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Physicochemical composition and fermentation kinetics of a novel Palm Sap-based Kefir Beverage from the fermentation of *Borassus aethiopum* Mart. fresh sap with kefir grains and ferments

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ABSTRACT

Palm sap collected (sugary juice) from palm trees is very widespread in the intertropical regions of Asia, America and Africa. This study aimed to evaluate the use of kefir grains and ferments as starters for the fermentation of fresh palm sap (PS) from *Borassus aethiopum* Mart. to produce new kefir-like beverages (KLBs). The batch fermentation was performed statically at room temperature (22 °C) during 48 h. Physicochemical analysis were performed using standard and HPLC methods. The KLBs (kefir-like beverages) from PS showed low ethanol and glycerol contents ranging from 0.84 ± 0.14 to 17.30 ± 2.06 g/L (0.07 to 1.38% v/v) and 0 to 0.67 g/L respectively. The pH value decreased significantly during 24 h of fermentation and ranging from 3.98 to 4.40 at the end of fermentation. The organic acids (lactate, acetate, propionate, citrate, succinate and pyruvate) were detected in KLBs from PS. There was an increase in the content of lactate, acetate, propionate and succinate during fermentation process, except citrate content. Lactate and acetate content reached maximum values ranging between 5.18 and 9.31 g/L, 0.94 and 1.69 g/L respectively. Sucrose concentration decreased significantly and reached a value ranging from 0 to 0.84 g/L, except in KLBs fermented using milk ferments (9.45 g/L). The study showed that water and milk kefir grains as well as kefir ferments were well adapted to ferment *Borassus aethiopum* Mart. fresh sap for KLBs production. Palm sap could be an ideal alternative base substrate to produce non-dairy probiotic fermented beverage with low ethanol and sugar contents.

Abbreviations: B. aethiopum, *Borassus aethiopum*; KLBs, kefir-like beverages; MF, milk kefir ferments; MKG, Milk kefir grains; PSKB, Palm Sap-based Kefir Beverage; PS, Palm Sap; PSKB MG, Palm Sap-based Kefir Beverage fermented using Milk Grains; PSKB MF, Palm Sap-based Kefir Beverage fermented using Milk Ferment; PSKB WG, Palm Sap-based Kefir Beverage fermented using Water Grains; PSKB WF, Palm Sap-based Kefir Beverage fermented using Water Ferment; WKG, water kefir grains; WF, water kefir ferments.

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Introduction

Kefir is fermented milk, produced mainly from cow, sheep or goat milks using "kefir grains". The region of origin of this drink is the South Caucasus, where it is nowadays prepared under a wide variety of names [1]. The kefir microflora is embedded in an exopolysaccharide matrix (EPS) like cauliflower containing a symbiotic community of yeasts and bacteria, called kefirane, as metabolic products of lactose milk acidifier and other substrates [2].

Antifungal, antibacterial, immunomodulatory, anti-inflammatory, anti-allergic, antioxidant, anti-carcinogenic, etc. are some of kefir grains' properties that have beneficial effects on the human health [3–9]. In addition, it is suggested that kefir contains some bioactive compounds (polysaccharides and peptides) that have great potential for inhibiting proliferation of various type of cancers such as colorectal, breast and lung, malignant T cells, and inducing apoptosis in tumor cells [3,8,9]. Cardoso et al. [10] conducted a study on the digestive benefits of kefir and reported an improvement in peristaltic activity in the intestinal tract of sweet kefir-treated rats for 15 days. Kefir can improve various health conditions in addition to its nutritional properties, so it can be considered as probiotic food [11].

Kefir grains grown in brown sugar solution and water (eg, molasses) are called sweet kefir, or water kefir [12]. The structure of sweet kefir grains looks very similar to milk kefir grains in terms of associated microorganisms and products formed during the fermentation process, but without the characteristic of cauliflower appearance [13]. This drink, extensively consumed in countries such as the United States, Japan, France and Brazil, represents a promising market for functional beverage cultures [12]. Kefir is widely recognized as an excellent source of probiotics that can have beneficial effects on health. Research on kefir mostly focused on milk substrates from cows, ewes, goats or other types of milk [14–16].

The consumption of milk kefir is however limited for some peoples such as lactose intolerants, vegetarians, and dairy allergic consumers. Thus, its adaptation to non-dairy substrates is another way to bring the beneficial effects of kefir on health for these consumers. The main alternative substrate used for the fermentation of kefir is the brown sugar solution [13]. The adaptation of kefir grains and the production of various functional beverages have been tested on other non-dairy substrates, such as fruits, vegetables and molasses [12,16–20]. There is few research on kefir fermentation using other matrices as raw material compared to their dairy counterpart. Kefir grains has a large microbial diversity (yeasts and bacteria) that can make its adaptation to different substrates much easier [12].

The sap producing palm trees (sugary juice) are very widespread in the intertropical regions of Asia, America and Africa. Palm sap collected from different palm trees is transparent, with a sugar (mainly sucrose) content between 10 and 18% w/v [21–24]. Palm sap contains mainly sugars (10–12% sucrose), organic acids, minerals, vitamins (B, C), soluble proteins, amino acids, amides, phenolic compounds and flavonoids [23,24–27]. Palm sap, is a rich substrate for growth of various types of microorganisms (bacteria and yeasts). The composition of sap and wine has been widely studied but there is no study on the use of sap for probiotic or functional food production. This study follows a study previously performed by Zongo et al. [24] on the biochemical and microbiological composition of fresh sap from *Borassus aethiopum* Mart. in Burkina Faso. In view of its physicochemical characteristics which is close to that of fruit juice, palm sap could be used as base substrate for the production of new probiotic or functional foods using probiotic strains. This study aimed to evaluate the use of fresh sap from palm *B. aethiopum* Mart. as raw material to produce a probiotic beverage using kefir grains and ferments as starters. The physicochemical composition and kinetics of fermentation were determined.

Materials and methods

Kefir grains and ferments origin and activation

Milk and water kefir grains were provided by *Kefiralia* firm (France) and lyophilized milk and juice kefir ferments by *Yalacta* firm (France). Milk kefir grains (MKG) and water kefir grains (WKG) were activated using sterile ultra-high temperature (UHT) skimmed milk and sterile mango juice pH (pH meter METTLER TOLEDO, Five easy) adjusted around 6.5 with 2 N NaOH respectively. The grains were activated during 48 h of fermentation at room temperature (22 °C) without stirring. Then, the grains were washed using sterile distilled water, preserved in UHT skimmed milk and sterile mango juice and stored at 4 °C, then subsequently used for sap inoculation [18]. The lyophilized milk kefir ferments (MF) were activated in 100 mL UHT skimmed milk with 0.5 g of lyophylisate (0.5% w/v) after 48 h of fermentation at room temperature (22 °C) without stirring and then stored at 4 °C. The microorganisms of the milk ferment provided by *Yalacta* are composed of *Lactococcus lactis, Leuconostoc mesenteroides*, *Lactobacillus acidophilus, Bifidobacterium* and *Kluyveroymyces marxianus*, as declared by the producer. Lyophilized water kefir ferments (WF) were reactivated using 100 mL of pasteurized sap (15 min at 85 °C) pH adjusted to 6.58 with 0.5 g of lyophylisate (0.5% w/v) after 48 h of fermentation at room temperature (22 °C) without stirring and then stored at 4 °C. The freeze-dried fruit or water ferment provided by *Yalacta* is composed of *Lactobacillus paracasei, Lactococcus lactis, Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* as declared by the producer.

Production of kefir-like beverages from palm sap

The palm fresh sap (PS) obtained from Palmyra *Borassus aethiopum* Mart. was used as raw material. Before the fermentation, the pH of the sap was adjusted to 6.5 and then pasteurized at 85 °C for 15 min, cooled at room temperature before processing. Kefir-like beverages (KLBs) were produced using 100 mL of PS. The PS was inoculated with 4% w/v activated MKG and WKG and 5% v/v for the active MF and WF. The batch anaerobic fermentation processes were performed during 48 h at room temperature (22 °C) in static conditions [18]. Beverage productions were performed in triplicate. The fermentation was monitored over 48 h at 0, 6, 16, 24, 30, 40, and 48 h to determine the change in physicochemical parameters over the fermentation period. Samples were taken at these different times for pH measurement and HPLC analysis.

MKG and MF were used for the production of milk kefir as a control. For kefir from mango juice WKG and activated WF were used. The fermentation took place under the same conditions (48 h at room temperature 22 °C, without stirring).

Preparation of samples and physicochemical characterization

Samples preparation

Before the analyses, the samples were first centrifuged (EPPENDORF Minispin) at 13000 rpm for 3 min and then 500 µL of the supernatant was removed and then diluted in 500 µL of 25 mM H_2SO_4. The mixture obtained was homogenized using Vortex (IKA, model Vortex 2) for 10 s and then centrifuged again at 13000 rpm for 3 min. Finally, the samples were filtered (0.20 μm filter) in vials for HPLC analysis. For ionic chromatography, the samples were centrifuged at 13000 rpm for 3 min and then 100 µL of supernatant was removed and then diluted in 900 µL of 200 mM NaOH. After homogenization, the mixture was centrifuged again at 13000 rpm for 3 min and then filtered (0.20 μm filter) in vials for analysis.

Organic acids, ethanol and glycerol analyses by HPLC

Organic acids, ethanol, and glycerol were quantified by HPLC as described by Zongo et al. [24]. The samples of KLBs first diluted ½ in 25 mM H_2SO_4 were then filtered with 0.20 µm pore size filter (Startorius CA, UK) and 10 µL of filtered samples were injected into HPLC system (Dionex Ultimate 3000) equipped with an ion-exchange column (Aminex HPX-87H, 300 × 7.8 mm, BIORAD, CA, USA) protected with a guard column (Aminex, 30 × 4.6 mm, BIORAD) and coupled to a RI detector and an UV detector ($\lambda = 210$ nm).

Analyses of sugars by high-performance ionic chromatography

The sugars (sucrose, glucose, fructose and lactose) were determined by high-performance ionic chromatography (HPIC) according to the modified method described by Zongo et al. [24]. The filtered samples (20 µL) diluted 1/1000 were injected in HPIC device (Dionex ICS-3000) equipped with CarboPac PA1 (4 × 250 mm) column preceded by a guard column CarboPac PA1 (4 × 50 mm) and coupled to an Amperometer detector (ED 40). The running was carried out in isocratic mode with a gradient of NaOH 50–200 mM as mobile phase at a flow rate of 1.0 mL/min at 30 °C.

Statistical analyses

Each fermentation was carried out in triplicate and results were expressed as mean values ± standard deviations. Statistical analysis was performed with SPSS 20 software using one way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The statistical difference between the beverages was considered to be significant when *P*-values < 0.05.

Results and discussion

pH value

The pH value of different Palm Sap-based Kefir Beverage (PSKB) obtained from palm sap significantly decreased during the process of fermentation (Table 1). The pH decreased from 6.55 to 3.98 for PSKB MG (Palm Sap-based Kefir Beverage fermented using Milk Grains) and 6.55 to 4.39 for PSKB WG (Palm Sap-based Kefir Beverage fermented using Water Grains). For the PSKB MF (Palm Sap-based Kefir Beverage fermented using Milk Ferment) and PSKB WF (Palm Sap-based Kefir Beverage fermented using Water Ferment), the pH decreased from 6.53 to 4.08 and 4.07 respectively after 2 days of fermentation at room temperature (22 °C). The difference was not significant between the final pH value of the PSKB MG, PSKB MF and PSKB WF. However, there was a difference between the final pH value of PSKB WG and the others KLBs (kefir-like beverages) obtained from palm sap ($p < 0.05$). The pH values of differents KLBs from PS measured in this study fall in the same range of the typical pH of dairy kefir beverages which is between 4.0 and 4.4 [28]. The values of the pH obtained at the end of fermentation in this study are close to those reported by Fiorda et al. [18] (~4.0) during honey, colostrum and soybean hydrolyzed extract fermentation with kefir grains. The reduction of pH along with the production of some compounds such as ethanol, carbon dioxide, organic acids, and other volatile substances resulted from the bacteria and yeast metabolism

Table 1
Sugars and pH contents of Palm Sap and Kefir-like Beverages fermented with Kefir grains and ferments.

Samples	pH	Glucose (g/L)	Fructose (g/L)	Sucrose (g/L)
PS	6.55 ± 0.01[b]	4.84 ± 0.66[a]	3.68 ± 0.58[b]	11.23 ± 0.42[c]
PSKB MG	3.98 ± 0.03[a]	5.89 ± 1.86[a]	4.54 ± 1.59[b]	0.19 ± 0.05[bc]
PS	6.53 ± 0.01[b]	7.5 ± 0.82[bd]	5.68 ± 0.75[c]	14.61 ± 0.64[a]
PSKB MF	4.08 ± 0.03[a]	7.33 ± 0.83[bd]	3.01 ± 0.33[b]	9.45 ± 0.23[d]
PS	6.55 ± 0.01[b]	4.84 ± 0.66[a]	3.68 ± 0.58[b]	11.23 ± 0.42[c]
PSKB WG	4.39 ± 0.005[ad]	1.61 ± 0.78[c]	0.93 ± 0.43[da]	nd
PS	6.53 ± 0.01[b]	7.5 ± 0.82[bd]	5.68 ± 0.75[c]	14.61 ± 0.64[a]
PSKB WF	4.07 ± 0.10[a]	8.45 ± 0.59[bd]	4.17 ± 0.14[b]	0.84 ± 0.07[bc]

nd: not detected. Values are expressed at means ± standard deviation of triplicate. independent experiments. Means with different lowercase letters are significantly different.
($P < 0.05$). PS: Palm Sap; PSKB MG: Palm Sap-based Kefir Beverage fermented using. Milk Grains; PSKB MF: Palm Sap-based Kefir Beverage fermented using Milk Ferment;. PSKB WG: Palm Sap-based Kefir Beverage fermented using Water Grains;. PSKB WF: Palm Sap-based Kefir Beverage fermented using Water Ferment.

Table 2
Organic acids, ethanol and glycerol contents of Palm Sap and Kefir-like Beverages fermented with Kefir grains and ferments (g/L).

Samples	Ethanol	Lactate	Acetate	Citrate	Succinate	Propionate	Pyruvate	Glycerol
PS	nd	2.83 ± 0.11 [a]	0.86 ± 0.06[b]	1.03 ± 0.04[c]	5.31 ± 0.22[b]	1.06 ± 0.06[b]	0.13 ± 0.01[bd]	nd
PSKB MG	16.61 ± 2.54[a]	9.31 ± 0.25[bd]	1.69 ± 0.16[c]	0.36 ± 0.02 [b]	6.10 ± 0.12[b]	1.72 ± 0.05[ab]	0.13 ± 0.02[bd]	0.45 ± 0.15[a]
PS	nd	2.18 ± 0.16[a]	0.6 ± 0.08 [b]	0.73 ± 0.02[c]	3.62 ± 0.18[ac]	1.04 ± 0.03[b]	0.11 ± 0.03[bd]	nd
PSKB MF	0.84 ± 0.14[bc]	5.66 ± 0.64[c]	1.49 ± 0.26[c]	0.41 ± 0.05[b]	3.35 ± 0.33[ac]	1.41 ± 0.14 [ab]	0.10 ± 0.01[bd]	nd
PS	nd	2.83 ± 0.11[a]	0.86 ± 0.06 [b]	1.03 ± 0.04[c]	5.31 ± 0.22[b]	1.06 ± 0.06[b]	0.13 ± 0.01[bd]	nd
PSKB WG	17.30 ± 2.06[a]	5.18 ± 0.19[c]	1.32 ± 0.07[c]	0.06 ± 0.01[ab]	4.17 ± 0.19 [ac]	1.01 ± 0.06[b]	0.03 ± 0.01[c]	0.67 ± 0.15[ac]
PS	nd	2.18 ± 0.16 [a]	0.6 ± 0.08[b]	0.73 ± 0.02[c]	3.62 ± 0.18[ac]	1.04 ± 0.03[b]	0.11 ± 0.03[bd]	nd
PSKB WF	9.90 ± 0.89[c]	6.12 ± 0.46 [c]	0.94 ± 0.19[b]	0.07 ± 0.00[ab]	5.04 ± 0.46 [b]	1.47 ± 0.13[ab]	0.11 ± 0.01[bd]	0.45 ± 0.10 [a]

nd: not detected. Values are expressed at means ± standard deviation of triplicate independent experiments. Means with different. lowercase letters are significantly different ($P < 0.05$). PS: Palm Sap ; PSKB MG: Palm Sap-based Kefir Beverage fermented using. Milk Grains; PSKB MF: Palm Sap-based Kefir Beverage fermented using Milk Ferment; PSKB WG: Palm Sap-based Kefir Beverage. fermented using Water Grains ; PSKB WF: Palm Sap-based Kefir Beverage fermented using Water Ferment.

[18,29]. Similarly, pH values of kefir beverage from Tibetan mulberry juice-whey based using kefir grains significantly decreased from 5.35 to 4.26 after 40 h of fermentation [30]. Corona et al. [17] also reported a pH value between 3.6 and 4.4 in kefir-like beverages produced from various vegetable juices using water kefir microorganisms as starter cultures.

Ethanol and glycerol contents

The results of ethanol and glycerol contents of PS and KLBs from PS are shown in Table 2. Ethanol was not detected in PS but its concentration in PSKB MG, PSKB WG and PSKB WF was 16.61 ± 2.54 g/L, 17.30 ± 2.06 g/L and 9.90 ± 0.89 g/L respectively at the end of fermentation. The lowest ethanol content was observed in PSKB MF at 0.84 ± 0.14 g/L. The difference was significant in the ethanol content of different KLBs from PS fermented with kefir grains and ferments ($p < 0.001$). These differences in ethanol content could be explained by the great diversity of yeasts in the kefir grains than kefir ferment [31]. Authors previously reported that during kefir fermentation, the conversion of sugar into ethanol was done by yeasts primarily [18,32]. The different KLBs from PS contained low glycerol content at 0.45 g/L for PSKB MG and PSKB WF, 0.67 g/L in PSKB WG. The difference was not significant in glycerol content between kefir beverages from PS. The glycerol production was likely the result of yeast metabolism during fermentation. The ratio ethanol/glycerol was found typically in the range obtained in ethanolic fermentation with *Saccharomyces cerevisiae* [33,34]. Glycerol was also reported in KLBs from Mediterranean fruit juices fermented with water kefir microorganisms [19,35].

Sugars and organic acids contents

The results of organic acids (lactate, acetate, citrate, succinate, propionate and pyruvate) contents of PS and different PSKB quantified by HPLC are presented in Table 2. There was an increase in the content of lactate, acetate, propionate and succinate during PS fermentation process. Lactate content reached maximum value of 9.31, 5.66, 5.18 and 6.12 g/L in PSKB MG, PSKB MF, PSKB WG and PSKB WF respectively at the end of fermentation. Significant increase was observed between PS and KLBs from PS ($p < 0.01$) for lactate content but little increase for acetate content (0.94 to 1.69 g/L). The decrease was significant in citrate content of all the fermented KLBs but there was no significant difference in pyruvate content of PS and KLBs from PS (0.13 g/L) except PSKB WG. Puerari et al. [36] reported similar observation in citrate content during

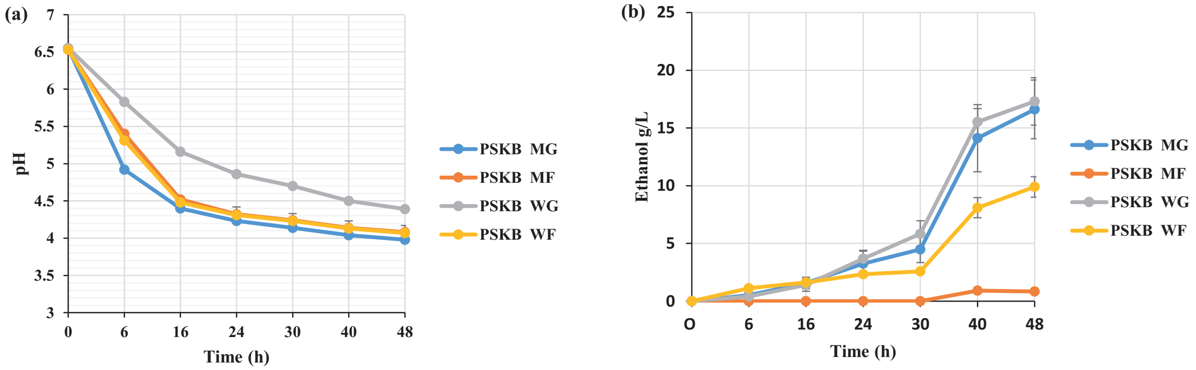

Fig. 1. Kinetic of pH and ethanol during the fermentation of palm sap with kefir grains and ferments.
PSKB MG: Palm Sap-based Kefir Beverage fermented using Milk Grains ; PSKB MF: Palm Sap-based Kefir Beverage fermented using Milk Ferment ; PSKB WG: Palm Sap-based Kefir Beverage fermented using Water Grains ; PSKB WF: Palm Sap-based Kefir Beverage fermented using Water Ferment.

fermentation of cocoa pulp-based kefir beverages. The production of lactic acid indicated the heterogeneity of lactic acid bacteria (LAB) metabolism [36]. The great majority of LAB uses glucose via the Embden–Meyerhof pathway for lactic acid production during fermentation but some species produce several components (lactic acid, acetic acid, ethanol, mannitol, glycerol and CO_2) via the hexose monophosphate pathway [14,37]. Study performed by Puerari et al. [36] showed that acetic acid was formed probably by heterolactic bacteria and acetic acid bacteria (*Acetobacter*), identified previously in kefir grains and beverages [36]. The lactic acid and acetic acid inhibit the development of undesirable or pathogenic microorganisms and provide a pleasant taste, due to the increase of acidity [36].

The sugars (glucose, fructose and sucrose) contents of PS and KLBs from PS are shown in Table 1. After 48 h of fermentation, glucose concentrations were 5.89, 7.33, 1.61 and 8.45 g/L in PSKB MG, PSKB MF, PSKB WG and PSKB WF respectively. Fructose contents ranged from 0.93 to 4.54 g/L. Differences were significant between the different PSKB ($p < 0.05$). The sucrose content decreased significantly during fermentation process. It decreased from 11.23 to 0.29 g/L, 14.61 to 0.84 g/L and 14.61 to 9.45 g/L in PSKB MG, PSKB WF and PSKB MF respectively but sucrose was completely consumed in PSKB WG. The differences observed in the composition of sugars contents of the different fermented KLBs from PS are related to the initial composition of sugars from PS. These results illustrate the capacity of yeasts present in kefir grains and ferments to transform the sucrose into glucose and fructose, which leads to a decrease in the concentration of the sucrose in KLBs. The kefir microbial consortium was able to use as carbon source sucrose, glucose or fructose present in the palm sap. This was consistent with the previous reports given by many researchers [12,20,38].

pH and ethanol kinetic

The change in pH and ethanol values of kefir beverages from PS were monitored during the period of fermentation (48 h) at room temperature (22 °C) (Fig. 1a and b). The pH value significantly decreased at the first 24 h from 6.55 to 4.2–4.3 for PSKB MG, PSKB MF, PSKB WF and 4.8 for PSKB WG. At the end of the fermentation, similar pH value of 4 was recorded in PSKB MG, PSKB MF, PSKB WF except in PSKB WG (4.4). Ethanol concentration increased during the kefir fermentation process in all KLBs from PS. The ethanol production increased significantly between 30 h and 40 h of fermentation for all KLBs from PS except PSKB MF, reaching maximum concentration of ~17 g/L (1.36% v/v) in PSKB MG and PSKB WG, ~10 g/L (0.8% v/v) in PSKB WF, ~0.9 g/L (0.07% v/v) in PSKB MF after 48 h of the fermenting process. Study performed by Puerari et al. [36] on kefir beverages from cocoa pulp after 48 h and 72 h of fermentation process at 25 °C reported higher ethanol content (~45.0 g/L, 3.6% v/v). Randazzo et al. [19] also reported a higher ethanol content (1.03–4.96% v/v) in KLBs from different fruit juices fermented by microorganisms of water kefir. The temperature has an important influence on the ethanol production. Lower temperatures slow down yeasts metabolic rate and subsequently ethanol production throughout fermentation as previously reported by Zajšek and Goršek [37]. However, studies performed by other authors showed low ethanol content in kefir beverages using different substrates, such as cow's milk (10.0 g/L) [37] and (0.05 g/L) [14], cheese whey (~11.0 g/L) and brown sugar solution (0.12 g/L) [14].

Organic acids and sugars kinetics

The kinetics of lactate, acetate, succinate and propionate are presented in Fig. 2a–d. All the organic acids contents, except citrate, increased during fermentation and a significant increase was observed at 30–40 h of fermentation reaching maximum value between 5 and 10 g/L, 0.9 and 1.7 g/L, 1 and 1.7 g/L, 3.4 and 6 g/L for lactate, acetate, propionate and succinate content respectively. The citrate content decreased during fermentation process as previously reported [36]. It could be explained by the fact that kefir microorganisms are able to metabolize citrate as carbon and energy sources. Indeed, *Saccharomyces cerevisiae* is able to ferment this organic acid, causing the increase of pH value and favouring the growth of

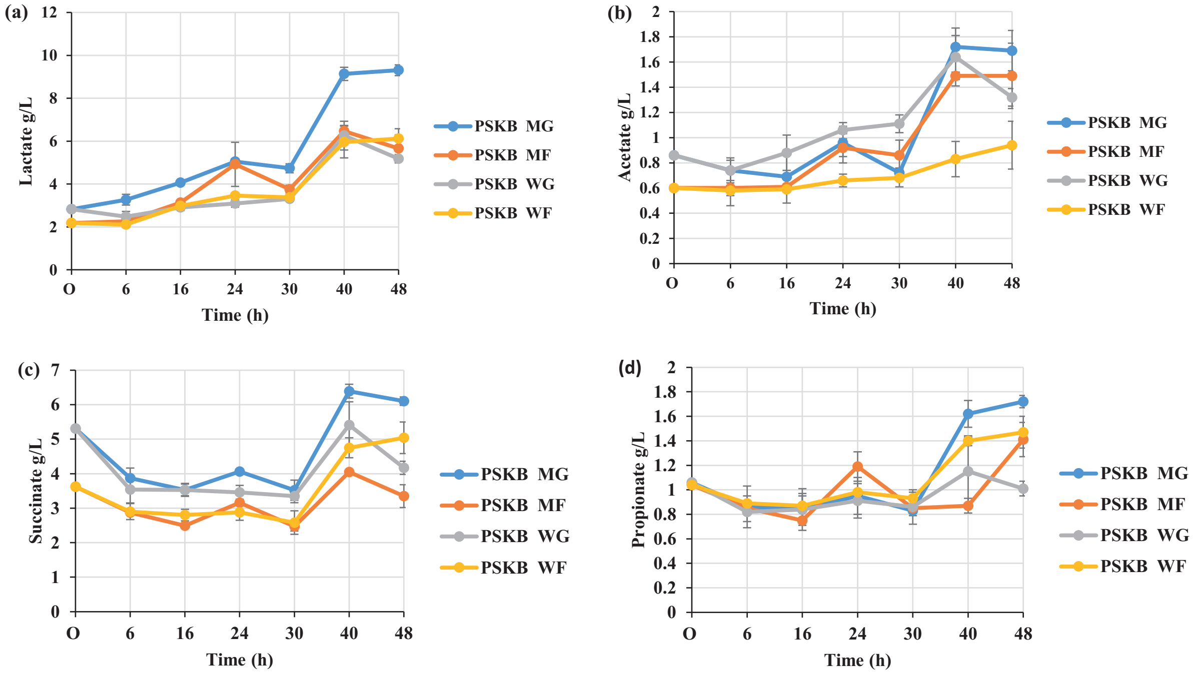

Fig. 2. Kinetic of organic acids during the fermentation of palm sap with kefir grains and ferments.
PSKB MG: Palm Sap-based Kefir Beverage fermented using Milk Grains ; PSKB MF: Palm Sap-based Kefir Beverage fermented using Milk Ferment ; PSKB WG: Palm Sap-based Kefir Beverage fermented using Water Grains ; PSKB WF: Palm Sap-based Kefir Beverage fermented using Water Ferment.

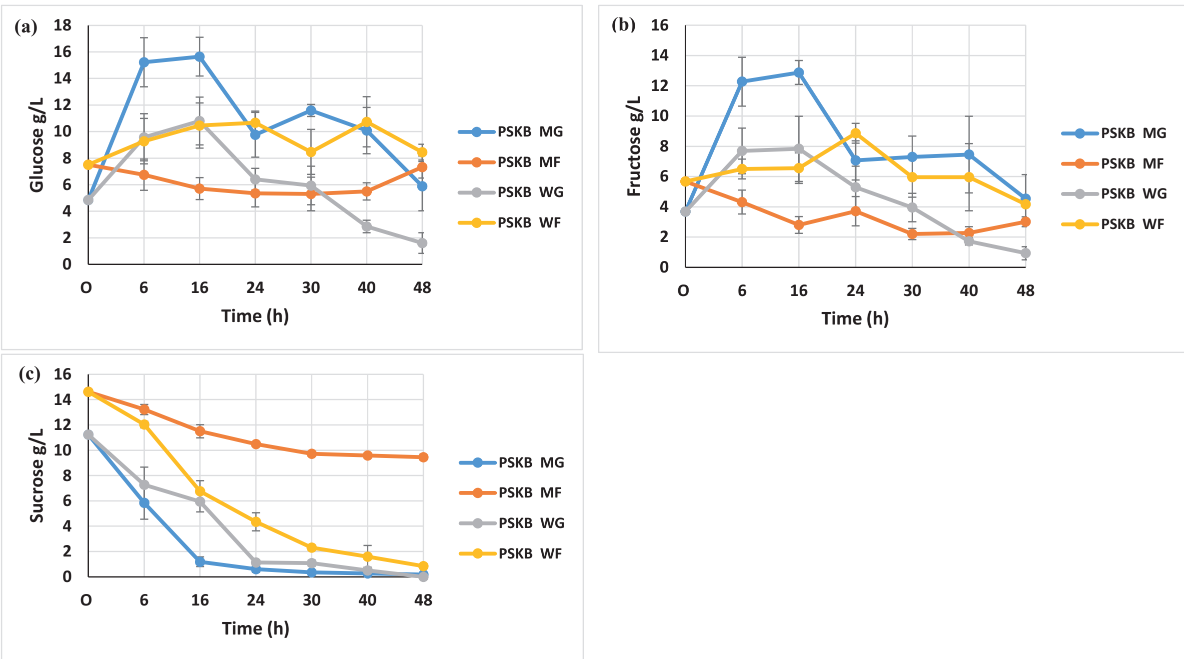

Fig. 3. Kinetic of sugars during the fermentation of palm sap with kefir grains and ferments.
PSKB MG: Palm Sap-based Kefir Beverage fermented using Milk Grains ; PSKB MF: Palm Sap-based Kefir Beverage fermented using Milk Ferment; PSKB WG: Palm Sap-based Kefir Beverage fermented using Water Grains ; PSKB WF: Palm Sap-based Kefir Beverage fermented using Water Ferment.

less acid-tolerant microorganisms (bacteria) [36,39]. According to Nualkaekul and Charalampopoulos [40] propionic acid, is an important odor-active compound in cocoa pulp. Also, in fruit fermented beverages, citric and malic acids are commonly found and act as beverage preservatives with antimicrobial properties.

The glucose (Fig. 3a) and fructose (Fig. 3b) concentrations increased during 16 h of PS fermentation with different kefir starters reaching values ranged from 10 to 16 g/L and 7 to 13 g/L respectively, except for PS fermented with MF which showed a decrease. After 16 h of fermentation there is a continuous decrease during the process of fermentation and the total residual glucose and fructose content was 1 to 8 g/L and 1 to 4 g/L at the end of the fermentation in the PSKB. The increase in glucose and fructose contents is due to sucrose conversion by yeasts present in kefir grains and ferments. Both decreased continuously during the fermentation process because they are metabolized into organic acids, alcohol and other products by kefir microorganisms. The concentration of sucrose in the PS decreased significantly during 24 h fermentation time reaching values ranged from 1 to 4 g/L except for PS fermented with MF (Fig. 3c). At the end of the fermentation (48 h) the residual sucrose content was less than 1.5 g/L in PSKB MG, PSKB WF, completely consumed in PSKB WG and 9 g/L in PSKB MF. Similar results were previously reported by Tu et al. [20] in sugars changes during soy whey KLBs fermentation using water kefir grains.

Conclusion

Analysis was performed to determine the physico-chemical characteristics and fermentation kinetics of PS KLBs. The results of this study showed that water and milk kefir grains as well as kefir ferments are well adapted to ferment *B. aethiopum* Mart. fresh sap for KLBs production. The present study provided evidence that fresh palm sap could be used as base substrate for kefir-like beverages production with functional and flavouring properties. These non-dairy kefir beverage offers alternatives for the consumption of probiotic products for people with special needs (dairy-allergic consumers, lactose intolerants, vegetarians and vegans particularly).

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Declaration of Competing Interest

There are no competing interests for this study.

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