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Scientific Research

Production of Traditional Sorghum Beer "*Ikigage*" Using *Saccharomyces cerevisae*, *Lactobacillus fermentum* and *Issatckenkia orientalis* as Starter Cultures

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Abstract

This study was carried out to evaluate the potential of the use of predominant yeast strains (*Saccharomyces cerevisiae* and *Issatkenkia orientalis*) and lactic acid bacteria (*Lactobacillus fermentum*) of Rwandese traditional sorghum beer "*ikigage*" as starter cultures to improve *ikigage* beer. The results show that *L. fermentum* has an influence on taste sour of *ikigage* beer and contributes also to generating ethyl acetate, ethyl lactate and higher alcohols such as 3-methylbutan-1-ol, 2-methylbutan-1-ol and 2-methylpropan-1-ol of this beer. *I. orientalis* contributed to the production of ethyl butyrate, ethyl caprylate, isobutyl butyrate and their corresponding acids, and to the generation of phenyl alcohols in *ikigage* beer. The association of *S. cerevisiae* with *I. orientalis* and *L. fermentum* produced *ikigage* beer with taste, aroma and mouth feel more similar to *ikigage* beers brewed locally by peasants. It is recommended to use *S. cerevisiae* in association with *L. fermentum* and *I. orientalis* as stater cultures to produce *ikigage* beer having the uniform organoleptic characteristics and a high ethanol content. This method also reduces the risk of contamination of the brew with food sanitary indicator and pathogenic microorganisms and will increase the chance of preservation of *ikigage* beer.

Keywords

Sorghum Beer; *Ikigage*; *Saccharomyces cerevisiae*; *Issatckenkia orientalis*; *Lactobacillus fermentum*

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1. Introduction

Fermented foods make up an important contribution to the human diet in many countries because fermentation is an inexpensive technology which preserves food, improves its nutritional value and enhances its sensory properties [1] [2]. Fermentation has also the potential of enhancing food safety by controlling the growth and multiplication of a number of pathogens in foods. Its importance in modern-day life is underlined by the wide spectrum of fermented foods marketed both in developing and industrialized countries [3].

In sub-Saharan African countries, traditional fermented beverages such as *ikigage* [4], *tchoukoutou* [5], *dolo* [6], *pito* [7], *bili bili* [8] and *merissa* [9] are prepared from sorghum and/or millet malts. The fermentation of these beverages is uncontrolled and the microorganisms that intervene come from the raw materials, equipment and local environments or from residues of previous fermentation batch. These microorganisms, by virtue of their metabolic activities, play an active role in physical, nutritional and organoleptic modification of starting material [10]. However, the wide variety of microorganisms present in a spontaneously fermented food gives a product with widely varying quality.

The use of starter cultures was proposed like a suitable approach to improving the African traditional fermented food [11] [12]. The use of suitable starter improves the fermentation process, facilitates the control over the initial phase of fermentation and the predictability of derivatives products [3] [12]. Similarly, the hygienic quality and the acceptability of African traditional food could be improved with the use of adequate starter [13]. The use of starter cultures also reduces the organoleptic variations and the microbiological instability of African fermented food [11]. The use of *S. cerevisiae* in combination with *Lactobacillus plantarum* as starter cultures was also used successfully for the production of "*pito*" sorghum beer having testa and aroma similar to that of the local *pito* beer [14]. Recently, N'Guessan *et al*. [15] used successfully *S. cerevisiae* and *Candida tropicalis* as starter cultures for the alcoholic fermentation of "*tchapalo*" sorghum beer.

Ikigage is a popular traditional fermented beverage in Rwanda and specially appreciated in various festivals and ceremonies (e.g., marriage, birth, baptism, dowery, etc.). But *ikigage* beer is declining because of poor hygienic quality, organoleptic variations and unsatisfactory conservation [4]. The fermentation of this beer is started by a traditional leaven "*umusemburo*", resulting from a previous spontaneous fermentation of sorghum wort. The dominant microorganisms involved in this fermentation have been identified as being yeasts (*Saccharomyces cerevisiae* and *Issatckenkia orientalis*) and lactic acid bacteria (*Lactobacillus fermentum*) [4]. The aim of the present study was to investigate the use of *S. cerevisiae*, *I. orientalis* and *L. fermentum* as starter cultures to produce *ikigage* beer of acceptable and consistent quality. Microbiological, physico-chemical, volatile compounds and organoleptic characteristics of pilot *ikigage* beers were compared to commercial *ikigage* beer from peasants.

2. Materials and Methods

2.1. Malting

The red sorghum grains (Kigufi variety) and *Eleusine coracana* "uburo" grains (Musama variety) were obtained from Rubona and Musanze stations of Rwanda Agriculture Board (RAB). The grains were sorted manually to remove broken kernels and debris and then used for malting. The grains selected for malting (5 kg) were steeped in distilled water (10 L) at 25˚C for 24 h. Before and after steeping, grains were sterilized by immersion in sodium hypoclorite solution (1% wt/v). After rinsing with sterile distilled water as described elsewhere [16], the grains were germinated at 30˚C for 3 days and then kilned at 50˚C for 24 h. The shoots and rootlets were removed manually and the malt kernels were ground in a hammer mill to pass through a sieve of pore size 1.0 mm.

2.2. Wort Production

The wort was produced by decantation mashing procedure developed for sorghum [17] [18]. 3.5 kg of milled malt (70% sorghum and 30% *Eleusine coracana*) were mixed with 12 L distilled water at 45˚C and left in decantation during 30 min. Thereafter, 6 L of the clear "enzymatic supernatant" was removed while a mash residue was heated at 90˚C for 30 min to gelatinize malt starch. After cooling below 50˚C, the clear "enzymatic supernatant" was re-added and then the mixture was brewed according to the following mashing program: 1 h at 63˚C, 10 min at 75˚C and cooled to 30˚C. The filtration was very poor so the mash was centrifuged at 4000 × g for 5

min and then the filtrate was heated until boiling for 1 h. The leaves of *Vernonia amygdalina* (2 g/L) were added 10 min before the end of boiling.

2.3. Yeast and Lactic acid Bacteria Strains

Two yeast strains (*Saccharomyces cerevisiae* RV6 and *Issatckenkia orientalis* RG1*)* and lactic acid bacteria strain (*Lactobacillius fermentum* CWBI-B552) used as starter cultures in this work were obtained from culture collection of Walloon Center of Industrial Biology (CWBI-Gemboux, Belgium). These strains were isolated from Rwandese traditional leaven "Umusemburo".

2.4. Preparation of Starter Culture

S. cerevisiae RV6 and *I. orientalis* RG1 strains were each sub-cultured on Yeast extract dextrose peptone (YEPD) agar at 30˚C for 48 h and then by successive sub-culturing on YEPD broth at 30˚C for 24 h and 18 h, respectively. *L. fermentum* CWBI-B552 strain culture was sub-cultured at 37˚C for 48 h on Man-Rogosa-Sharpe (MRS) agar followed by two successive rounds of sub-culturing in MRS broth with incubation at 37˚C for 24 h and 16 h, respectively. Yeast and LAB strains were each harvested by centrifugation at 4000 × g for 20 min and pellets were added in 50 ml of sterile sorghum worts and then incubated at 30˚C for 24 h in order to initiate fermentation. The cell concentrations were checked using a Bürker counting cell.

2.5. Fermentation

Seven or ten liters of sterile wort were transferred into fermenter kit (30 L, Brewferm, Belgium), equipped with airlock bubbler and tap, and pitched with starter cultures to obtain 10^6 cfu/mL followed by the incubation at 30˚C for 72 h. In parallel, 2 L sterile wort transferred into sterile Erlenmeyer flasks (5 L) were pitched with *L. fermentum* culture to obtain 10^7 cfu/mL, and then incubated at 30˚C for 22 h and again boiled (30 min) and cooled. Four fermentation systems were constituted as follows:

1) 10 L wort was inoculated with *S. cerevisaie* alone or *I. orientalis* alone;
2) 10 L wort was inoculated with *S. cerevisaie* (60%) in combination with *L. fermentum* (40%);
3) 10 L wort was inoculated with *S. cerevisaie* (60%) in combination with *I. orientalis* (40%).

2.6. Enumeration of Microorganisms

Duplicate aliquots of *ikigage* beer (10 mL) were diluted in 90 mL sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, and 1000 mL distilled water, pH = 7.0) and homogenised. Decimal dilutions were plated. Total counts of aerobic mesophilic flora, yeasts, Lactic acid bacteria (LAB), coliform, *Escherichia coli*, fecal streptococci and *Staphylococcus aureus* were enumerated as described by Lyumugabe *et al*. [4].

2.7. Physicochemical Analysis

The pH was measured using a pH meter 781 (Metrohm Herisau). Titratables acidity, expressed as a percentage lactic acid, was determined by titrating the samples with 0.1 N NaOH. Ethanol was determined by enzymatic method using the Boehringer Kit (R-Biopharm AG, D-64293 Darmastadt). The reducing sugars (glucose, maltose and maltotriose) were determined by High-Performance Liquid Chromatography (HPLC) on an Agilent 1100 series apparatus (Agilent Technologies, Massy, France) equipped with a refractometric detector. Sugars were separated on a C-610-H ion exchange column (300 mm × 7.8 mm, supelco, Bellefonte, PA) preceded by a pre-column H (5 cm × 4.6 mm, supelco, Bellefonte, PA).

2.8. Determination of Volatile Compounds

The analysis of the volatile compounds of *ikigage* beers was performed with Headspace solid phase microextraction (HS -SPME) and an Agilent 7890 GC system equipped with a 5975 C. inert XL EI/ CI. Mass Selective Detector (Agilent Technologies, Santa Clara, CA, USA). The samples analyzed were extracted using a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Inc., Bellefonte, PA). 10 ml of beer were enclosed in a 20 mL SPME glass vial together with 2.5 g of sodium chloride and 5 µL of the internal standard 3-octanol (100 mg/L in absolute ethanol). The samples were equilibrated at 40˚C for 10 min and

then the fiber was exposed in the headspace of the vial for 30 min and the extracted analytes from fiber were automatically desorbed in injection port of the GC-MS system at 250˚C. A HP—5 ms column (30 m × 0.25 mm I.D) with a film thickness of 0.25 µm was applied to extract the volatile compounds from the headspace of above-prepared glass vial. The GC was equipped with a split—splitless injector which was held at 250˚C. After starting at 30˚C, the oven temperature was raised in 3 steps after 2 min: 30˚C - 70˚C at 10˚C/min followed by 1 min at 70˚C; 70˚C - 220˚C at 4˚C/min and 220˚C - 280˚C at 20˚C/min and was finally held at 280˚C for 6 min. During this program a constant flow rate (1.0 mL/min) of the carrier gas (Helium) was maintained. Mass spectra were obtained by electronic impact (E.I) scan mode (Low mass: 30.0; High mass: 500.0; Threshold: 150) and temperature source (230˚C) were generated. The identification was achieved by comparing mass spectra obtained from the sample with those from the NIST and Pal1600k.L. libraries Database and by comparing the kovats index estimated for each compounds on both chromatographic column with the values given in the literature [19] (www.pherobase.com/database/kovats). Selective ion monitoring was used for the integrations of all chromatogram peaks and the semi-quantitative concentrations of volatiles in *ikigage* beers were calculated according to the method proposed by Zhao *et al*. [20].

3. Results and Discussion

3.1. Physicochemical and Microbiological Characteristics of Sorghum Beer "*Ikigage*"

The pH, total acidity (expressed as lactic acid), reducing sugars and ethanol content of pilot *ikigage* beers produced with starter cultures are given in **Table 1**. Pilot *ikigage* beers produced with *S. cerevisiae* in combination with *L. fermentum* or *S. cervisiae* in combination with *I. orientalis* and *L. fermentum* have pH and total acidity more similar to commercial *ikigage* beer from peasants than those produced without association of *L. fermentum*. Higher ethanol content and lower reducing sugars were observed in *ikigage* produced with *S. cerevisiae* in combination with *I. orientalis* following by *ikigage* produced with *S. cerevisiae* alone and *ikigage* obtained by the association of *S. cerevisiae* with *I. orientatilis* and *L. fermentum*. Except *I. orientalis*, all starter cultures used produced higher ethanol content than that observed in local *ikigage* beers from peasants. Contrary to *ikigage* beers from peasants, *S. aureus* and the food sanitary microorganisms were absents in pilot *ikigage* beers made with starter cultures.

The production of lactic acid by LAB can explain the lower and higher total acidity values observed in *ikigage* beers produced by peasants and those produced with association of *L. fermentum*. The similar total acidity level and low pH value were also observed by Orji *et al*. [14] in Nigerian traditional sorghum *pito* beers produced by *Lactobacillus plantarum* in combination with *S. cerevisiae*. Light increase of ethanol content obtained with *S. cerevisiae* in combination with *I. orientalis* compared to *S. cerevisiae* alone shown that *I. orientalis* could have to play an influence, for example by facilitating the synthesis of α-glucosidase by the fast glucose consumption, on maltose and maltotriose fermentation by *S. cerevisiae*.

Lower ethanol content obtained with *I. orientalis* alone could be explained by the no maltose fermentation by this yeast strain. Similar studies on fermentation of grape wort [21] reported also that *I. orientalis* produced a low ethanol content compared to *S. cerevisae*, but when the co-fermentation of *S. cerevisaie* and *I. orientalis*

Table 1. Physicochemical and microbiological characteristics of sorghum beers "*ikigage*".

	SC*	SCLF	IO	SCIO	SCIOLF	IKp
pH	5.04	4.01	4.87	4.56	4.16	3.90
Titrable acidity (%)	0.86	1.12	0.81	0.89	1.07	1.37
Reducing sugars (g/l)	24.10	26.33	75.37	17.40	20.78	24.34
Ethanol (% v/v)	4.34	3.04	1.49	4.56	3.99	2.25
Yeast (cfu/ml)	6.5×10^7	12.4×10^5	9.8×10^6	32.4×10^7	67.2×10^5	10.15×10^6
LAB (cfu/ml)	-	2.4×10^5	-	-	-	35.35×10^4

*SC: *S. cerevisiae* alone; IO: *I. orientalis* alone; SCIO: *S. cerevisiae* in combination with *I. orientalis*; SCLF: *S. cerevisiae* in combination with *L. fermentum*; SCIOLF: *S. cervisiae* in association with *I. orientalis* and *L. fermentum*; Ikp: traditional *ikigage* beer from local peasants.

were used, the ethanol production and reducing sugars consumption were similar to those obtained by *S. cerevisiae* alone.

3.2. Volatile Compounds of Sorghum Beer "*Ikigage*"

The results of GC-MS analyses of the volatile compounds obtained from the pilot *ikigage* beers produced with starter cultures and traditional sorghum beers *ikigage* made by peasants are outlined in **Table 2**. Traditional sorghum beer *ikigage* made by peasants differs from those produced in laboratory with starter cultures by relatively high concentrations of certain alcohols (propan-1-ol, 2-methylpropan-1-ol and 2,3-butanediol), esters (ethyl acetate, isobutyl acetate, propyl acetate, ethyl lactate and ethyl valerate), acids (acetic acid and heptanoic acid) and carbonyls (acetaldehyde). These compounds were also found in great quantity in the *ikigage* beers produced with *S. cerevisiae* in association with *L. fermentum* and *I. orientalis.*

High concentrations of 3-methylbutan-1-ol, 2-methylbutan-1-ol, ethyl acetate, acetic acid and ethyl lactate were also observed in the *ikigage* beers produced with *S. cerevisiae* in combination of *L. fermentum* without association of *I. orientalis.* However, *ikigage* beers produced with *I. orientalis* alone or in combination with *S. cerevisiae* were characterized by high concentration of ethyl butyrate, ethyl caprylate, isobutyl butyrate, ethyl nonanoate, ethyl dec-9-enoate, 3-methylbutyl decanoate, capric acid, butyric acid and 2-phenyl alcohols. These compounds were also found in great quantity in the *ikigage* beers produced with *S. cerevisiae* in association with *L. fermentum* and *I. orientalis*. High concentrations of 3-methylbutan-1-ol, 2-methylbutan-1-ol, ethyl acetate, acetic acid and ethyl lactate were also observed in the *ikigage* beers produced with *S. cerevisiae* in combination of *L. fermentum* without association of *I. orientalis*. However, *ikigage* beers produced with *I. orientalis* alone or in combination with *S. cerevisiae* were characterized by high concentration of ethyl butyrate, ethyl caprylate, isobutyl butyrate, ethyl nonanoate, ethyl dec-9-enoate, 3-methylbutyl decanoate, capric acid, butyric acid and 2-phenyl alcohols. Ethyl acetate (and its corresponding acid) and ethyl lactate were also considered as some of the main bacterial volatile compounds [22], and the high concentrations were found in the *ikigage* beers produced by association of triple strains (*S. cerevisiae*, *I. orientalis* and *L. fermentum*). As Belgian Lambic and gueuze beers [23], ethyl acetate, ethyl lactate and ethyl caprylate can be the characteristic compounds of *ikigage* beer. Important amounts of ethyl butyrate (and its corresponding acid) found when *I. orientalis* is used, were also observed in a Bavarian pilsner-type beer, where this odorant was suggested one of the key contributors to the overall aroma [24].

The high concentration of 3-methylbutan-1-ol, 2-methylbutan-1-ol and 2-methylpropan-1-ol, observed in *ikigage* beers from peasants and those produced when *L. fermentum* was associated, were also found in fermented dough with mixed culture of *L. fermentum* and *S. cerevisiae* [25]. This observation can be explained by the amino acids released by the proteolytic activity of lactic acid bacteria and their use by *S. cerevisiae* [26]. Phenethyl alcohol, known to have intense odour of roses and a burning taste, is produced by enzymatic conversion of phenylalanine by yeast cells, particularly by *S. cerevisiae* [27], but in this work, *I. orientalis* seems to produce high concentration of this compound more than *S. cerevisiae.*

3.3. Sensory Characteristics of Sorghum Beer "*Ikigage*"

The results of sensory evaluation of *ikigage* beers produced with starter cultures are indicated in **Figure 1**. These results shown that *ikigage* beers produced with *S. cerevisiae* in association with *I. orientalis* and *L. fermentum*, following those produced with *S. cerevisia* in co-cultures with *L. fermutum*, have the taste, aroma and mouth feel much more similar to that the *ikigage* beer brewed locally by peasants. *S. cervisiae* in co-cultures with *I. orientalis* produced *ikigage* beers with aroma is also more comparable to local *ikigage* beers than *S. cerevisiae* or *I. orientalis* alone.

The lack of sour taste in the *ikigage* produced without *L. fermentum* may explain their unacceptability by the consumer panelists. The *ikigage* beers are characterized by the sour taste due to acidity produced during fermentation, mainly by *L. fermentum*. However, the acidity produced by *I. orientalis* may have contributed also to the taste and mouth feel of *ikigage* beers. By comparing the aroma scores with the volatile compounds observed in this work, it is obvious that esters and alcohols have contributed to the similarity between aroma of *ikigage* beers produced with the mixture cultures and those produced locally by peasants. Other studies reported also that the sour taste of Nigerian sorghum *pito* beers [7] [14] and *kaffir* sorghum beer of South Africa [28] was due to the lactic acid produced by the lactic acid bacteria during the fermentation.

Table 2. Volatile compounds of traditional sorghum beer *ikigage*.

Compounds	RI	ID[a]	Concentration in beers (µg/L calculated by internal standard equivalent)					
			IKp	SC	IO	SCIO	SCLF	SCIOLF
Esters								
Ethyl acetate	609[b]	MS/RIL	749.3	286.8	38.6	151.5	338	512.7
Propyl acetate	712	MS/RIL	160	1.0	-	-	-	-
Ethyl isobutyrate	740	MS	-	-	69.2	18.2	-	7.5
Isobutyl acetate	773	MS/RIL	49.3	3.4	15.5	11.6	0.3	-
Ethyl butyrate	801[b]	MS/RIL	9.8	1.6	468.7	153.4	-	2.0
Ethyl lactate	815	MS/RIL	205	-	-	-	82.2	31.5
Ethyl isovalerate	871	MS	38.9	-	-	-	-	-
Isoamyl acetate	877	MS/RIL	47.2	41.9	40.3	72.5	67.7	79.0
2-Methylbutyl acetate	879	MS/RIL	17.7	-	-	-	-	-
Ethyl valerate	900	MS	44.1	-	-	-	8.2	0.6
Ethyl caproate	998[b]	MS/RIL	75.6	56.8	12.1	95.1	43.4	66.8
Hexyl acetate	1017	MS/RIL	1.8	1.7	0.1	1.9	1.7	1.3
Ethyl heptanoate	1096	MS/RIL	4.4	43.3	5.9	35.2	0.2	7.7
Ethyl benzoate	1169	MS/RIL	1.3	-	-	-	-	-
Methyl salicylate	1192[b]	MS/RIL	11	69.5	40.8	69.2	60.5	47.8
Ethyl caprylate	1196[b]	MS/RIL	201.8	62.8	664.8	227	47. 6	88.8
Isobutyl caprylate	1386	MS	2.3	-	-	-	-	-
phenethyl acetate	1257	MS/RIL	4.5	2.6	-	0.6	0.6	1.0
Ethyl nonanoate	1294	MS/RIL	3.4	9.3	275	161.6	56.0	92.4
Ethyl dec-9-enoate	1382	MS/RIL	10.3	-	38.2	19.7	-	-
Ethyl caprate	1395[b]	MS/RIL	34.1	35.7	9.4	42.0	5.5	22.0
3-Methylbutyl octanoate	1447	MS	4.8	-	-	-	-	-
Ethyl laurate	1597	MS/RIL	1.6	3.6	4.8	4.4	4.0	0.9
Isopropyl laurate	1631	MS	0.9	0,6	-	0.7	-	-
3-Methylbutyl decanoate	1648	MS	1.3	-	2.2	1.6	-	-
Ethyl merystate	1797	MS/RIL	2.5	1.7	2.8	4.7	1.0	1.3
Ethyl palmitate	>1900	MS	1.0	1.7	5.4	2.4	1.3	1.1
Isopropyl palmitate	>1900	MS	5.0	0.7	5.3	-	-	-
Alcohols								
Propan-1-ol	>600[b]	MS	1041.6	890.7	275.1	810.3	385.1	443.3
2-Methylpropan-1-ol	>600[b]	MS	194.3	170.4	53.3	90.2	156.9	77.4
3-Methylbutan-1-ol	704	MS/RIL	603.8	1042	772.6	948.7	1233.1	1055.3
2-methylbutan-1-ol	709	MS/RIL	22.5	181,6	155,3	182.9	356.7	113.0
2,3-butanediol	804	MS/RIL	56.2	38.1	-	30.4	-	45.9
Hexano-1-l	871	MS/RIL	18.8	24.0	0.3	14.7	17.2	21.3
Heptan-1-ol	971	MS/RIL	65.1	72.0	9.4	21.3	12.2	61.2
Octan-1-ol	1072	MS/RIL	4.3	21.7	16.4	33.3	0.4	3.0

Continued

2-Phenethyl alcohol	1115	MS/RIL	120	21.6	84.5	62.0	21.9	32.3
Nonan-1-ol	1172	MS/RIL	-	11.0	16.8	42.5	14.4	9.9
Nonan-2-ol	1099	MS/RIL	-	54,1	-	18,0	-	7,1
Decan-1-ol	1273[b]	MS/RIL	-	17.5	0.8	5.2	-	6.2
Acids								
Acetic acid	700	MS/RIL	2333.7	633.2	82.1	433.1	941.1	1015.2
butyric acid	775	MS/RIL	6.1	-	14.0	9.8	-	1.0
Propionic acid	772	MS	12.4	-	-	-	-	4.4
Heptanoic acid	1064	MS/RIL	63.1	-	26.2	28.0	-	13.5
Caprylic acid	1186[b]	MS/RIL	11.2	0.5	8.2	6.6	8.1	16.9
Capric acid	1380[b]	MS/RIL	3.7	-	65.1	49.0	12.3	12.9
Carbonyl								
Acetaldehyde	<600	MS	76.2	21.3	36.3	6.7	18.8	66.3
2-Butanone	<600	MS	-	88.7	-	-	-	-
3-Methylbutanal	<600	MS	34.4	39.1	43.2	31.5	23.1	8.3
2-methylbutanal	600	MS	11.3	20.1	18.3	12.5	11.4	0.4
1-Hexanal	797	MS/RIL	4.2	7.5	0.6	5.2	0.2	6.0
Phenylacetaldehyde	1048	MS/RIL	0.5	-	-	-	-	-
2-Nonanone	1090	MS/RIL	-	22.2	-	-	-	-
Nonanal	1101	MS/RIL	-	9.6	10.5	3.3	8.0	0.3
2-Decanone	1203	MS	-	1.22	-	-	-	-

IKp: traditional sorghum beer *ikigage* made by Rwandese peasants. SC: pilot *ikigage* beer made with *S. cerevisiae* alone; IO: pilot *ikigage* beer made with *I. orientalis* alone; SCIO: pilot *ikigage* beer made with *S. cerevisiae* in combination with *I. orientalis*. SCLF: pilot *ikigage* beer made with *S. cerevisiae* in combination with *L. fermentum.* SCIOLF: pilot *ikigage* beer made with *S. cervisaie* in association with *I. orientalis* and *L. fermentum.* [a]ID: Identified by mass spectra (MS) and by comparison of retention index (RI on HP—5 ms) calculated and retention index from literature (RIL); [b]Identification confirmed by pure standard injection.

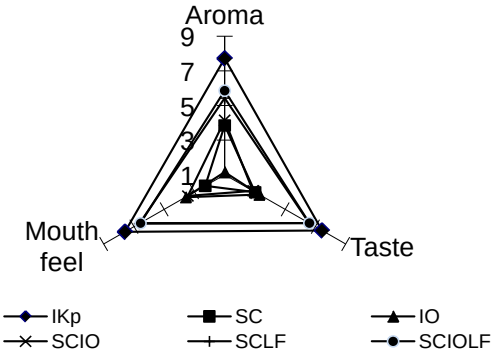

Figure 1. Sensory evaluation of sorghum beers "*ikigage*". 1: extremely different. 9: not different. IKp: traditional sorghum beer *ikigage* made by Rwandese peasants. SC: pilot *ikigage* beer made with *S. cerevisiae* alone; IO: pilot *ikigage* beer made with *I. orientalis* alone; SCIO: pilot *ikigage* beer made with *S. cerevisiae* in combination with *I. orientalis*. SCLF: pilot *ikigage* beer made with *S. cerevisiae* in combination with *L. fermentum.* SCIOLF: pilot *ikigage* beer made with *S. cervisaie* in association with *I. orientalis* and *L. fermentum.*

4. Conclusion

The present study has provided information on the use of *S. cerevisiae*, *I. orientalis* and *L. fermentum* as starter cultures for the production of Rwandese traditional sorghum beers "*ikigage*". *L. fermentum* has an influence on sour taste of *ikigage* beer and contributes also to generating ethyl acetate, ethyl lactate and higher alcohols such as 3-methylbutan-1-ol, 2-methylbutan-1-ol and 2-methylpropan-1-ol of this beer. *I. orientalis* contributed to the production of ethyl butyrate, ethyl caprylate, isobutyl butyrate and their corresponding acids, and to generation of phenyl alcohols in *ikigage* beers. *S. cerevisiae* in co-culture with *I. orientalis* produced higher ethanol than *S. cerevisiae* alone or *I. orientalis* alone. The association of *S. cerevisiae* with *I. orientalis* and *L. fermentum* produced *ikigage* beer with taste, aroma and mouth feel much more similar to that of *ikigage* beer brewed locally by peasants. It is recommended to use *S. cerevisiae* in association with *I. orientalis* and *L. fermentum* as starter cultures to produce *ikigage* beer having the uniform organoleptic characteristics and a high ethanol content. This method also reduces the risk of contamination of the brew with food sanitary indicator and pathogenic microorganisms and will increase the chance of the conservation of *ikigage* beer. However, complementary studies on *ikigage* beer conservation are needed.

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