided into 2 groups, whose are hydrolysable tannins (HT) and condensed tannins (CT). While hydrolysable tannins are gallic or ellagic acid esters linked to glucose, the condensed tannins or proanthocyanidins consist of flavan-3-ol subunits linked together to form oligomers and polymers. Altogether, HT and CT are defined as astringent, medium to high molecular weight polyphenolic compounds that bind and precipitate soluble proteins. So, their actions are important in the body. Moreover, Mueller-Harvey *et al.* (2019) reported that, due to bacteria resistance to antibiotics, condensed tannins are alternatives for animals' gut health. Better, when the forages distributed to ruminants is rich in condensed tannins, the feed improved growth, milk, wool production, and fertility (Harvey *et al.*, 2019). Most important is the ability of such forages to combat the effects of gastrointestinal parasitic nematodes (Harvey *et al.*, 2019).

Like the global behavior during polyphenols assessments, peels are richer in condensed tannins (CT) than the combined peels-pulps portions (Table 3, Figure 3). The condensed tannins behave like flavonoids. Of course, condensed tannins or proanthocyanidins are flavonoids that consist of two or more flavan-3-ol such as catechin, epicatechin or gallic catechin (Hue *et al.*, 2012). In fact, they were heat sensitive, from 60 to 75°C. In details, from 60 to 65°C, looking at the peels, the CT extracts dropped from 0.248±0.002 to 0.153±0.002 µg CatE/g. This gap was 38.31% loss (p<0.0001). Again, from 70 to 75°C, the peels CT extracts declined from 0.190±0.002 to 0.096±0.002 µg CatE/g, and it was 49.47% drop (p<0.0001). Progressively, the best extracts from the combined peels-pulps portion appeared at 60, 70 and 75°C, with a unique 0.058±0.002 µg CatE/g value.

Rashmi *et al.* (2017) concluded that the peels had 125-155% more tannin than the pulp, among mango varieties. Tannins values varied between 8 and 14 mg/g based on dry weight in the peel among the mango varieties, while in the pulp it was 3.5 to 10.6 mg/g based on dry weight. At different levels of fruit maturity, tannins were observed more in the peels than in the pulp. Elsewhere, Morales *et al.* (2017) found that condensed tannin contents of the different mango varieties ranged from 84.3 to 161.5 mg EC/100g dry weight. Moreover, Suleria *et al.* (2020) showed that avocado peels contained a slightly higher tannin content than its pulp.

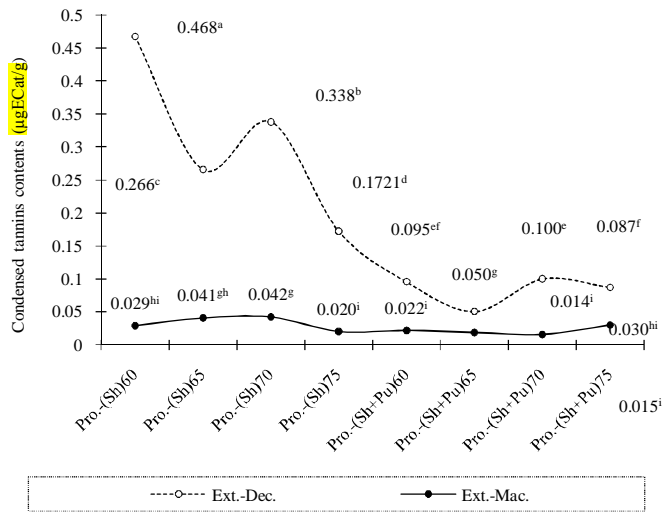
**Table 3.** Products condensed tannins contents (µg CatE/g)

Products	µ ±SE (µg CatE/g)	Products comparison	p-values
(Pe)60	0.248±0.002 <sup>a</sup>		
(Pe)70	0.190±0.002 <sup>b</sup>	(Pe)60 vs (Pe)70	<0.0001
(Pe)65	0.153±0.002 <sup>c</sup>	(Pe)70 vs (Pe)65	<0.0001
(Pe)75	0.096±0.002 <sup>d</sup>	(Pe)65 vs (Pe)75	<0.0001
(Pe+Pu)60	0.058±0.002 <sup>e</sup>	(Pe)75 vs (Pe+Pu)60	<0.0001
(Pe+Pu)75	0.058±0.002 <sup>e</sup>	(Pe+Pu)60 vs (Pe+Pu)75	0.9677
(Pe+Pu)70	0.058±0.002 <sup>e</sup>	(Pe+Pu)75 vs (Pe+Pu)70	0.8132
(Pe+Pu)65	0.034±0.002 <sup>f</sup>	(Pe+Pu)70 vs (Pe+Pu)65	<0.0001

µ: Least square mean, SE: standard error, µg CatE/g: microgram catechin equivalent per g, Pe: Peel, Pu: Pulp, Pe+Pu: Peel + Pulp (together)  
<sup>a, b, c, d, e, f</sup>: Least square means bearing different superscripts in the column [µ ±SE (mg EQ/g)]

Abbassia *et al.* (2018) work on *Silybum marianum* leaves showed that the solvent water had the highest contents of condensed tannins than methanol, ethanol, and acetone. Indeed, the condensed tannins gave a value of 3.86 mg TAE/g for the decoction and 2.30 mg TAE/g for the

maceration with solvent water (Abbassia *et al.*, 2018). So, distilled water was a good medium for extraction. Maceration is not an appropriate method to assess condensed tannins (Figure 3). Without a doubt, 60°C was the best drying temperature. Because CT extracts were  $0.468 \pm 0.003 \mu\text{g CatE/g}$ , with decoction while with maceration this result was  $0.029 \pm 0.003 \mu\text{g CatE/g}$ , 16.14 times lower than the precedent ( $p < 0.0001$ ). Besides 60°C, the following good drying temperature was 70°C. Again, from maceration to decoction, the CT extracts went from  $0.042 \pm 0.003 \mu\text{g CatE/g}$  to  $0.338 \pm 0.003 \mu\text{g CatE/g}$ , an increase of 8.05 times ( $p < 0.0001$ ).



**Figure 3.** Condensed tannins' contents ( $\mu\text{gE Cat/g}$ ) according to the extraction mode ( $\sigma = 0.003$ ; Sh: shell (peel); Sh+Pu: shell+pulp (peel+pulp); 60, 65, 70, and 75 (drying temperatures, °C).  
<sup>a, b, c, d, e, f, g, h, i</sup>: Least square means bearing same superscripts do not differ significantly ( $p < 0.05$ ).

**Comment [pp11]:** 1) You should still use Pe instead of Sh.  
 2) scale in the x-axis should be in line with condensed tannin values and with the name of the samples.

#### Antioxidant activities

The antioxidant activities were higher with peels than that of peels combined with pulps (Table 4). Particularly, on peels level, two drying temperatures delivered similar results. For instance, when the peels were dried at 65 and 70°C, the antioxidant activities were  $3.237 \pm 0.053$  and  $3.347 \pm 0.053 \mu\text{mol TE/g}$ , respectively ( $p = 0.1543$ ). Following, drying the peels at 75°C destroyed their phytochemicals by burning them. So, the extracts dropped from  $3.347 \pm 0.053$  to  $1.898 \pm 0.053 \mu\text{mol TE/g}$ , meaning 43.29% loss, equivalent to  $1.449 \pm 0.053 \mu\text{mol TE/g}$  ( $p < 0.0001$ ). The combined peel-pulp parts' best drying temperature were 65 and 75°C. In effect, at 65 and 75°C, the peels combined with the pulps' antioxidant extracts were  $2.027 \pm 0.053$  and  $1.995 \pm 0.053 \mu\text{mol TE/g}$ , respectively (similar,  $p = 0.2137$ ). Already, Arizo *et al.* (2011) indicated that *Gaillonia tinctoria* fruits peel extracts had the highest antioxidant capacity and reached a maximum value of  $2.7 \mu\text{M}$  at  $10 \mu\text{g/mL}$ , compared to their pulps.

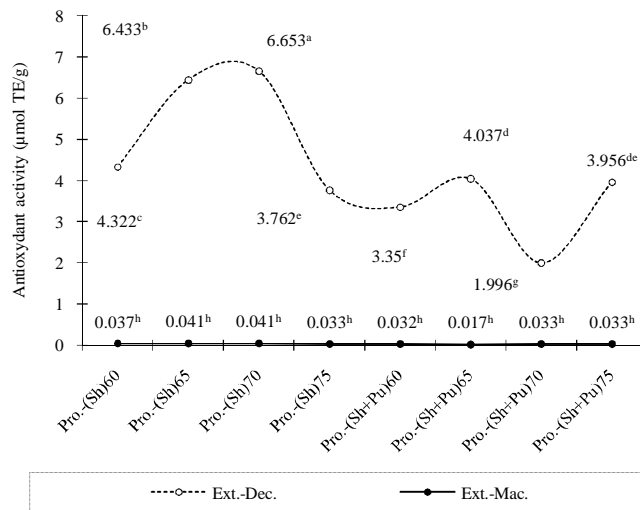
**Table 4.** Products antioxidant activities ( $\mu\text{mol TE/g}$ )

Products	$\mu \pm \text{SE}$ ( $\mu\text{mol TE/g}$ )	Products comparison	p-value
(Pe)70	$3.347 \pm 0.053^a$		
(Pe)65	$3.237 \pm 0.053^a$	(Pe)70 vs (Pe)65	0.1543
(Pe)60	$2.180 \pm 0.053^b$	(Pe)65 vs (Pe)60	<0.0001
(Pe+Pu)65	$2.027 \pm 0.053^{bc}$	(Pe)60 vs (Pe+Pu)65	0.0502
(Pe+Pu)75	$1.995 \pm 0.053^{bc}$	(Pe+Pu)65 vs (Pe+Pu)75	0.2137
(Pe)75	$1.898 \pm 0.053^c$	(Pe)60 vs (Pe)75	0.0038
(Pe+Pu)60	$1.691 \pm 0.053^d$	(Pe+Pu)75 vs (Pe)75	0.2067
(Pe+Pu)70	$1.014 \pm 0.053^e$	(Pe)75 vs (Pe+Pu)60	0.0099
		(Pe+Pu)60 vs (Pe+Pu)70	<0.0001

For example, Stojanovic *et al.* (2016) tested peels and pulps of several peach varieties. They concluded that, all peach varieties peels' extracts showed significantly higher scavenging activities ranging from 0.2 to 0.4  $\mu\text{mol TE}/100\text{g}$  fresh weight compared to pulp extract which ranged from 0.1 to 0.3  $\mu\text{mol TE}/100\text{g}$  fresh weight. In the same way, Fratianni *et al.* (2019) analyzed the peels and pulps extracts of three Citrus varieties were. The results showed that the peels of the different varieties of Citrus have a trapping activity much higher than that of the pulp (Fratianni *et al.*, 2019). Similarly, the radical scavenging activity of the quince peel was higher than that of the flesh, with the lowest IC 50 value of  $52.34 \pm 7.3 \mu\text{g/mL}$  (Babashpour-Asl, and Piryaei, 2021). Again, Costanzo *et al.* (2022) worked on *Citrus reticulablanco* and showed that the ability to trap free radicals is very significant. Specifically, the highest antioxidant capacity was observed in the peels compared to the pulps with respectively 17  $\mu\text{mol TE/g}$  fresh weight and 4.8  $\mu\text{mol TE/g}$  fresh weight (Costanzo *et al.*, 2022).

Admittedly, the extraction method was important. Since all extractions lasted 30 min, the decoction was more effective (Figure 4). In similarity with polyphenols, and condensed tannins, extracting through the decoction gave the best results for each dried product. Increasing the drying temperature from 65 to 70°C, and extracting the phytochemicals through decoction, significantly impacts the peels' antioxidant activity. Succinctly, the antioxidant activity increased by 3.42%, equivalent to a gain of  $0.22 \pm 0.075 \mu\text{mol TE/g}$ , from 6.433 to  $6.653 \pm 0.075 \mu\text{mol TE/g}$  ( $p=0.0473$ ). Not only did the decoction improve the extraction, but also revealed that the peels phytochemicals' contents were higher than those of the combined peel-pulp parts.

While the maceration outputs lined up with the x line, the decoction results rose, and fluctuated between 2 and 7  $\mu\text{mol TE/g}$ . So, the product obtained at 60°C extracts were  $0.037 \pm 0.075 \mu\text{mol TE/g}$  and  $4.322 \pm 0.075 \mu\text{mol TE/g}$ , respectively for maceration and decoction. Thus, the decoction permitted 116.81 folds the peels' extracts obtained through the maceration. Likewise, the extracts from the peels dried at 70°C, went from 0.041 to  $6.653 \pm 0.075 \mu\text{mol TE/g}$ , respectively for maceration and decoction. Equally, the decoction result was 162.27 times higher than that of maceration.



**Figure 4.** Antioxidant activity ( $\mu\text{mol TE/g}$ ) according to the extraction mode  $\sigma=0.075$ ; Sh: shell (peel); Sh+Pu: shell+pulp (peel+pulp); 60, 65, 70, and 75 (drying temperatures, °C).  
<sup>a, b, c, d, e, f, g, h.</sup> Least square means bearing same superscripts do not differ significantly ( $p < 0.05$ ).

**Comment [pp12]:** 1) You should still use Pe instead of Sh.  
 2) scale in the x-axis should be in line with TE/g values and with the name of the samples.

### Conclusion and perspectives

*Borassus aethiopus* fruits can be classified among the natural resources that provide the rural people food and off-farm incomes. This fruit has a high-water content and has the particularity of being good sources of sugars, vitamin C, minerals, and fiber. Locally, people use *Borassus aethiopus* products for different purposes such as therapeutic, drinking, fruit consumption, etc. Drying the mature fruits' pulps and peels is a good preservation method to avoid different losses. When it comes to assessing the nutritional value of *Borassus aethiopus* mature fruits' dried products, the drying temperature and the extraction method are very important. To sum up, at the first stage, the drying temperatures should range between 65 and 70°C. Thereafter, at the second stage, when the extractions are performed with distilled water, the

**Comment [pp13]:** What is this??

decoction is the best, compared to the maceration. The best polyphenols extracts were obtained with the peels dried at 70°C and 65°C for 0.082 and 0.077±0.001 mg GAE/g, respectively (p<0.0001). When the portions of peel-pulp were dried, the best drying temperature was 75°C, and the output was 0.072±0.001 mg GAE/g. This gap of 0.010±0.001 mg GAE/g was significant (p<0.0001). Naturally, these important extracts were sustained by high antioxidant activities. So, dried at 70 and 65°C, the peel decoction extracts' antioxidant activities were 6.653 and 6.433±0.075 µmol TE/g, respectively (p<0.0001). Due to condensed tannins (CT) effects on gastrointestinal parasitic nematodes, they were examined in the extracts. Interestingly, the products extracts were rich in condensed tannins. Particularly, the peels' extracts were richer than the combined peel-pulp portions. Furthermore, the decoction was the best method to extract the condensed tannins. So, the dried peels at 60, 65 and 70°C allowed the extraction of 0.468, 0.338 and 0.266±0.003 µg CatE/g, respectively. Moreover, within these drying temperatures, from a step to another, the difference was significant (p<0.0001). To conclude, firstly *Borassus aethiopicum* mature fruits are good sources of polyphenols. Moreover, when the fruits' parts are dried at a temperature between 65 and 70°C, they may provide important condensed tannins. Secondly, mature fruits contain some fibrous orange-yellow pulp, thus rich in carotenoids. So, this natural resource can be a good fiber source for rabbits, and the dried fruits' parts could be beneficial for rabbit gut and gastrointestinal health. In perspective, the peels may be sliced in fine parts, dried at room temperature, and assess its nutritional value.

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**Comment [pp14]:** Too many versions of reference style, please check and correct them.

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