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Fermentation Effects on Physicochemical, Nutritive and Antinutritive Characteristics of Oranges (*Citrus sinensis*) and Pomelos (*Citrus paradisi*) Seeds in Cote D'ivoire

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ABSTRACT

Citrus fruit residues, composed of peel, mesocarp, and seeds, represent more than 58% of the total mass of the fruit. Therefore, the high consumption of citrus fruits, especially oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*), generates enormous quantities of residues, including the seeds that were the subject of this study. Also, the seeds would be sources of nutritive compounds. Unfortunately, they are not valued in food because of their composition in antinutrients such as phytates and oxalates which prevent the body from absorbing nutrients. Hence the interest of this study aims to contribute to the valorization of oranges and pomelos seeds by fermentation. The study was carried out on oranges and pomelos purchased in Abidjan, Côte d'Ivoire. The data show statistically identical physicochemical characteristics by species. Overall, the seed batches show a pH close to 6 and therefore acidic. Protein contents vary between 10 and 15.5 ± 0.05 g/100 g; polyphenols contents are between 0.588 ± 0.05 g/100 g and 0.641 ± 0.08 g/100 g. Lipids constitute the highest fraction with contents between 38.8 ± 0.2 g/100 g and 45 ± 0.40 g/100 g. thus, oranges and pomelos seed fermentation influence the physicochemical, nutritive, and antinutritive characteristics. It increases their titrable acidities and certain macronutrient levels reduce the contents of phytates and oxalates by 0.15 g/100 g and 0.10 g/100 g, respectively. The results of this study would enhance oranges and pomelos alimentary valorization.

1. INTRODUCTION

Oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) are fruits of the genus *Citrus* belonging to the *Rutaceae* family. Côte d'Ivoire produced 108,000 tons of citrus fruits in 2017 including 40,000 tons of oranges and 24,000 tons of pomelos **[1]**. These citrus fruits are rich in various nutrients such as vitamins (A and C), folic acid, and dietary fiber. They are sources of bioactive compounds such as flavonoids, coumarins, limonoids, and carotenoids**[2 ; 3; 4]**.

These compounds are important in a balanced diet. Like other citrus fruits, oranges and pomelos pulp is consumed fresh or processed into juices and various dehydrated products. They are sometimes used in cosmetic and therapeutic fields. As a result, enormous quantities of residues, consisting of seeds, peels, and mesocarps are discarded during consumption or processing. According to **Marin[5]**, seeds are an important source of bioactive compounds. Moreover, studies on the biochemical characteristics of *Citrus sinensis* (sweet oranges) seeds oil have shown that it is rich in essential fatty acids such as linoleic and linolenic acids, as well as vitamin E **[6; 7]**. Other studies have shown that bitter oranges (*Citrus aurantium*) seed flour significantly reduces blood glucose levels **[8]**.

However, some studies on seeds composition reveal the presence of antinutritional factors such as phytates, oxalate, and tannins **[9]**. These are chemical compounds that interfere with nutrients absorption in humans and animals **[10]**. They can reduce the bioavailability of certain compounds or inhibit enzymes needed for digestion. Thus, technological treatments such as fermentation, soaking, and cooking could reduce their teneusr**[11; 12]**. Fermentation is an ancestral process and one of the most widely used methods in the preservation and processing of food raw materials. It prolongs their shelf life and increases the bioavailability of certain minerals and vitamins. Also, it eliminates toxic elements and reduces anti-nutritional factors while improving nutritional properties **[13]**.

In Côte d'Ivoire, the food use of seeds from the high consumption of oranges and pomelos is limited because of their composition in antinutrients. Hence, the interest of this study whose objective is to determine the influence of fermentation on the nutritive and antinutritive composition of seeds. More specifically, the nutritional composition of fermented oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) seeds will be determined, their antinutritive composition will be determined and compared to that of unfermented oranges and pomelos seeds.

2. MATERIAL AND METHODS

2.1 Material

The studied seeds were obtained from oranges (*Citrus sinensis*) of blond variety and mature pomelos (*Citrus paradisi*) of red bush variety (red bush).

2.2 Methods

2.2.1 Sampling

Mature oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) fruits, showing no visible signs of disease, were collected from October to November 2019 at the wholesale market in Adjamé, the largest fruit market in Abidjan. Thus, 3 traders of oranges and pomelos were considered. From each trader, 150 oranges and 150 pomelos were collected. That is a total of 450 oranges and 450 pomelos. The samples were conveyed to the Laboratory of Biochemistry and Food Sciences (LaBSA) and a pool was made by mixing the collected fruits of the same species before extracting their seeds.

2.2.2. Analyses

The analyses concerned the proportions in seeds, the contents in dry matter, pH, titrable acidity, fat, proteins, sugars, total polyphenols, and antinutritional compounds of the fermented or unfermented seeds. All the tests were carried out in triplicate.

The seed proportions were calculated by weighing the seeds extracted from oranges or pomelos and calculating their proportions with the total mass of fruit.

The dry matter content was determined according to the method of BIPEA **[14].** The results were expressed in mg per 100 g of fresh matter.

The pH is determined using a pH meter. This determination is carried out according to the AOAC**[15]** method. A mass of 10 g of crushed seeds is homogenized in 100 ml of distilled water and filtered through filter paper (Whatman). The filtrate was used to determine the pH value using a pH meter (Hanna). Subsequently, 10 ml of the filtrate was titrated with a solution of NaOH (0.1 N) with the addition of phenolphthalein to pink for titrable acidity determination.

Fat content was determined by the AOAC**[16]**method using a Soxhlet type extractor.

Protein was determined by the AOAC **[17]** method. The determination of protein content was reduced to that of total nitrogen in the sample by Kjeldahl.

The extraction of ethanol-soluble sugars was carried out according to Agbo **[18]**; the determination of total sugars was determined by the method described by Dubois**[19]**using phenol and sulphuric acid.

reducing sugars content was determined by the method described by Bernfeld**[20]** using 3,5-dinitrosalicylic acid (DNS).

Phenolic compounds are extracted with methanol and determined by the method of Singleton**[21]**.

Antinutritives compounds were tested by phytate and oxalate assays. The oxalates were determined according to the method described by Day and Underwood**[22]** using potassium permanganate. Phytates were quantified according to the method of Latta and Eskin**[23]** based on the decoloration of Wade's reagent by phytates.

3. RESULTS

3.1. Physicochemical characteristics of studied seeds

The different proportions of oranges and grapefruit seeds obtained with the total fruit masses are small (Fig. no.1). However, oranges are more seeded (7%), relative to the mass of the fruit, than the pomelos (5%).

All dry matter contents of fermented and unfermented seeds are less than 50%. They range from 46.22% to 47.85%. Pomelos seeds have statistically identical dry matter contents regardless of the treatment. Concerning the pH, all the values are close to 6. Indeed, the pH of oranges seeds varies from 5.34 to 5.35 while those of pomelos vary between 5.44 and 5.99. Also, fermented oranges and pomelos seeds have lower pH values with 5.34 for oranges and 5.44 for pomelos. As for titratable acidity, statistical analysis has not revealed any significant difference between batches of each Citrus species studied. The titrable acidity of unfermented oranges seeds is 0.29 meq/100 g and that of fermented oranges seeds is 0.77 meq/100 g. The titrable acidity of fermented pomelos (*Citrus paradisi*) seeds is 0.7±0.01 meq/100 g higher than that of unfermented seeds. The ash contents of oranges and pomelos seeds are statistically identical. Those of oranges are between 2.73 and 2.83%, while those of pomelos seeds, fermented or not, are between 2.93 and 2.96%.

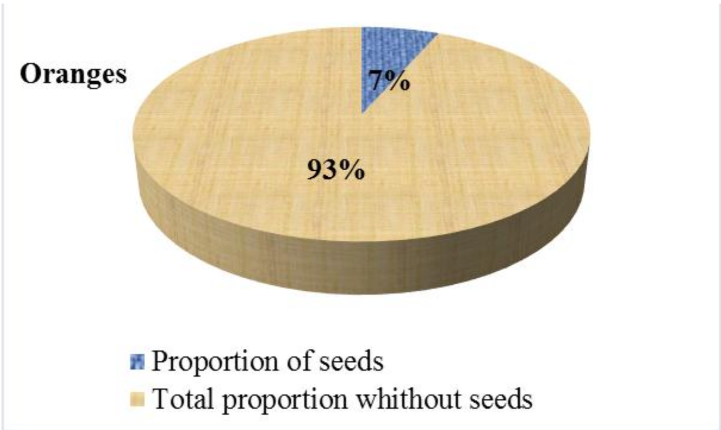

Figure No. 1: Proportion of seeds per contribution to the mass of oranges (a)

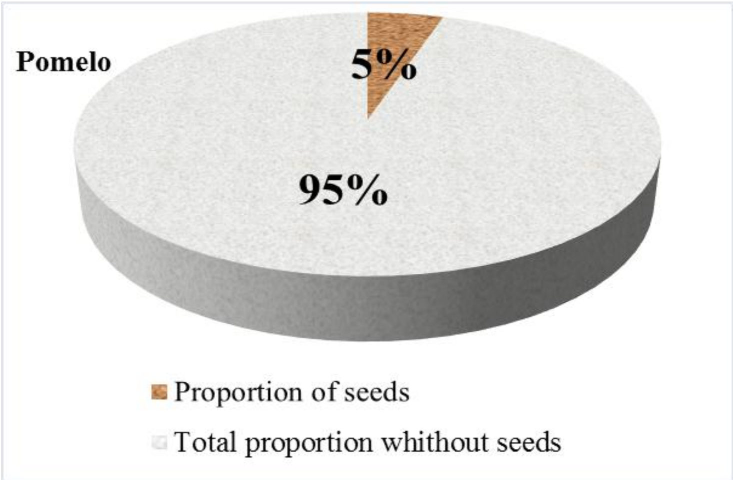

Figure No. 2: Proportion of seeds per contribution to the mass of a pomelo (b)

Table No. I: Physicochemical characteristics of oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) seeds

Traitement		Dry matter Sèche (%)	pH	Titrable acidity (meq/100 g)	Ash (%)
Oranges (*Citrus sinensis*)	Unfermented	$47,85\pm0,42^{a}$	$5,35\pm0,36^{a}$	$0,29\pm0,1^{a}$	$2,73\pm0,23^{a}$
	Fermented	$47,25\pm0,42^{b}$	$5,34\pm0,06^{b}$	$0,77\pm0,01^{a}$	$2,83\pm0,15^{a}$
Pomelos (*Citrus paradisi*)	Unfermented	$46,22\pm0,06^{A}$	$5,99\pm0,09^{A}$	$0,39\pm0,01^{A}$	$2,93\pm0,12^{A}$
	Fermented	$46,31\pm0,06^{A}$	$5,44\pm0,07^{B}$	$0,70\pm0,01^{A}$	$2,96\pm0,06^{A}$

Means and standard deviations followed by the same superscript lowercase or uppercase letter in the same column are not significantly different at 5%, according to the Newman-Keuls test.

3.2 Nutritional characteristics of the studied seeds

The nutritional characteristics of oranges and pomelos seeds are presented in Table II. Ash contents range from 2.73±0.23% to 2.96±0.06%. They are not statistically different among unfermented and fermented seeds of two Citrus species studied.

Total sugar content don't show significant differences between unfermented and fermented samples. Thus, they are respectively 1.66±0.75 g/100 g and 1.42±0.61 g/100 g for unfermented and fermented oranges seeds. Those of pomelos are 1.71±0.78 g/100 g, for the unfermented seeds and 1.23±0.48 g/100 g, for the fermented batch.

On the other hand, the contents of reducing sugars of studied seeds are statistically different. Indeed, unfermented oranges and pomelos seeds have higher contents than fermented seeds. They are estimated at 0.75±0.1 g/100g for unfermented oranges seeds and 0.90±0.12 g/100g for those of unfermented pomelos. Fermented pomelos seeds contain 0.58±0.04 g of reducing sugars and 0.66±0.05 (unfermented oranges seeds) per 100 g of DM.

The protein content of fermented oranges seeds is higher than that of unfermented ones with a value of 14.5±0.05 mg/100 g. On the other hand, those of pomelos seeds, fermented or not, are the same with a value of 15.5 mg/100 g.

Lipids represent the largest nutritional fraction of oranges and pomelos seeds studied, ranging from 41 to 45%. The lipid content of fermented pomelos seeds is the lowest with a value of 38.8%.

Table No. II: **Nutritional composition of oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) seeds expressed in g/100g MS**

	Traitements	Total sugars	Reducing sugars	Proteins	Fat	Polyphénols*
Oranges *Citrus sinensis*	unfermented	1,66±0,07^{a}	0,75±0,1^{ab}	10,5±0,05^{a}	43,6±0,4 ^{b}	0,60±0,04^{a}
	Fermented	1,42±0,06 ^{a}	0,66±0,05^{b}	14,5±0,05 ^{a}	45±0,40 ^{a}	0,59±0,05^{b}
Pomelos *Citrus paradisi*	unfermented	1,71±0,07 ^{A}	0,90±0,12^{A}	15,5±0,73^{A}	41,7±0,10^{A}	0,64±0,08^{A}
	Fermented	1,23±0,04 ^{A}	0,58±0,04^{B}	15,5±0,05^{A}	38,8±0,20^{B}	0,59±0,09^{B}

*gEAG/100g DW

Means and standard deviations followed by the same superscript lowercase or uppercase letter in the same column are not significantly different at 5%, according to the Newman-Keuls test.

3.3. Antinutrient composition of studied seeds

Oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) seeds contain antinutritional factors such as phytates and oxalates, whose levels are generally lower in fermented samples than in unfermented ones (Figure 3).

For each citrus fruit studied, the fermented or unfermented seeds phytate contents, are not statistically different and are higher than those of the oxalates.

On the other hand, the oxalates contents of fermented oranges seeds (0.50 mg/100 g) are lower than those of unfermented oranges seeds (0.60 mg/100 g).

The phytates contents of pomelos seeds decrease with fermentation from 0.74 mg/100 g to 0.59 mg/100 g. Similarly, their oxalates contents decrease significantly, from 0.55 mg/100 g MS for unfermented batches to 0.45 mg/100 g MS for fermented batches.

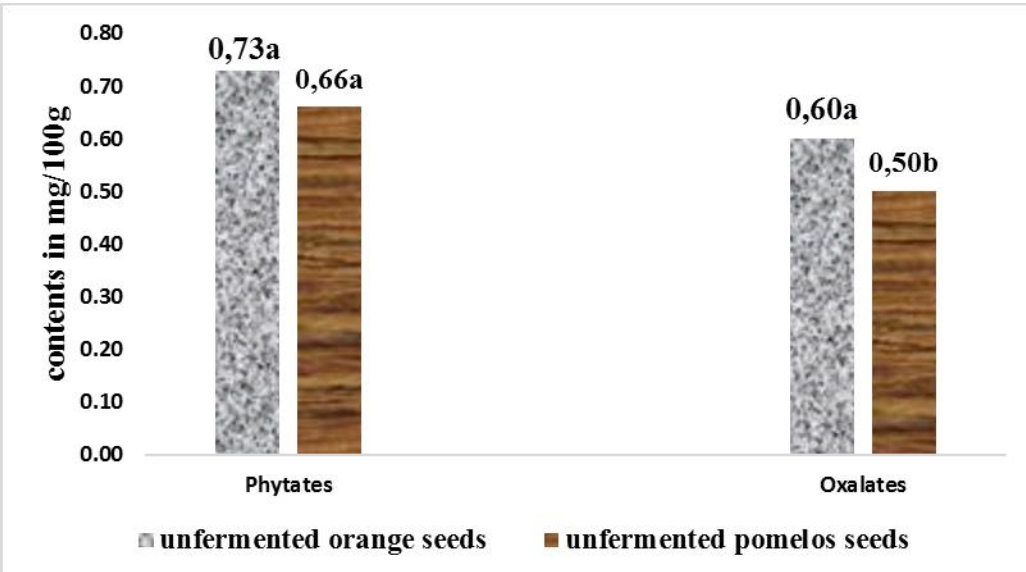

Figure No. 3: Phytates and oxalates content in oranges seeds.

4. DISCUSSION

Oranges and pomelos seeds constitute a small proportion of the total fruit mass. The difference in the dry matter content of the seeds may be because the two fruits are not of the same species of the genus Citrus. Oranges are of *Citrus sinensis* species while pomelos fruits are of Citrus paradisi. Dry matter contents below 50% mean that they are rich in water. Water activity being thus important, it would favor the development of microorganisms making the preservation of seeds complex. Different pH values are lower than 6 and therefore indicate the acidic character of the Citrus seeds studied. However, the results suggest a negative correlation between pH and titrable acidity. Indeed, pH results form hydronium ions (H_3O^+) whereas the titratable acidity depends on the content of acid volatile organic compounds such as amino acids and fatty acids **[24]**.

Oranges and pomelos seeds contain many nutritive compounds such as sugars, proteins, lipids, and polyphenols.

Protein contents of the oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) seeds studied are high. Results are globally similar to those of Lagou**[25]**. Also, fermentation increases the amino acid level through the action of enzymes synthesized by fermenting microorganisms. These results give the seeds major importance because proteins are necessary for the proper functioning of the organism. They play a structural role in the body (muscular or skin), but proteins are also involved in many processes such as the immune response (antibodies), oxygen transport in the body (hemoglobin), or digestion (digestive enzymes) **[26]**.

Our results indicate that fat constitutes the highest fraction of the nutrient compounds in the seeds. This has been proven by several studies **[25; 27; 28]**. Total lipid contents obtained are consistent with those determined by El-Adawy**[27]** on oranges (*Citrus sinensis*) seeds. However, oranges seeds contain more fat than those of pomelos. This is contrary to the data of Anwar**[29]**, on oranges seeds (*Citrus sinensis*) and pomelos (*Citrus paradisi*) in Pakistan. According to their results, pomelos seeds are more lipidic than those of oranges. This difference can be explained by the study areas divergence and the difference in environmental factors such as climate, soil fertility, and ripeness. These results constitute a way to valorize seeds of the Citrus. Indeed, according to some previous studies, lipids contained in citrus seeds are a real matrix of essential fatty acids such as linoleic acid and linolenic acid respectively omega-3 (ω-3) and omega-6 (ω-6) **[29; 30]**. Indeed, omega-3 and omega-6 are essential fatty acids that cannot be synthesized by the body and are provided by the diet. They are important components of the cell membrane and give rise to many other compounds in the body, such as those involved in the regulation of blood pressure and inflammatory responses **[31; 32]**.

The presence of phenolic compounds in the studied seeds represents an advantage for health. According to Wang **[33]**, polyphenols are recognized for their effectiveness in reducing many risks such as cancer, muscle degeneration, skin damage due to sunburn, and cardiovascular disease. They could therefore be incorporated into certain foods to make them functional. They could combat oxidative stress in people whose free radical/antioxidant balance is disrupted in favor of free radicals **[34; 35]**.

The fermentation of oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) seeds influenced the characteristics studied. The difference is weakly noticeable for physicochemical characteristics. It is only observed at the pH level at which fermented pomelos seeds have more acidic pH than unfermented ones. This could be explained by the growth of the biomass in the pomelos juice used to ferment the seeds. Akin **[36]** showed that the decrease in pH was related to the assimilation of the nitrogen source by yeasts contained in the must.

In terms of biochemical characteristics, reducing sugars contents in fermented seeds are lower. Indeed, biochemically, fermentation is a reaction that consists generally of transforming sugars into alcohols, acids, or gas.

The fermentation has reduced the antinutritional compounds such as phytates and oxalates in studied seeds. This was demonstrated by Salah*et al***[37]** who have proved in their study, that fermentation reduced phytates to 57%. This process is thus, a way of using citrus seeds with their numerous virtues.

5. CONCLUSION

The objective of this work was to determine the influence of fermentation on the nutritive and anti-nutritive composition of oranges and pomelos seeds. The results show that oranges (*Citrus sinensis*) and pomelos (*Citrus paradisi*) seeds have enormous nutritional potentialities such as proteins and lipids. These properties, therefore, favor their use in nutrition. Finally, this study shows that despite their nutritional properties, oranges and pomelos seeds contain antinutritive compounds such as phytates and oxalates. However, fermentation influenced the characteristics of the seeds studied. It increases their titrable acidities and certain macronutrient levels. Also, it reduced the levels of phytates and oxalates in the seeds studied. Another, an optimization of oranges and pomelos seeds fermentation would allow an efficient reduction of antinutritive factors. It will enhance, therefore a better use in food sciences to contribute to populations' food security.

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