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Contribution to the Reduction of Post-Harvest Losses in the Orange Sector in Senegal through the Production of Quality Orange Vinegar

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Abstract

This work aims to study the feasibility of producing vinegar from oranges. Two types of matrices were used: pasteurized juice from fresh oranges and unpasteurized juice from overripe oranges. After juice extraction, parameters such as pH, soluble dry matter content, and total and reducing sugar contents were determined. The juices were then subjected to alcoholic fermentation following three batches: FPJ (from the pasteurized juice of fresh oranges), FNJI and FNJ (from the unpasteurized juice of overripe oranges). FPJ and FNJI were both inoculated with *Saccharomyces cerevisiae*, while FNJ was not inoculated. After the alcoholic fermentation, acetous fermentation followed for vinegar production. Finally, the mineral profile of the best vinegar product was determined and then compared to those of four vinegars marketed in Senegal. The results showed that the best ethanol contents were obtained in FNJI and FNJ with a maximum of 74.44 ± 0.03 g/L for FNJI, followed by 61.817 ± 0.05 g/L for FNJ. The lowest ethanol content, 43.132 ± 0.1 g/L, was obtained with FPJ. Following the same trend, the best acetic acid content, 6.43% ± 0.03%, was obtained with FNJI, followed by FNJ at 5.13% ± 0.01%, then FPJ at 0.67% ± 0.02%, with respective productivities of 0.17, 0.13, and 0.017 g/L·h. The evaluation of the mineral profile of vinegar from FNJI has shown that it has the highest levels for all the minerals sought (Iron, Potassium, Calcium, Phosphorus, Magnesium, Manganese) compared to four other vinegars marketed in Senegal (alcohol based vinegar). These results show the importance of the beneficial contribution of native orange microorganisms during the overripening process and confirm the feasibility of manufacturing a quality vinegar with oranges in an overripening state, thus contributing to the reduction of post-harvest losses in the orange sector in Senegal.

Keywords

Oranges, *Saccharomyces cerevisiae*, Vinegar, Acetic Acid Bacteria, Mineral Content

1. Introduction

Throughout the world, in both developed and underdeveloped countries, the issue of food loss and waste is a critical challenge with profound consequences for food security, the economy, and the environment [1] [2]. Food waste, defined as food intended for human consumption that is discarded or lost, occurs throughout the supply chain, from post-harvest processing, storage, handling, and transportation, up to consumer waste from unconsumed food [3]. According to the Food and Agriculture Organization (FAO), each year about one third of global food production, or 1.3 billion tons, is lost [4]. In Senegal, up to 60% post-harvest losses are recorded in certain areas of the country, with an annual post-harvest loss cost estimated at more than 100 billion, therefore requiring urgent and concerted action [5]. Fruits, which are very perishable due to their richness in water and carbohydrates, are among the foods most affected by this phenomenon [6]. In Senegal, estimates indicate that post-harvest losses can reach more than 30% for fruits [7].

Orange is one of the most abundant fruit crops in the world due to its attractive color, aroma, and flavor [8]. In Senegal, certain varieties of orange are produced in large quantities and are highly valued for their flavor. Nevertheless, just like other fruits such as mango, they suffer from significant post-harvest losses, particularly due to the lack of storage infrastructure and fresh fruit processing industries, unsophisticated agricultural practices making products not very competitive on the international market and limiting exports, and unsuitable transport methods causing significant mechanical damage [9] [10]. Traditional strategies to eliminate these losses, such as landfilling or incineration, can cause soil and water pollution and contribute to greenhouse gas emissions [11]. On the other hand, weathered oranges with their high humidity levels can serve as a substrate for the growth of flies, pathogenic bacteria, and molds capable of producing mycotoxins, thus correlating with environmental risks [12]. It is therefore imperative to find innovative ways of valorization that are in line with the dietary habits of the population. Thus, the development of new products such as orange vinegars seems to be a good way to reduce these losses.

Vinegars are widely used in the food industry; as an ingredient in condiments like ketchup and mayonnaise; and traditionally as a food seasoning and preservative [13], although for their flavor and functional properties, mainly those derived from fruit fermentation [14]. Vinegar is obtained following a two-step biochemical process, which first involves the transformation of fermentable carbohydrates into ethanol through the action of yeasts, usually the genus *Saccharomyces*, fol-

lowed by the oxidation of ethanol into acetic acid under aerobic conditions by acetic acid bacteria [15].

Oranges, rich in sugars (8% - 15%), are therefore eligible to be transformed into vinegar [16]. Several studies report on fruit vinegars around the world: date vinegar [17], apple vinegar [18], pineapple [19], mango vinegar [20], etc. In Senegal, the vinegar market is dominated by alcohol vinegars, often imported or derived from the dilution of acetic acid of petrochemical origin, and often does not meet food standards [21]; only mango vinegar is produced as a fruit-based vinegar [22]. Market diversification through the production of orange vinegar would represent added value in this sector, with potential economic benefits for processors, particularly in terms of job creation and the sale of quality products.

This study explores the feasibility of transforming oranges into good quality vinegar to reduce post-harvest losses. The objective of this work was therefore to evaluate the production of vinegar from oranges, by comparing the use of juices from fresh oranges with those from over-ripe oranges, the effect of adding yeasts (*Saccharomyces cerevisiae*) on the ethanol content produced, and consequently on the acetic acid yield. Finally, the quality of the best vinegar produced was evaluated by determining its mineral profile, then compared to that of four other vinegars marketed in Senegal. To our knowledge, this is the first time such a vinegar has been developed and characterized in Senegal.

2. Material and Methods

2.1. Material

2.1.1. Vegetal Material

The vegetal material consisted of oranges of the variety *Citrus Tangelo*, purchased in the city of Dakar, Senegal.

2.1.2. Biological Material

It consisted of yeasts and acetic acid bacteria.

❖ **Yeasts**

For the alcoholic fermentation, yeasts of the species Saccharomyces cerevisiae of the brand Saf-instant® (France) were used.

❖ **Acetic Acid Bacteria**

Acetic Acid Bacteria (AAB) were used for the second step, which is acetous fermentation. These bacterial strains come from the mother of a mango vinegar made in the Laboratory of Applied Microbiology and Industrial Engineering (MAGI) at the "Ecole Supérieure Polytechnique of Dakar".

2.2. Methods

2.2.1. Juice Extraction

The fruits were first sorted and divided into two batches; a first batch composed of fresh oranges and a second batch composed of oranges at a very advanced stage of ripening. For the first batch, the fruits were cleaned, washed, peeled, and then

cut into small quarters. The same process was adopted for the second batch, except that the oranges were not peeled. This approach was chosen in order to verify the hypothesis that native microorganisms and compounds on orange peel contribute positively to the efficiency of fermentation and the quality of the finished product. Indeed, vinegars show differences depending on the presence and absence of peel on the original fruit [23]. For the two batches, the fruit pieces were crushed using a Su Tai brand blender to extract the juice. This was followed by filtration using a 0.1 mm mesh sieve to remove solid particles. The content of soluble dry extracts was then determined using a refractometer, then the concentration was increased by adding sucrose powder until reaching a soluble dry extract content of 18˚Brix. The pH, total and reducing sugars have also been determined. The juice from the first batch was then pasteurized at 90˚C for 5 minutes, while that of the second batch underwent no heat treatment. The two juices were then packaged in sterile bottles and kept at 4˚C for the following steps (**Figure 1**).

Figure 1. From left to right, fresh oranges and overripe oranges.

2.2.2. Alcoholic Fermentation

❖ **Preparation of the inoculum**

The yeasts used in our study (of the brand Saf-instant®) brand are in lyophilized form. Before use, they are regenerated in a sucrose solution and then incubated at 30˚C for 1 hour.

❖ **Process**

Three types of trials were conducted in 250 mL flasks:

- The first batch, named FPJ, involved the fermentation of pasteurized orange juice inoculated with 5% yeast (v/v);
- Second batch named FNJI involves the fermentation of unpasteurized orange juice inoculated with 5% yeast (v/v);
- The third batch, named FNJ, involved the fermentation of unpasteurized orange juice but was not inoculated with yeasts to promote only natural fermentation.

The three flasks were then incubated at 30˚C and fermentation was conducted until the decrease in soluble dry extract content reached a constant value. Samples were taken from each batch in order to evaluate the ethanol content produced.

2.2.3. Acetous Fermentation

❖ **Preparation of the inoculum**

The strains of acetic bacteria from the mother of vinegar were cultured in YG broth (Yeast-extract, Glucose), containing 3% (w/v) yeast and 3% (w/v) glucose, respectively. Then, the medium was incubated at 30˚C until the optical density (OD600 nm) of the suspension reached 0.4.

❖ **Fermentation process**

At the end of the alcoholic fermentation, the three previous batches were in each case inoculated with 10% (v/v) pre-culture of the acetic acid bacteria broth. The flasks were then incubated at 35˚C with shaking at 150 rpm for a total of 16 days. Samples were taken periodically to determine the evolution of the acetic acid content produced.

❖ **Calculation of acetic acid productivity**

The productivity in g/L·h is determined according to the following formula.

$$P = M(Ac)/Tf$$

P: Productivity; $M(Ac)$: Content of produced acetic acid; *Tf*: Time of fermentation.

2.2.4. Determination of the Mineral Profile

Elemental analysis was performed for the best orange vinegar produced and for four other vinegars marketed in Senegal, including two locally produced and two imported from France. The average content of Fe, Mn, K, Ca, Mg, P, Pb, Se, Hg, and Cd elements was determined by the ICP atomic absorption method.

2.2.5. Analytic Methods

❖ The pH, dry matter, reducing and total sugars, and ethanol content were evaluated according to the AFNOR standard methods [24].

❖ **Estimate of acetic acid production**

1 ml of culture is taken from the fermentation medium and then diluted to one hundredth with distilled water. After homogenization, 20 mL of this solution is placed in an Erlenmeyer flask. 2 - 4 drops of phenolphthalein are added to assess the pH variation. The solution is then titrated against a 0.1 N NaOH solution. The amount of acetic acid produced in g per 100 mL was calculated using the following formula:

$$d^{\circ} = \frac{1000 \times C_b \times V_b \times P_m}{V_a \times m_v}$$

- C_b: Concentration of NaOH solution
- V_b: Average volume of NaOH needed to reach the endpoint
- P_m: Molar mass of acetic acid
- V_a: Volume of the sample
- m_v: Volumetric mass of acetic acid

2.3. Statistical Analyses

All experiments were conducted in triplicate, and the mean and standard devia-

tion were calculated. An honest significant difference (HSD) was detected using the Tukey test. The results were considered statistically significant if $p < 0.05$, using SAS JMP Statistical Discovery Pro 16.0.0.

3. Results and Discussion

This present study was conducted in order to contribute to the reduction of post-harvest losses in the orange sector, by transforming them first into juice, then into ethanol, and finally into acetic acid for vinegar production.

3.1. Chemical Properties of the Orange Juices and Orange Alcohol

After extraction, the orange juices obtained were characterized. Table 1 highlights the results obtained for total and reducing sugar concentrations, pH, and soluble dry extract content. The values of total and reducing sugars obtained for juice from fresh oranges are respectively 12% ± 0.14% and 9.5% ± 0.03%. As for the juice from overripe oranges, the values are respectively 11% ± 0.01% and 9.3% ± 0.05%. The values obtained for fresh orange juice are in accordance with those declared by [25]. Similarly, the soluble dry extract content of the juice from fresh oranges is 10.98 ± 0.01˚Brix, in line with that declared by [26]. The juice from overripe oranges has a dry extract content of 10 ± 0.03˚Brix.

The sugar content is a very important parameter to consider when producing vinegar; indeed, it is directly related to the concentration of ethanol produced during alcoholic fermentation, and consequently to the final acidity of the vinegar produced during the acetic fermentation [27]. The reducing sugar content represents about 95% of total sugars, which means that the majority of the sugars present in the juice are simple sugars (glucose and fructose), which would promote alcoholic fermentation, because these sugars are directly metabolizable by yeasts such as *Saccharomyces cerevisiae* [28]. *S. cerevisiae* is a preferred producer due to its high ethanol yield and robustness [29]. It strongly undergoes the Crabtree effect, which means that the majority of the carbon source is directed towards ethanol production [30].

The values of juice from overripe oranges are slightly lower than those of fresh orange juice, with significant differences ($p < 0.05$). This could be explained by the beginning of degradation of the carbohydrate components of the peel of oranges by surface microorganisms, thus leading to a decrease in sugar content and Brix level during the overripening process. Indeed, some studies have highlighted the presence of *Saccharomyces cerevisiae* on orange peels [31].

The pH of both juices was also determined. The values obtained are, respectively, 4.17 ± 0.03 for the fresh juice and 4.09 ± 0.02 for the juice from overripe oranges. These values are conducive to the growth of our yeast. Due to its acidophilic nature, yeast often grows best in acidic environments. pH levels between 4 and 6 are ideal for its growth and metabolism. Indeed, it has been described that the activity of biological components, including enzymes, transport proteins, and proteins bound to plasma membranes, depends on the optimal pH of yeast and

influences yield and productivity [32].

Table 1. Characterization of orange juices.

Samples	Total sugar content (%)	Reducing sugar content (%)	pH	Soluble dry extract (°Brix)
Juice from fresh oranges	12% ± 0.14%	9.5% ± 0.03%	4.17 ± 0.03	10.98 ± 0.01
Juice from overripe oranges	11% ± 0.01%	9.3% ± 0.05%	4.09 ± 0.02	10 ± 0.03

3.2. Ethanol Production

For the alcoholic fermentation, three batches were carried out; the results obtained (Table 2) show a statistically significant difference ($p < 0.05$) in ethanol production depending on the quality of the juice used and whether or not *Saccharomyces cerevisiae* yeasts are added to the juice. The highest ethanol content, 74.44 ± 0.03 g/L, is obtained with FNJI, followed by that obtained with FNJ, 61.817 ± 0.05 g/L, and finally, the lowest ethanol content, 43.132 ± 0.1 g/L, is obtained with FPJ.

The alcohol content obtained with FPJ reflects the activity of the *Saccharomyces cerevisiae* strains used in this study. The alcohol content obtained with FNJ (without yeast inoculation) shows that even without yeast inoculation there is bioconversion of sugars. The microbial flora naturally present in oranges therefore allowed alcoholic fermentation to occur. The amount of alcohol obtained with FPJ is lower than that obtained with FNJ. This could be due, on the one hand, to the higher efficiency of native yeast strains than those marketed; indeed, some studies have been able to isolate *Saccharomyces cerevisiae* strains from food waste, exhibiting thermotolerant and ethanol-tolerant properties [33]. On the other hand, we can assume that the natural microbial flora present on oranges have had time to develop and reach a high non-critical density during the overripening process, so their concentration becomes higher than that of our inoculum. Indeed, it is proven that the concentration of inoculum has a considerable impact on the yield of ethanol produced [34]. In both cases, the hypotheses can be verified in subsequent studies by increasing the concentration of inoculum on the one hand, and on the other hand by isolating and identifying microbial strains naturally present on our oranges. The best result obtained with FNJI can be explained by the fact that there was a very good synergy between the native microorganisms of oranges and the inoculated *Saccharomyces cerevisiae* strains, resulting in a higher ethanol content produced. Conducting more detailed studies could provide further explanation for the observed phenomenon of synergy.

Table 2. Ethanol content.

Samples	Ethanol content (g/L)
FPJ	43.132 ± 0.1
FNJ	61.817 ± 0.05
FNJI	74.44 ± 0.03

3.3. Acetic Acid Production

Following the alcoholic fermentation, the second step of the process was initiated, specifically the acetous fermentation. The results obtained are illustrated in Figure 2.

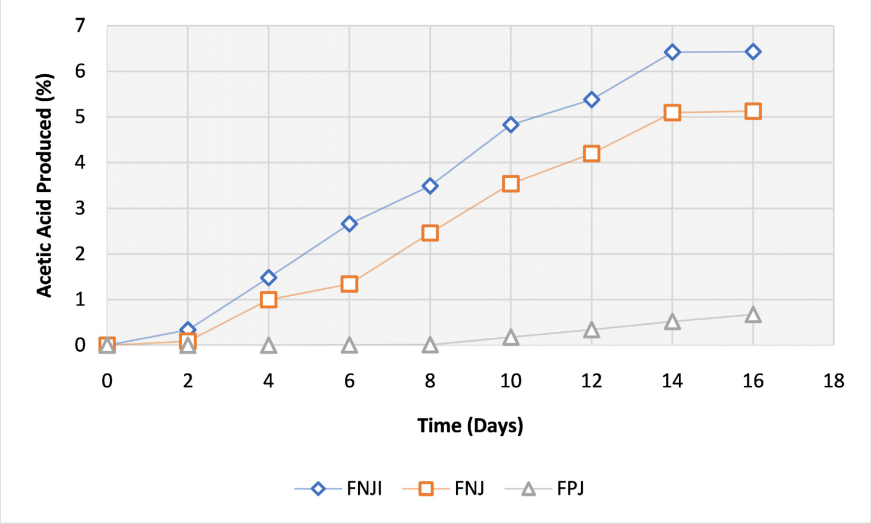

Figure 2. Acetic acid production.

The figure shows that in 16 days of production, the highest concentration of acetic acid, 6.43% ± 0.03%, is obtained with FNJI, followed by that with FNJ, 5.13% ± 0.01%. Finally, the lowest acetic acid content, 0.67 ± 0.02, is obtained with FPJ. These results follow a consistent trend, given that ethanol is the main substrate of acetic acid bacteria, which they convert into acetic acid by bioconversion to obtain vinegar; therefore, the concentration of acetic acid produced is proportional to the ethanol content in the medium as long as it does not reach a concentration that could inhibit bioconversion metabolism [35] [36].

Acetic acid production starts with difficulty in the FPJ batch, with a latency time of 6 days, unlike in the FNJI and FNJ batches. The productivities obtained are respectively 0.17; 0.13; 0.017 g/L·h. The very low acetic acid content and productivity obtained with FPJ for the production time considered, unlike in batches FNJI and FNJ, despite its initial ethanol content, would indicate that the acetic bacteria used as inoculum exhibit poor performance in the considered time. This could be due to several factors, considering that the activity of acetic acid bacteria is governed by several factors such as temperature, ethanol concentration, acetic acid accumulation, and availability of dissolved oxygen [37]. On the other hand, the high acetic acid contents obtained with FNJ and FNJI would testify to the presence and good performance of native acetic acid bacteria present on oranges. Indeed, several studies report acetic acid bacteria isolated from overripe fruits and having shown strong performance for vinegar production [38]. These results then highlight the importance of the fruit's state of maturity, whether advanced or not, to ensure sufficient acidification in a short time (about 2 weeks). The high acetic

degrees obtained with FNJ and FNJI are within the range of the acetic acid rate recommended by the vinegar regulation [39] [40]. This therefore confirms that the manufacture of orange vinegar with a good acetic acid content is possible and feasible.

3.4. Mineral Profile of Orange Vinegar Produced and Comparison with Other Vinegars

The mineral content of the best vinegar produced, FNJI, as well as that of four other vinegars marketed in Senegal, was determined. The quantification of mineral elements present in vinegars is important due to the significant role of metals in the body and also the potential toxicity of certain elements. Table 3 highlights the results obtained.

Table 3. Mineral content of vinegars.

SAMPLE	Units	Pb	Se	Hg	Cd
FNJI	ppm (mg/L)	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001
E4	ppm (mg/L)	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001
E3	ppm (mg/L)	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001
E2	ppm (mg/L)	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001
E1	ppm (mg/L)	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001	<LOD = 0.001
SAMPLE	**Fe**	**Mn**	**Ca**	**K**	**P**
FNJI	0.389	0.540	19.157	30.940	97.702
E4	0.019	0.046	13.152	29.970	40.771
E3	0.038	0.042	13.236	19.463	54.266
E2	0.025	0.052	14.747	26.428	30.970
E1	0.028	0.048	12.488	24.835	27.473

The tables demonstrate that the mineral composition varies from sample to sample. The iron (Fe) content ranges from 0.389 to 0.019 mg/L; manganese (Mn) from 0.540 to 0.042; calcium (Ca) from 19.157 to 12.488; potassium (K) from 30.940 to 19.463; phosphorus (P) from 97.702 to 27.473; magnesium (Mg) from 28.736 to 5.261 mg/L. Other elements such as lead (Pb), selenium (Se), mercury (Hg), and cadmium (Cd) were reported as "<LOD" (below the limit of detection, which was 0.001 mg/L), indicating that they were not detected at measurable levels in these samples. The mineral composition of vinegar is influenced by both the natural composition of the raw materials and the constituents created during fermentation [41]. The FNJI vinegar produced in this study has the highest levels for all the minerals sought. It contains about 10 times more iron and manganese and is richer in calcium, phosphorus, potassium, and magnesium than the four vinegars marketed in Senegal. These values are lower than those declared by [42] and [43] for iron and potassium, but are much higher for calcium, magnesium, and manganese. Similarly, the levels of calcium, magnesium, and phosphorus obtained

in this study are greater than those obtained by [44].

The quantification of mineral elements present in vinegars is important due to the role of metals in metabolism, the potential toxicity of certain metals, the detection of product adulteration, and the characterization of vinegar [45]. Calcium and phosphate are essential macrominerals for neuromuscular function and skeletal mineralization [46]. Phosphorus is the second most abundant mineral in the body after calcium, with many important functions such as tissue and cell repair [47]. Magnesium has been recognized as a cofactor in more than 300 enzyme systems that regulate various biochemical reactions in the body [48]. Potassium (K^+) plays a role in the transmission of neural stimulation and the regulation of cardiac and muscle functions [49]. Iron plays a crucial role in the formation of hemoglobin [50]. Mn is essential for a variety of metabolic functions, including the activation of certain metalloenzymes [51].

Macrominerals are typically required at levels above 100 mg/day, including calcium (Ca), phosphorus (P), magnesium (Mg), sulfur (S), sodium (Na), and potassium (K), and microminerals are required in amounts less than 100 mg/day, including Fe, Zn, Mn [52]. Due to their toxic effects, heavy metals Pb and Cd require special attention; their content should not exceed the respective values of 0.02 mg/L (Cd) and 0.2 mg/L (Pb) [53]. The mineral contents determined in this study therefore do not exceed the recommended daily values.

4. Conclusions

This work was able to evaluate the feasibility of producing quality vinegar from oranges by comparing the use of juices derived from fresh oranges with those derived from overripe oranges, as well as the effect of pasteurization of the juice and the addition or not of an inoculum of *Saccharomyces cerevisiae* on the yield of ethanol and biological acetic acid.

The results obtained showed that the best ethanol contents were obtained with unpasteurized juices from overripe oranges, with a maximum obtained with the inoculated batch FNJI (74.44 ± 0.03 g/L), followed by the non-inoculated one FNJ (61.817 ± 0.05 g/L). The lowest ethanol content, 43.132 ± 0.1 g/L, is obtained with pasteurized inoculated juice from fresh oranges FPJ. Following the same trend, the best acetic acid content, 6.43 ± 0.03%, is obtained with FNJI, followed by FNJ at 5.13% ± 0.01%, then FPJ at 0.67 ± 0.02, with respective productivities of 0.17, 0.13, and 0.017 g/L·h. The evaluation of the mineral profile of vinegar from FNJI has shown that it has the highest levels for all the minerals sought compared to four other vinegars marketed in Senegal. It is therefore entirely possible to produce high-acetic vinegar of good quality from overripe oranges, thus contributing to the reduction of post-harvest losses in the orange sector in Senegal. Through future research, the identification of native strains of yeasts and acetic bacteria could provide valuable direction to complete this work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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