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Valorization of Orange Zest by Lactic Fermentation to Increase the Nutritional Value of Millet Products in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CF and DKM were responsible for study design and supervision of work. Authors BKMJP, TBYC and KKA were responsible for laboratory work data analysis and manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

In order to exploit and control the technological properties for transformation of substrates tropical and other waste of household (orange Zest) in a food improved, a study of the technological properties of *Lactobacillus plantarum* and *Lactobacillus coprophilus* isolated from grape must and sour of the tchapalo has been undertaken. It clear from this study that these two LAB have exercised an antibacterial activity on *Escherichia coli, Bacillus subtilis*, *Staphylococcus aureus and Pseudomonas aeruginosa*, which could be related to the inhibitory effect of bacteriocins. They have owned a good capacity acidifier in the sprouted millet extract and orange zest extract with about a

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rate acidification of 4% after 24 hours (h) of fermentation. Similarly, these lactic strains have a good rate saccharification in the orange zest extract with a maximum of 38.57 ± 6.77% for *L. plantarum* and 32.51 ± 9.53% for *L. coprophilus* after 16 h of fermentation. Vitamin C synthesis was more remarkable in the orange zest extract than in the sprouted millet extract.

Keywords: Orange zest extract; sprouted millet extract; fermentation; Lactobacillus coprophilus; Lactobacillus plantarum.

1. INTRODUCTION

Cereals occupy a prominent place in the human diet. The main cereals cultivated in the world are; soft wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), rice (*Oryza sativa*), *millet (*Panicum *miliaceum L.*), sorghum (Sorghum) and maize (*Zea mays*). In the West African sub-region, the most consumed of local cereals are maize, rice, millet, and sorghum. The diet of the West African population depends on the products resulting from the processing of these cereal grains [1]. These products resulting from the processing of cereals bring the necessary energy and some protein in the diet of these populations [2]. In spite of this energy and input of proteins, these products are deficient in lysine and vitamins A, C, and D. They also have a low in calcuim, iron and zinc content compared with legumes and fruits [3]. To palliate this deficiency into these essential nutritional elements, several methods have been employed to improve the nutritional quality of cereals. These include genetic improvement, amino acid fortification, and supplementation or complementation with protein concentrates and isolates or other protein-rich sources, such as grain legumes and defatted oil seed meals [3]. The processing technologies employed include cooking, sprouting, milling, and fermentation. More recent innovations to improve the nutritional quality from cereal products and kefir-like beverages into vitamins and proteins are practiced in some countries [4]. They involve supplementation or complementation of cereal products and kefir-like beverages with legumes or oil seeds [3,4]. Considering the potential embedded in these grains, it is important to explore this crop through the application of appropriate modern fermentation and malting technologies. This will ensure the availability of ready to eat (RTE) and ready to use (RTU) food products and to a large extent address the incessant food security challenges plaguing Africa [5]. Among these technologies, the fermentation technology is widely used in Africa to transform cereals into edible products as well as to preserve and enhance the nutritional and safety aspects of cereals. In general, in Africa, this process is not controlled and is devoid of good manufacturing practices (GMPs). However, spoilage and/or pathogenic microorganisms may compromise the final quality when the fermentation is not controlled. Taking into account the increasing demand for cereals, in particular maize and millet, which contains undesired microorganisms, fermentation can play an important role in creating food security and safety [2]. Among these spoilage and/or pathogenic microorganisms, we may be mentioned among others *Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa* [6-8]. These pathogenic microorganisms coexist in these foods with LAB which are predominant. LAB observed in cereal products are generally not pathogenic microorganisms but bacteria which play several important roles in the production of these fermented foods. On the one hand, they allow changing the flavor and texture of food. And secondly, they produce peptides and molecules as the acetoin, acetaldehyde, diacetyl or ethanol generated via the heterofermentative pathway for that are important to the flavor of foodstuffs [9]. Another role of LAB is to inhibit the development of the undesirable bacterial flora whether it is pathogenic or spoilage. This inhibition is due to two factors: the acidification due to the production of organic acids that inhibit growth of some acid sensitive bacteria and the synthesis of antibacterial molecules such as the bacteriocins and hydrogen peroxide [10]. In food industry, LAB is used to ferment vegetables, fruit juices or vegetables and wine. Acidification of these foods by LAB has several advantages: it avoids the secondary fermentation, makes protein precipitation, which makes the food more digestible and extends their shelf life by inhibiting the activity of undesirable microorganisms; it also contributes to flavor development, adjusts the pH and activates the vitamin C. Natural processes such as sprouting and controlled fermentation with natural microflora or pure cultures for a limited period have proved to be very beneficial in improving the quality of cereal-based foods [3]. These processes would be even more innovative

with controlled fermentation of cereal-based foods supplemented with protein-rich substrates such as orange zest by LAB strains inhibiting spoilage and/or pathogenic bacteria of these products. It's in this perspective that signs up this study. It aims to transform cereal products (millet) for the improvement foods by using nutrient-rich such as orange zest for the production of news foods by lactic fermentation. The success of such a study will contribute to the valorization of household waste and will be a real economic opportunity in terms of added value.

2. MATERIALS AND METHODS

2.1 Materials

Material used for this study was constituted of orange zest (*Citrus sinensis*) collected from an orange saleswoman in Yopougon (Abidjan, Côte d'Ivoire) and the sprouted millet prepared at the laboratory. Two strains of LAB (*Lactobacillus plantarum* and *Lactobacillus coprophilus*) belonging to the collection of microorganisms of food interest from Biotechnology Laboratory and Microbiology of Food from UFR-STA (University of Nangui Abrogoua) were used during this study. These bacterial strains were isolated from sort wort of sorghum beer (tchapalo) produced in the city of Abengourou (East of Côte d'Ivoire) and identified using API 50 CH et multiplex PCR methods [11]. In that study, they were reported as one dominant LAB species in the sort wort of sorghum beer. The bacterial strains such as *Escherichia coli* ATCC 25922*, Bacillus subtilis* ATCC 6633*, Staphylococcus aureus* ATCC 25913*, Pseudomonas aeruginosa* ATCC 27853 were used for the antibacterial test. These strains are often implicated in food spoilage and diseases.

2.2 Methods

2.2.1 Antibacterial activity of the LAB strains

Antibacterial activities of *Lactobacillus plantarum* and *Lactobacillus coprophilus* were studied using the agar-well diffusion assay as described by Labioui et al. [12] with some modifications. LAB strains were grown twice in MRS broth for 24 h at 37°C and for 18 h at 37°C. The broth (20 ml) obtained after incubation was centrifuged at 4000 tours / min for 20 min with a mini refrigerated centrifuge HW 12. LAB cell free supernatants obtained were filtered through a 0.22 µm (0.22 Am, Schleir and Schuell, GmbH, Dassel, Germany). Two portions (10 mL each) of the sterilized cell-free filtrates (CFF) were transferred aseptically into sterile sample bottles. One of these portions was neutralized using 0.1 M NaOH (pH 6.5) and treated with peroxidase (5 mg/mL) to eliminate respectively organic acid and H2O2 and the other portion was untreated. Strains used for antibacterial tests (*Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25913, *Pseudomonas aeruginosa* ATCC 27853) were on one hand pathogenic bacteria and on the other hand spoilage. These strains were grown in Brain HeartIn-fusion (BHI) at 37°C for 18–24 hours. Nutrient broth was tempered to about 40-50°C and inoculated with 100 µL of a fresh culture (18 to 24 h at 37°C in brain heart broth) of each pathogenic strain. The plates were poured and allowed to solidify. Once solidified, wells 5 were then made using a sterile Pasteur pipette with four (4) wells per Petri dish. Wells were filled using 80 µL of CFF of LAB or Neutralized CFF (pH 6.5). Spread of antimicrobial agents in the agar medium was improved by a pre-incubation for 24 h at room temperature. After incubation, antibacterial activity of LAB strains was shown by the appearance of inhibition zones around wells. The diameter of the inhibition zone was measured (average of two perpendicular diameters) with a transparent ruler. The results were expressed in mm, including the diameter of the wells. The results are presented as the means of duplicate tests.

2.2.2 Preparation of the substrates

Three types of substrate were used in this study. These include extract of orange zest, extract of sprouted millet and sprouted millet extract supplemented with orange zest extract. For the preparation of the extract of orange zest, two hundred (200) grams of orange zest, collected from an orange saleswoman in Yopougon were first washed with cold tap water and were rinsed with distilled water. Two (2) liters of distilled water were then added and the mixture was heated at 100°C using a hotplate for 1 hour and a half. The liquid was filtered, and then centrifuged at 4000 tours/min for 10 min with refrigerated centrifuge (TG-16M, Loncare Medical Technology Co, Chine) to separate the cellulosic debris from supernatant. The supernatant collected was brought to the autoclave, after having lowered the pH to 6 for sterilizing at 121C for 15 min with an autoclave of the type Steam disinfecting apparatus Modele: BKQ-Z50 (Biobase Biodustry, Shandong). As to the preparation of the extract of sprouted millet, it

has necessitated one (1) kilogram of millet bought at Locodjro market. Grains of millet was spread on a jute bag and were then sprayed with 100 mL of ditilled water and then covered by another bag of the same nature in order to produce a sprouting at room temperature in the laboratory. After three (3) days, the sprouted grains were washed cold tap water and then ground using a mechanic grinder to Williamsville market. Two hundred (200) grams of the non-sifted flour were mixed with 2 L of distilled water. After settling, the supernatant was filtered and centrifuged at 4000 tours/min for 10 min. The supernatant collected was brought to the autoclave after adjusting the pH to 6, for sterilization at 121C for 15 min.Sprouted millet extract supplemented with orange zest extract was obtained by mixing 10 mL of the orange zest extract with 90 mL of the sprouted mille extract (10%).

2.2.3 Fermentation conditions

Lactobacillus plantarum and *Lactobacillus coprophilus* were used in single starter for fermentation of sprouted millet extracts, orange zest extracts and the mixture (sprouted millet extracts and orange zest extracts). For this, a series of 8 cap test tubes containing 19 mL of each pre-sterilized substrate was used for each controlled fermentation. Each tube was inoculated with 1 mL of the inoculum containing 108 cells/mL of each isolate (5%). The fermentations were performed in duplicate and the tubes were incubated at 35C for 48 hours. During fermentation, the samples were taken every 4 h for analysis. The witness tube (eighth tube) contained only 19 mL of each substrate.

2.2.4 Determination of cellular biomass

The turbidimetric determination of the cellular biomass was conducted using the method described by Miniatis et al. [13]. Immediately after the fermentation, (1) mL of each substrate was sampled and read at 600 nm using a colorimeter SPECTRONIC 20D brand.

2.2.5 Biochemical analysis

Titrable acidity, total and reducing sugars and vitamin C contents of each sample were determined immediately to the laboratory after fermentation. Total titrable acidity was determined by titrating samples against 0.1 N NaOH using 1% phenolphthalein as indicator as described by Amoa-Awua et al. [14] and expressed as percentage of lactic acid. Total sugars in samples were determined using phenol sulphuric acid method according to Dubois, Gilles,Hamilton, Rebers, and Smith [15], while reducing sugars were quantified as previously described by Bernfeld [16]. Two independent measurements were made on each sample and results were expressed in g/L. For the assay of vitamin C, a volume of ten (10) mL sample of each substrate (orange zest extract, sprouted millet extract and the mixture) was added to 10 mL of metaphosphoric acid/acetic acid to stabilize vitamin C. The mixture of each sample was centrifuged at 4000 tours/min for 20 min. One (1) mL of the supernatant obtained was taken and titrated with 2.6-dichlorophenol indophenol (DCPIP) contained in a graduated burette with tap. For each sample two trials were performed. A blank assay was performed with 1 mL of metaphosphoric acid/acetic acid as well as the dosage of vitamin C standard. The dosage ends when it appears a champagne pink color.

2.2.6 Determination of the saccharification rate

Saccharification rates in different samplings were function to the rate of reducing sugars and total sugars. Total sugars in samples were determined using phenol sulphuric acid method according to Dubois, Gilles, Hamilton, Rebers, and Smith [15] while reducing sugars were quantified as previously described by Bernfeld [16]. Two independent measurements were made on each sample and results were expressed in (%).

Thus, Saccharification rate was obtained from the following relationship:

$$\%\ Saccharification\ rate = \frac{\text{rate of reducing sugars}}{\text{rate of total sugars}} \times 100$$

2.3 Statistical Analysis

The analysis of variance (ANOVA) and the Duncan's test were performed with STATISTICA software, 99th Edition to compare variables analyzed on different selected isolates. The significance level is $\alpha = 0.05$. Differences were considered significant for values of $\alpha < 0.05$. This software also allowed to calculating means and standard deviations of the parameters analyzed.

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity

Antibacterial activity of two strains LAB (*Lactobacillus plantarum* and *Lactobacillus coprophilus*) isolated from sour wort of sorghum beer has been studied in order to use them in the fermentation of orange zests extract, sprouted millet extract and sprouted millet extract supplemented with 10% of orange zest extract. Thus, the antibacterial activity tested on *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* has indicated that the *Lactobacillus coprophilus* suspension neutralized to pH 6.5 without peroxidase, inhibited the growth of *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* with diameters included 15 and 12 mm. As for *L. plantarum*, it has inhibited the growth of *E. coli*, *P. aeruginosa* and *S. aureus* with respective zones of inhibition of 17; 20 and 8 mm (Table 1). The inhibition of these pathogenic strains is due to the hydrogen peroxide produced by these two strains of LAB, because these bacteria have not been sensitive against the neutralized suspension. Indeed, the hydrogen peroxide produced in the presence of oxygen tends to accumulate due to the absence of catalase in LAB. It has an oxidative potential capable to inhibit certain microorganisms by altering the nucleic acids and oxidizing lipids [9]. However, the sensitivity of *E. coli* was stronger than other pathogenic strains. Furthermore, inhibition of *P. aeruginosa*, *S. aureus* and *B. subtilis* may also be attributed to bacteriocin production by *L. plantarum* and *L. coprophilus*. When the pH of the medium was reduced to 6.5 in the presence of peroxidase, the inhibitory action of any organic acid was neutralized; peroxidase, as for it, has neutralized that of hydrogen peroxide. In the presence of peroxidase at pH 6.5, the residual inhibitory activity of the lactic strain may be related to the presence of bacteriocins12 but some tests (proteases effects, temperature) shall be performed for the confirmation. Under these conditions, only *P. aeruginosa* has presented sensitivity to lactic bacteria strains with inhibition zones of 12 and 11 mm diameter (Table 1). The antimicrobial activity and probiotic bacteria has been attributed to the production of metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, other low molecular mass compounds with antimicrobial activity and bacteriocins [17]. These characteristics make LAB the potential probiotic strains. In addition to these characteristics, their use as a potential probiotic LAB will depend to their adhesion to the epithelial tissue, the GIT colonization, a host immune response stimulation, influence metabolic activities such as vitamin production and compete with pathogenic microorganisms, thereby preventing their survival in the GIT [18]. Inded, acid and bile tolerance are two fundamental properties that will demonstrate the ability of these potential probiotic microorganisms to survive passage through the upper gastrointestinal tract.

3.2 Growth of *Lactobacillus plantarum* and *Lactobacillus coprophilus* in the Extracts

Orange zest extract can be an excellent culture medium for *L. coprophilus* and *L. plantarum*. The biomass produced in the orange zest extract inoculated with *L. coprophilus* was $4.9x10^9 \pm 8.4x10^6$ cells/mL in 24 h and that inoculated with *L. plantarum* produced a $4.3x10^9 \pm 8.5x10^6$ cells/mL for the same period of fermentation (Fig. 1). This is due to the fact that orange zest is rich in amino acids, peptides and vitamins [19]. Indeed, the growth of LAB was stimulated by these compounds for which, they have complex nutritional exigency [20,21]. Orange zest extract thus provides to lactic acid bacteria the exigency nutrients. Biomass produced during the sprouted millet extract fermentation was significantly less than that produced in the orange zest extract. This difference may be related to factors limiting growth in the extract of millet germinated. The extract of sprouted millet supplemented with 10% orange zest slightly improved growth of the strains (Fig. 1). This slight improvement can be explained by an insufficient intake of nutrients, or through the presence of elements in the sprouted millet extract that slow down the growth of lactic acid bacteria.

3.3 Evolution of Titratable Acidity during Fermentation of the Substrates

Good acidification was observed in the orange zest extract and sprouted millet extract. This is due to the richness of these media in carbohydrates. These media therefore promote the production of metabolites at the expense of cell multiplication; then, these media can be so used for the organic acid and intermediary metabolites production. The sprouted millet extract supplemented with 10% orange zest extract contains more lactic acid during the fermentation. The titratable acidity of the

sprouted millet extract supplemented with 10% of orange zest extract inoculated with *Lactobacillus coprophilus* varies from 0.1 ± 0.01 to 0.56 ± 0.00% and that inoculated with *L. plantarum* ranges from 0.17 ± 0.01 to 0.57 ± 0.01% after 48 hours of fermentation (Fig. 2). According to this ascertainment, the sprouted millet extract supplemented with 10% orange zest extract can be used in the preparation of millet porridge especially infantile. This is an excellent medium for the production of lactic acid. Indeed, lactic bacteria capable to acidify quickly by producing organic acids in the meduim are going to participate in the fermentation of sprouted millet extract supplemented with orange zest extract to improve taste, texture and shelf life of the finished product duration conservation of the finished product [22].

3.4 Evolution of the Saccharification Rate

This production of organic acids results in the consumption of reducing sugars. So the saccharification rate in the orange zest extract, in the sprouted millet extract and the sprouted millet extract supplemented with 10% orange zest extract varies depending on the strain of lactic bacteria which ensures the fermentation. Indeed, the variation of sugars depends two phenomena. The decrease in sugar rate due to the consumption of sugars by lactic acid bacteria and the increase in the sugar level due to the breakdown of complex sugars to simple sugars. Yet the saccharification rate is higher in orange zest extracts than in sprouted millet extracts. This rate reaches a maximum value of 25.03 ± 1.44% in the orange zest extract inoculated with *Lactobacillus coprophilus* and 24.03 ± 2.06% in the orange zest extract inoculated with *L. plantarum* after 16 hours of fermentation. However this rate ranged from 10.66 ± 0.72 to 5.03 ± 0.34% in the sprouted millet extract supplemented with 10% of orange zest extract inoculated with *L. coprophilus*. Similarly in the sprouted millet extract supplemented with 10% of orange zest extract inoculated with *L. plantarum*, the saccharification rate increased from 8.9 ± 0.015 to 4.6 ± 0.05% at the end of fermentation (Fig. 3). The high rate of saccharification in the orange zest extract could be due to an intense activity of α-amylase and β-glucosidase enzyme system of *Lactobacillus plantarum* or *L. coprophilus* [23].

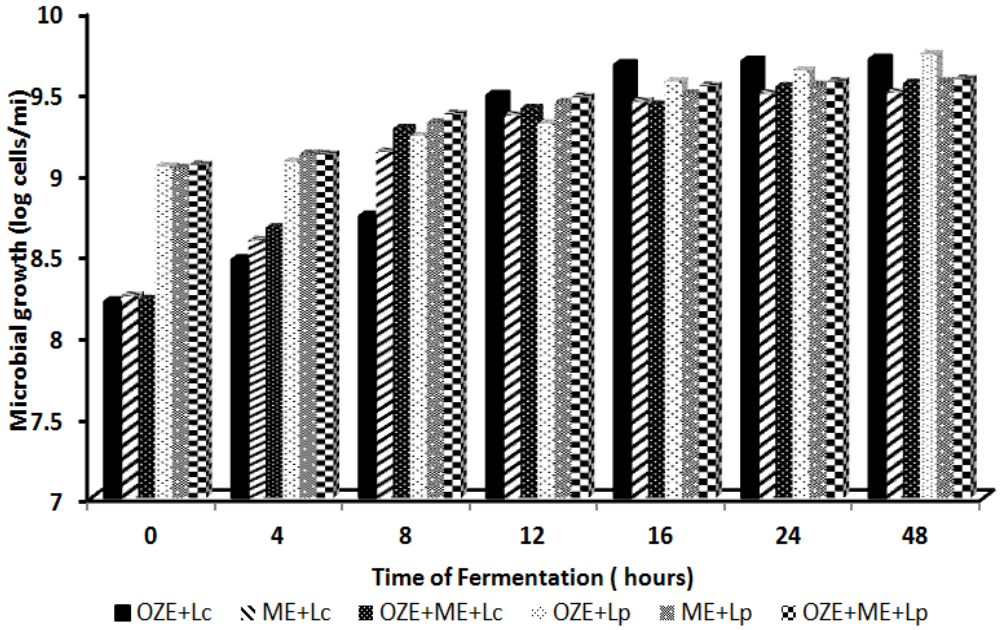

Fig. 1. Growth *Lactobacillus coprophilus and Lactobacillus plantarum* in the three media (Orange zest extract, millet extract and Orange peel extract +millet extract)

OZE+ME+Lc: Orange zest extract +millet extract + Lactobacillus coprophilus; OZE+Lc: Orange zest + Lactobacillus coprophilus; ME+Lc: Millet extract + Lactobacillus coprophilus; OZE+ME+Lp: Orange zest extract +millet extract + Lactobacillus plantarum; OZE+Lp: Orange zest extract + Lactobacillus plantarum; ME+Lp: Millet extract + Lactobacillus plantarum

Table 1. Diameter (mm) of the Inhibition Zone of *Lactobacillus coprophilus* and *Lactobacillus plantarum* on pathogens by the diffusion method on nutrient agar after 24 hours incubation

	pH 6,5 without peroxydase				**pH 6,5 with peroxydase**			
	***Escherichia coli* ATCC 25922**	***Pseudomonas aeruginosa* ATCC 27853**	***Bacillus subtilus* ATCC 6633**	***Staphylococcus aureus* ATCC 25913**	***Escherichia coli* ATCC 25922**	***Pseudomonas aeruginosa* ATCC 27853**	***Bacillus subtilus* ATCC 6633**	***Staphylococcus aureus* ATCC 25913**
Diameter (mm) of inhibition of *Lactobacillus coprophilus*	15	14	12	12	0	12	0	0
Diameter (mm) of inhibition of *Lactobacillus plantarum*	17	20	0	8	0	11	0	0

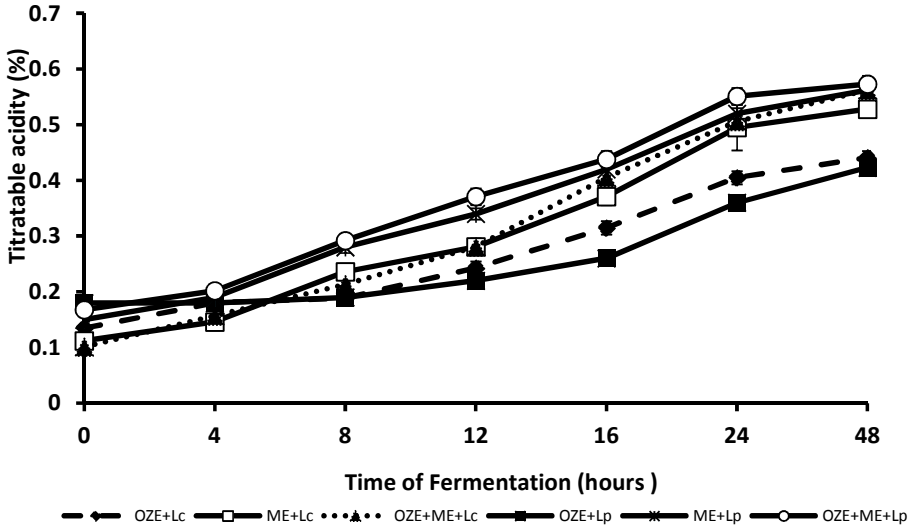

Fig. 2. Evolution of titratable acidity during growth *Lactobacillus coprophilus and Lactobacillus plantarum* in the three media (Orange zest extract, millet extract and Orange peel extract +millet extract)

OZE+ME+Lc: Orange zest extract +millet extract + Lactobacillus coprophilus; OZE+Lc: Orange zest + Lactobacillus coprophilus; ME+Lc: Millet extract + Lactobacillus coprophilus; OZE+ME+Lp: Orange zest extract +millet extract + Lactobacillus plantarum; OZE+Lp: Orange zest extract + Lactobacillus plantarum; ME+Lp: Millet extract + Lactobacillus plantarum

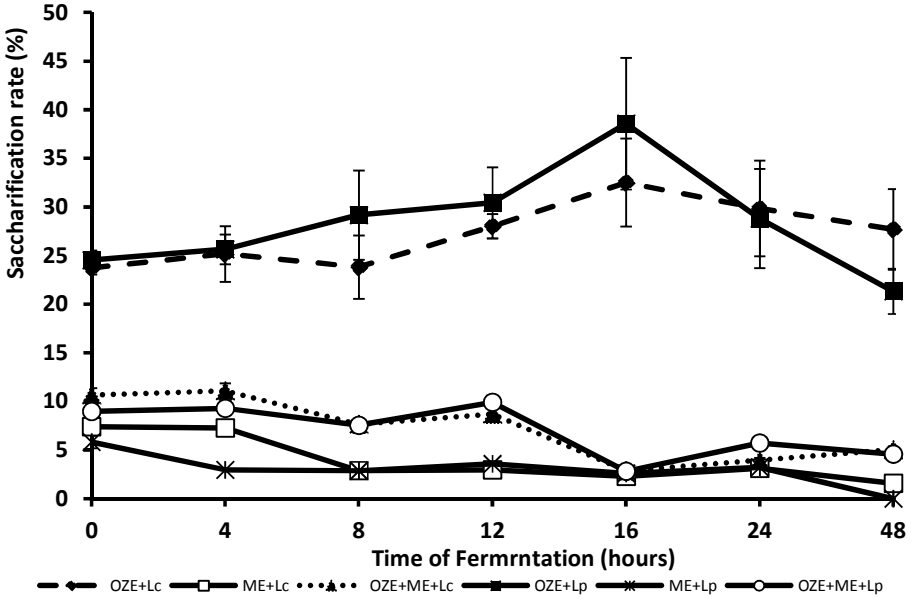

Fig. 3. Evolution of saccharification rate during growth *Lactobacillus coprophilus and Lactobacillus plantarum* in the three media (Orange zest extract, millet extract and Orange peel extract +millet extract)

OZE+ME+Lc: Orange zest extract + millet extract + Lactobacillus coprophilus; OZE+Lc: Orange zest + Lactobacillus coprophilus; ME+Lc: Millet extract + Lactobacillus coprophilus; OZE+ME+Lp: Orange zest extract +millet extract + Lactobacillus plantarum; OZE+Lp: Orange zest extract + Lactobacillus plantarum; ME+Lp: Millet extract + Lactobacillus plantarum

3.5 Evolution of the Vitamin C Rate

Similarly, the production of vitamin C in different substrates studied is more pronounced in the orange zest extract than in the sprouted millet extract. In the orange zest extract inoculated with *L. coprophilus*, the vitamin C amount increased from 3.85 ± 0.00 to 37.18 ± 1.81 mg/100 mL either an increase rate of 89.64%. Whereas the vitamin C amount produced by *L. plantarum* under the same fermentation conditions increased from 3.85 ± 0.00 to 28.21 ± 0.00 mg/100 mL either an increase rate of 86.35% after 48 h of fermentation (Fig. 4). Moreover, the vitamin C amount produced by each of these strains was very lower in the sprouted millet extract than from that obtained in the orange zest extract inoculated with *L. coprophilus* or *L. plantarum* under the same fermentation conditions. However, a significant increase was observed in the sprouted millet extract inoculated either with *L. plantarum* or with *L. coprophilus* when we added 10% of orange zest extract to each of the sprouted millet substrates. The analysis of variance shows that there is no significant difference (P> 0.05) between the vitamin C productions by the lactic acid bacteria strains in different substrates studied (Fig. 4). Indeed, the average amount of vitamin C in the crude orange pulp being 53 mg/100 g [19] is reduced to about 3.5 mg/100 g after the orange zest extract preparation. Much of the vitamin C is degraded during the extract preparation. Degraded vitamin C molecules are therefore precursors of vitamin C for the lactic bacteria. This reflects the ability of the lactic acid bacteria studied to synthesize Vitamin C in their growth. Thus orange zest may be used as a food additive in the manufacture of foods poor in vitamin C. In this case, an influence of the dose of the orange zest in sprouted millet extract has to be tested for the constitution of a millet porridge rich in vitamin C.

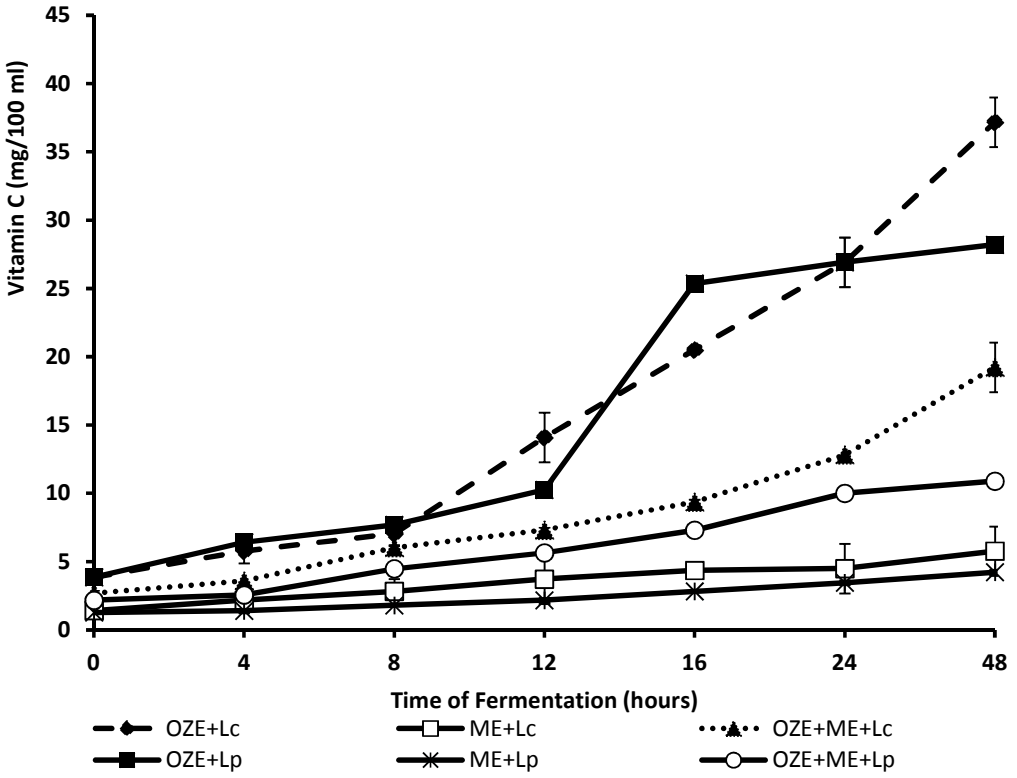

Fig. 4. Evolution of vitamin C during growth *Lactobacillus coprophilus and Lactobacillus plantarum* in the three media (Orange zest extract, millet extract and Orange peel extract +millet extract)

OZE+ME+Lc: Orange zest extract +millet extract + Lactobacillus coprophilus; OZE+Lc : Orange zest + Lactobacillus coprophilus; ME+Lc: Millet extract + Lactobacillus coprophilus; OZE+ME+Lp: Orange zest extract +millet extract + Lactobacillus plantarum; OZE+Lp: Orange zest extract + Lactobacillus plantarum; ME+Lp: Millet extract + Lactobacillus plantarum

4. CONCLUSION

On completion of this study, it appears that both LAB, *Lactobacillus plantarum* and *Lactobacillus coprophilus* almost have the same fermentary potentiality. However, the antibacterial activity of *Lactobacillus coprophilus* can be attributed to a bacteriocins. For Lactobacillus plantarum, his antibacterial activity observed on some indicator strains is attributed to the presence of hydrogen peroxide in its culture medium. In addition, the three environments studied (orange zest extract, sprouted millet extract, sprouted millet extract supplemented with orange zest extract) not having the same physico-chemical constituents do not offer the same conditions of crops bacteria. The best acidification was carried out in the sprouted millet extract supplemented with orange zest because of its sugar content. As for the extract of millet germinated, it can be used for the production of organic acids and intermediate metabolites. However, the best producing cellular biomass and best production of vitamin C were observed in orange zest extract in view of its wealth in growth factors (amino acids, peptides, vitamins, etc.). Thus, the orange zest extract can be used in the manufacture of millet porridge, especially infantile because cereals were deficient in some basic components (essential amino acids). So orange zest, rich in amino acids and vitamin C may be added to these grains to increase their nutritional value.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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