



## Comparative study of the anti-radical activity of different citrus fruits and ginger cultivated in Sub-Saharan Africa and in Europe

N'Negue ép Mezui-Mbeng M A<sup>1</sup>, Edou Engonga P<sup>2</sup>, Ella Ndong J G<sup>1</sup>, Nguema Edzang R W<sup>1</sup>, Mezoukou Malho O<sup>3</sup>

<sup>1</sup> Laboratory of the Chemistry-Biochemistry, Faculty of Medicine, University of Health Sciences, Libreville, Gabon

<sup>2</sup> Laboratory of the Chemistry, Ecole Normale Supérieure, Libreville, Gabon

<sup>3</sup> Laboratory of the Biology, Ecole Normale Supérieure, Libreville, Gabon

### Abstract

Antioxidants help fight the harmful effects of free radicals. Although it is well accepted that fruits and vegetables are typical foods rich in antioxidants, no study has however compared the antioxidant capacity of different fruits and vegetables quite well consumed in sub-Saharan Africa and Europe. Also in this work, we propose to evaluate the anti-radical capacity of the juices of some fruits and a spice vegetable widely consumed in Gabon and in Europe, namely: lemon, orange, grapefruit, tomato, pineapple and ginger. The anti-radical activity was measured by scavenging the radical cation of 2, 2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS<sup>+</sup>) with gallic acid as the benchmark antioxidant.

The results obtained showed the following variations in anti-radical activity: Local products; ginger > orange and pineapple > grapefruit > tomato > lemon. For imported products; ginger > lemon > grapefruit and orange > tomato. Comparison between local and imported products: local ginger > imported ginger; imported grapefruit ≥ local grapefruit; imported lemon > local lemon; local orange > imported orange; local tomato > imported tomato.

In general, the evolution of the anti-radical activity of all fruit juices and spices is as follows: Local ginger > Imported ginger > Imported lemon > local orange > imported grapefruit ≥ imported orange > Local grapefruit > Local tomato > local lemon > Imported tomato.

In conclusion, we can say that the fruit and ginger juices from Gabonese soil have an anti-radical activity equivalent or even superior to that of their European counterparts. Ginger juice, whether local or imported, remains the compound endowed with the higher anti-radical activity.

**Keywords:** ABTS, antioxidant activity, local ginger, imported ginger, local fruits, imported fruits

### Introduction

Antioxidants are the health allies of our body that allow it to fight against the harmful effects of free radicals and other reactive oxygen species (ROS) along with nitrogen (RNS) (Aguilar. 2020) <sup>[1]</sup> Indeed, complex cellular or tissue metabolic reactions produce very unstable and aggressive oxygen derivatives. Under normal conditions, the production of ROS is counterbalanced by the enzymatic antioxidant systems (catalase, glutathione peroxidase, the family of superoxide dismutases, glutathione reductase etc.) and not enzymatic ( $\alpha$ -tocopherol, ascorbic acid, carotenoids, flavonoids etc.) (Sies. 1992) <sup>[2]</sup>, (Halliwell. 1994) <sup>[3]</sup>, (Sharifian. 2009) <sup>[4]</sup> and (Kozakiewicz. 2019) <sup>[5]</sup>. This balance is upset under certain conditions such as an increase in the production of activated forms of oxygen (exposure to high concentrations of O<sub>2</sub>, bacterial infection, consumption of tobacco or alcohol, intense physical activity, uptake of certain drugs, etc.) or a decrease in the efficiency of antioxidant systems (Kozakiewicz. 2019) <sup>[5]</sup>.

An increase in the intracellular concentrations of ROS responsible for oxidative stress is then observed (Finkel. 2000) <sup>[6]</sup>, (Nordberg. 2001) <sup>[7]</sup> and (Seis. 2015) <sup>[8]</sup>. In humans, oxidative damage to cellular components (lipids, proteins and DNA) is associated with certain chronic pathologies such as diabetes (Luc. 2019) <sup>[9]</sup>, cardiovascular diseases (Choi. 2018) <sup>[10]</sup>, and certain types of cancers

(Klaunig. 2018) <sup>[11]</sup>, inflammatory diseases, Alzheimer's disease and other diseases Neurodegenerative (Tönnies. 2017) <sup>[12]</sup> as well as the aging process (Kozakiewicz. 2019) <sup>[5]</sup>. Antioxidants are molecules naturally present in many foods consumed by humans, such as fruits and vegetables. They are found in these foods as phenolic compounds, flavonoids, carotenoids, anthocyanins or vitamins (Kozłowska. 2014) and (Eggerdorfer. 2018). According to WHO recommendations, a daily consumption of five fruits and vegetables would be beneficial to human health. An application of the WHO recommendations would therefore provide the human body with a high level of non-toxic natural antioxidants allowing it to fight against the harmful effects of ROS and free radicals. Although it is well accepted that fruits and some vegetables are foods endowed with high level of antioxidants, no study, however, has compared the antioxidant capacity of different fruits and vegetables fairly well consumed in sub-Saharan Africa and Europe. Also in this work, we propose to compare on the one hand, the anti-radical activity of some fruits and a spice vegetable produced in sub-Saharan Africa (Gabon), and on the other hand for the majority of them, to compare this activity to that of their counterparts imported from Europe. They are: lemon, orange, grapefruit, tomato, pineapple and ginger.

The anti-radical activity was measured by scavenging the

radical cation of 2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS<sup>•+</sup>) according to the method developed by Re *et al.* (Re. 1999) and optimized by N'negue *et al.* (N'negue. 2020) <sup>[16]</sup> with gallic acid as the benchmark antioxidant.

## Materials and Methods

### Materials

The different fruits evaluated and their origin are: grapefruit, lemon and tomato grown in Gabon and imported from Europe (France); Orange cultivated in Gabon and orange imported from Europe (Spain); pineapple grown in Gabon. Ginger grown in Gabon and imported from Europe (France) was the only spice evaluated in this study. All this biological material was purchased either in an air-conditioned supermarket for the imported material, or at the large popular open-air market in Libreville. ABTS (2, 2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]), gallic acid, potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) and hydrated sodium dihydrogen phosphate were purchased from Sigma-Adrich (Saint -Quentin Fallavier, France). The water used was distilled by the equipment of the "Milli-Q Labo" laboratory (Millipore Japan, Tokyo, Japan). All these products are quality for analysis. The anti-radical activity was determined by UV spectrophotometry: V-200 spectrophotometer (BOECO, Germany). The optical density was read at 734 nm, the maximum absorption wavelength of the radical cation ABTS<sup>•+</sup>.

### Preparation of "reference antioxidant" gallic acid solutions

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is an aromatic organic compound, used as a reference anti-radical compound.

Ten working solutions, of decreasing concentrations, ranging from 0.5 to 5 μM, were prepared by diluting gallic acid in distilled water.

### Preparation of the juices of the different fruits

Grapefruit, lemon, and orange juices imported and not imported from Europe were obtained after manual pressing of the fruits. The same is true for imported tomato juice. As for tomatoes grown in Gabon, pineapple, local and imported ginger, the juice is obtained after cutting into pieces and blending in an electric mixer.

## Measurement of anti-free radical activity

The principle of the test for measuring the anti-radical activity by the ABTS method is based on the decrease in the absorbance at 734 nm of the radical cation ABTS<sup>•+</sup> (blue-green coloration) in the presence of a potentially anti-radical compound which reduces the radical cation. The reduction in the radical form of ABTS<sup>•+</sup> leads to a discoloration of the solution. The ABTS<sup>•+</sup> radical ion is obtained by reacting the ABTS molecule (7 mM) with potassium persulfate (2.45 mM), in distilled water for 16 hours at room temperature and under cover light. The ABTS<sup>•+</sup> solution obtained is diluted with sodium phosphate buffer (5 mM, pH = 7.4), in order to obtain a stock solution having an initial absorbance value at 734 nm between 0.65 and 0.70. The radical cation (ABTS<sup>•+</sup>) is stable for more than 2 days when stored at room temperature and protected from light. All the assays were carried out three times and the anti-radical activity is calculated according to the formula below: Anti-radical activity (%) = [1 - (Ar-Ab) / (Ai-Ab)] x 100. With Ar = remaining activity of ABTS<sup>•+</sup>, Ai = initial activity of ABTS<sup>•+</sup> and Ab = Activity of blank.

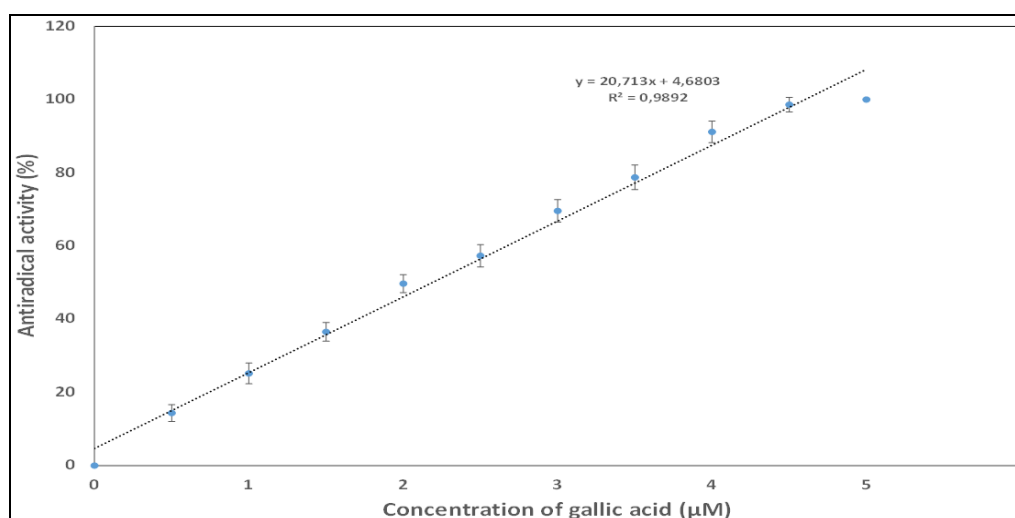
In fact, the reduction of the radical cation ABTS<sup>•+</sup> therefore yields to determining the anti-radical activity and in total, the antioxidant properties of whole blood, serum and plasma, compared to the antioxidant properties of gallic acid (standard). The anti-radical activity was determined by UV spectrophotometry in cuvettes with an optical path of 1 cm (reaction volume of 2 mL).

The incubation time is 6 minutes at room temperature.

## Results

### Anti-radical activity of gallic acid depending on the concentration

The percentage (%) of anti-radical activity increases linearly with the concentration of gallic acid (Figure 1). The ABTS<sup>•+</sup> Radical disappears in the presence of the standard antioxidant. Indeed, an anti-radical activity of 14.34 ± 2.31% was recorded for a concentration of 0.5 μM of gallic acid. This activity increases to 98.6 ± 1.98% for a concentration of 4.5 μM. According to our results, the IC<sub>50</sub> of gallic acid, which is the concentration required to reduce the anti-radical activity by 50%, is 2 μM (0.37 μg.mL<sup>-1</sup>).

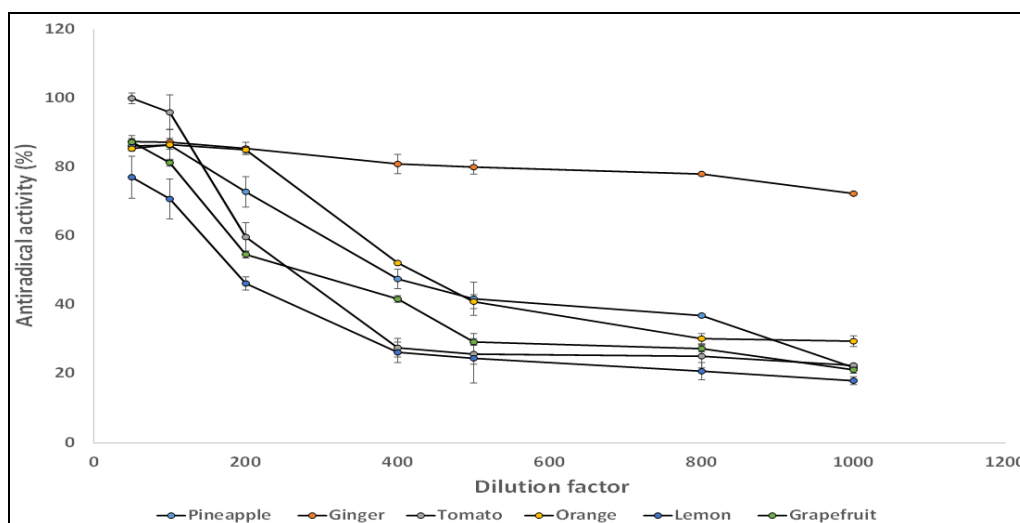


**Fig 1:** Anti-radical activity as a function of the concentration of gallic acid after 6 minutes of incubation. The proportion ABTS<sup>•+</sup> transformed into ABTS<sup>+</sup> in the presence of gallic acid is calculated from the change in absorbance at 734 nm measured by spectrophotometry. The equation on the right is:  $y = 110.28x + 4.9$  ( $R^2 = 0.98$ );  $n = 3$ .

### Anti-radical activity of fruits and ginger grown in Gabon

The results of the evaluation of the anti-free radical activity of grapefruit, lemon, orange, pineapple, tomato and ginger juices grown in Gabon are shown in Figure 2. According to

these results, ginger juice has the strongest anti-free radical activity, followed by orange juice and pineapple juice whose anti-free radical activities are more or less equal. Then comes grapefruit juice followed by tomato juice. Lemon juice was the one with the lowest activity.

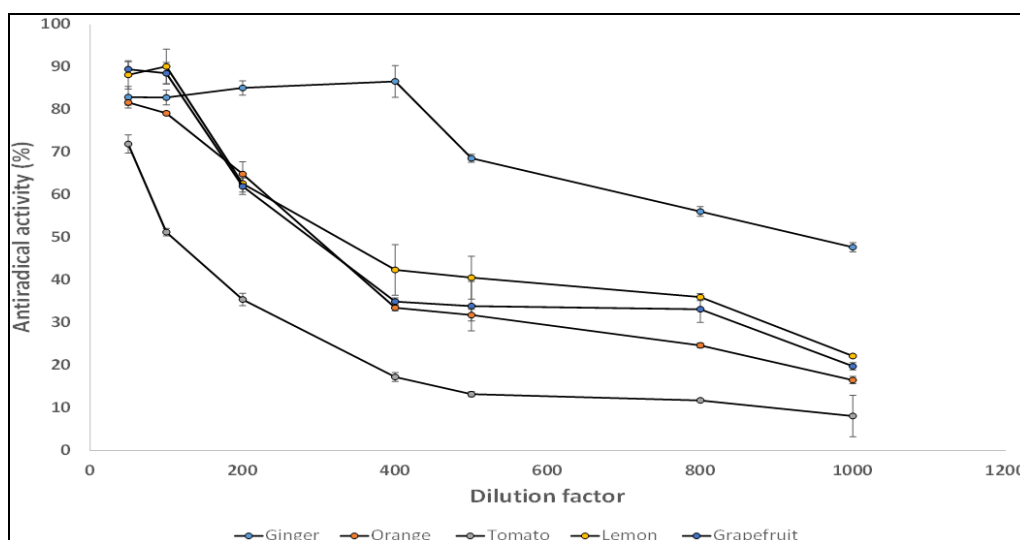


**Fig 2:** Anti-radical activity according to the dilution of fruit and ginger juices grown in Gabon after 6 min of incubation. The proportion  $ABTS^{\bullet+}$  transformed into  $ABTS^+$  in the presence of juice samples is calculated from the change in absorbance at 734 nm measured by spectrophotometry;  $n = 3$ .

### Anti-radical activity of fruits and ginger imported from Europe

According to the results presented in Fig. 3, ginger juice has an anti-radical activity much greater than that of other

imported fruit juices. Then comes lemon juice, whose anti-radical activity is slightly better than grapefruit and orange juice, both of which have more or less equivalent activities. Tomato juice has the lowest potency.



**Fig 3:** Anti-radical activity according to the dilution of imported fruit and ginger juice after 6 min incubation. The proportion  $ABTS^{\bullet+}$  transformed into  $ABTS^+$  in the presence of orange juice samples is calculated from the change in absorbance at 734 nm measured by spectrophotometry;  $n = 3$ .

### Comparison of the anti-radical activity of fruit juice and ginger juice grown in sub-Saharan Africa and those imported from Europe

#### Grapefruit juice

According to the results obtained (Figure 4), grapefruit juice cultivated in Africa (Gabon) and grapefruit juice imported from Europe have quite similar anti-free radical activities, however with a slight advantage for imported grapefruit juice. Indeed, according to Figure 4, the two trend curves representing the anti-radical activity as a function of the

dilution of the two grapefruit juices are superimposed. The curve for imported grapefruit juice is slightly higher for the lowest dilutions. In terms of percentage of anti-radical activity (% ARA), our results showed that for a dilution factor of 50 for example, the values of the two juices are practically the same. They are 87.33% for local fruit juice and 89.53% for grapefruit juice imported from Europe.

#### Lemon juice

The results obtained (Figure 4) show that the lemon

imported from Europe has a higher ARA percentage of 10 to 15% (depending on the dilution factor) than that of the lemon produced in Gabon. Indeed, for a dilution factor of 50 for example, the % ARA is  $77.08 \pm 1.19\%$  for local lemon juice and  $88.21 \pm 0.31\%$  for imported lemon juice. They are  $26.27 \pm 2.09\%$  for local lemon and  $42.42 \pm 5.9\%$  for imported lemon, for a dilution factor of 400.

### Orange juice

According to the results (Figure 4), orange juice imported from Europe has an anti-radical activity 4 to 9% lower than that of local orange juice for certain dilutions. This difference can be up to 20% for other dilutions.

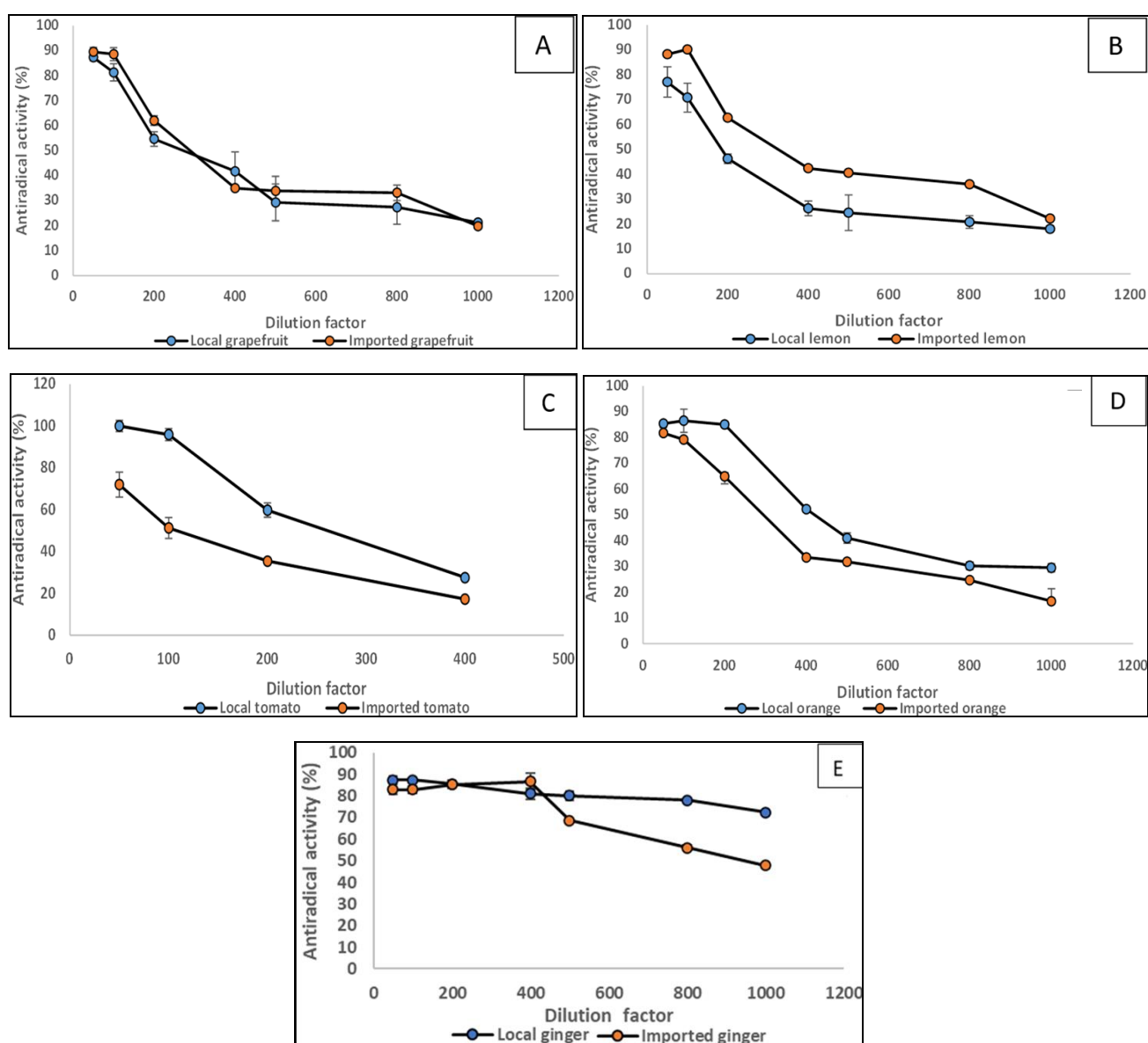
### Tomato juice

The results obtained (Figure 4) showed an anti-radical activity of local tomato juice greater than that of imported tomato juice. Indeed, for a dilution to the thousandth (1/1000), the percentage of anti-radical activity is  $22.40 \pm$

$1.56\%$  for local tomato juice, and only  $8.08 \pm 2.15\%$  for imported tomato juice. For the dilution factors of 100 and 50, the anti-radical activities are  $95.90 \pm 3.40\%$  and  $100\%$  respectively, for local tomato juice, against only  $51.26 \pm 0.05\%$  and  $71, 98 \pm 4.88\%$  respectively for tomato juice imported from Europe. That is a strong difference in anti-radical activity of about 40% for a hundredth dilution.

### Ginger juice

According to the results obtained (Figure 4), local ginger juice exhibits better anti-radical activity at high dilutions (1/500; 1/800; 1/1000). However, for lower dilutions and therefore for a more concentrated ginger juice, the anti-free radical activities of the local and imported juice are almost identical. Indeed, according to the results, the anti-radical activity of local ginger juice is  $72.34 \pm 1.76\%$  for a thousandth dilution against  $47.73 \pm 2.59\%$  for imported ginger juice.



**Fig 4:** Anti-radical activity according to the dilution of local fruit juices imported from Europe after 6 min of incubation. The proportion  $ABTS^{\bullet+}$  transformed into  $ABTS^+$  in the presence of samples of fruit juice and ginger is calculated from the change in absorbance at 734 nm measured by spectrophotometry;  $n = 3$ .

### Discussion

The antioxidant activity of the fruits was evaluated by

scavenging free radicals with the radical cation  $ABTS^{\bullet+}$  according to the method of Re *et al.* (Re, 1999) optimized

by N'negue *et al.* (N'negue. 2020)<sup>[16]</sup> with gallic acid as the benchmark antioxidant. The results of the antioxidant activity of gallic acid validate the chosen method. Indeed, according to our results, the IC50 value of gallic acid was 2  $\mu\text{M}$ . This value is more or less equivalent to that recorded by Sadat *et al.* (Sadat. 2011)<sup>[17]</sup> or 2.5  $\mu\text{M}$  and N'negue *et al.* (Re. 1999), (N'negue. 2021)<sup>[18]</sup> or 2.5 and 2  $\mu\text{M}$ . These authors worked under the same conditions. Regarding the comparison of the anti-radical activity of the different fruit and ginger juices grown locally in Gabon, the results obtained (Figure 2) showed that ginger juice was the most active, followed by orange and pineapple juices whose activities overlap. Then comes grapefruit juice followed by tomato juice. Lemon juice seems to be the least active among all the juices of the local products tested namely: ginger, orange, pineapple, grapefruit, tomato and lemon. The anti-radical activity of ginger juice and the juices of all the fruits tested is linked to the presence in variable amounts in these local products of antioxidant compounds such as vitamins C and E, polyphenols, carotenoids, flavonoids, etc. (Diallo. 2016)<sup>[19]</sup>. Indeed, studies have described the presence in ginger of high levels of polyphenols and flavonoids responsible for its free radical scavenging activity of DPPH and ABTS<sup>•+</sup> (Lu. 2017)<sup>[20]</sup>, (Kim. 2018)<sup>[21]</sup>, (Mustapha. 2019)<sup>[22]</sup> and (Ademosun. 2021)<sup>[23]</sup>. The presence of phenolic compounds, phenolic acid, anthocyanin and Vitamin C in orange juice is described (Galani. 2017)<sup>[24]</sup>. According to some authors, pineapple juice and even co-products from the industrialization of pineapple contain phenols which are responsible for their antioxidant activity (Frontela-Saseta. 2011)<sup>[25]</sup> and (Martinez. 2016). A good amount of antioxidant bioactive flavonoids are found in grapefruit peel (Castro-Vazquez. 2016)<sup>[27]</sup>.

In addition, grapefruit juice contains anti-radical phenolic compounds such as bergaptol (Girenavar. 2007)<sup>[28]</sup>, vitamin C and carotenoids (Zacarias-García. 2021)<sup>[29]</sup>. Studies show that the tomato contains antioxidant compounds such as phenolic compounds, flavonoids, vitamin C, anthocyanins (Aguiar. 2020)<sup>[1]</sup>. Lemon contains phenolic compounds, vitamin C and even flavonoids (Nakajima. 2019)<sup>[30]</sup> and (Kang. 2020)<sup>[31]</sup>. The level of anti-free radical compounds would therefore be higher in ginger juice compared to the other juices tested and less important in lemon juice.

Regarding the comparison of the anti-radical activity of different fruit and ginger juices imported from Europe, the results obtained (figure 3) showed that ginger juice has the highest anti-radical activity, followed by lemon juice, then grapefruit and orange juice which showed more or less similar anti-radical activities. Tomato juice exhibited the lowest anti-radical activity. We can therefore say from these results that ginger juice imported from Europe contains a higher rate of anti-radical compounds than that of lemon juice, which is higher than that of grapefruit and orange juice.

The imported tomato is said to have the lowest level of anti-radical compound of any imported product. Note that lemon juice, which is the least active juice among local fruits, has been found among the most active of imported juices. This could be explained by a difference at the phenotypic or genotypic level of the lemon tree. Indeed, according to the literature, the chemical composition of the pulp of a fruit can be very different depending on the color of the fruit (Zacarias-García. 2021)<sup>[29]</sup>.

Comparison of the anti-radical activity of local fruit and ginger juices imported from Europe (Figure 4) showed that:

Local grapefruit juice and imported grapefruit juice have quite similar anti-radical activities, with a slight advantage over imported grapefruit juice (Figure 4A). We can therefore say that the level of anti-free radical compounds in the juice of the two grapefruit species remained the same despite the difference in temperature or the nature of the cultivation soil.

However, lemon juice imported from Europe showed a percentage of anti-free radical activity 10 to 15% higher (depending on the dilution factor) than that of local lemon (Figure 4B). We could explain this result either by the effect of the European climate, the temperature and the phenotype and genotype of the European lemon tree which would produce lemons with a level of anti-radical compounds higher than those of its African counterpart. Another hypothesis would be the better preservation of imported fruits and vegetables in air-conditioned supermarkets respecting the cold chain, local fruits being most often sold in popular markets where they are strongly exposed to the sun. We may think that prolonged exposure to UV rays from the sun could alter the chemical and therefore anti-free radical properties of local fruits. Moreover, according to the literature, UV rays activate oxidative stress (Xue. 2016)<sup>[32]</sup> and (Nakashima. 2017)<sup>[33]</sup>.

Interestingly, while focusing on orange, tomato and ginger juices, the results obtained (Figures 4C, 4D and 4E) showed that local orange juice had an anti-radical activity greater than 4 to 20% depending on the dilutions to that of imported orange juice. Likewise, local tomato and local ginger juices exhibited superior radical activity than their imported counterparts. We can make different hypotheses to explain this result. The first would be that the hot and humid climate in sub-Saharan Africa (Gabon) and the genetic factors (genotypes and phenotypes) of the plants and shrubs producing these fruits and this spice vegetable would promote the synthesis of the bioactive compounds responsible for their anti-free radical activity in comparison to the Mediterranean climate of Europe. The second hypothesis would be that the more or less long time and the conditions of transport by boat from Europe to Gabon could be at the origin of a deterioration in the quality of the imported fruits with the consequence of a decrease in the anti-radical capacity of these. Moreover, according to the literature, the antioxidant power of fruits and vegetables varies with the type of storage (Nakashima. 2017)<sup>[33]</sup>.

## Conclusion

Ginger, grapefruit, lemon, orange and tomato juices from Gabonese and European soils are all endowed with anti-radical activity. Maximum activity was observed with ginger juice cultivated in Gabon (sub-Saharan Africa) then ginger juice produced in Europe (France) then with European lemon juice. A number of studies have revealed the involvement of free radicals in the etiology of several pathologies such as diabetes, certain types of cancers, cardiovascular diseases, atherosclerosis, etc. A high consumption of ginger and fruits could slow down the onset of these diseases linked to oxidative stress. In addition, lyophilized ginger and fruits rich in antioxidants could be used to increase the shelf life of preparations in the food and pharmaceutical industries. Finally, these results valorize the fruits and spices cultivated in Gabon which have shown anti-radical activities which may be equal to or greater than those from Europe. This should encourage the population to

consume local fruits that are by far much cheaper.

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