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MANUFACTURING OF *KIVUGUTO* MILK AND STABILITY IN STORAGE UNDER REFRIGERATION

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Abstract

The *kivuguto*milk was processed in a 20 liters bioreactor with three bacteria previously selected in *kivuguto* traditional milk. The work aimed to study the association of three bacteria previously selected in traditional *kivuguto* in order to reproduce it in a controlled fermentation, and thereafter to understand its stability during storage under refrigeration. Post-acidification, viability, proteolysis, flavor compounds as well as rheological characteristics were monitored for 36 days. The ph decreases from 4.54 to 4.45 and the titratable acidity grew from 73°d to 79°d. The final biomass after storage was 0.60 10^8 cfu.g^{-1} which is far higher than the recommended 10^6 cells.g^{-1}before consumption. The proteolysis was at a range of 3.0 to 7.0 mg.$l^{-1}$of lysine equivalent, which is too low so that it can't produce bitter peptides. The evolution of flavor compounds in storage showed that no change found with 3-methylbutan-1-ol, acetic acid and furan-2(5h)-one, whilst pentan-1-ol and furanmethan-2-ol increased slightly upon 24 days' storage. The complex viscosity decreased from 4 - 5.3 pas before storage to 2.9 - 4.0 pas corresponding respectively to the ratio g"/g' of about 0.3-0.4 with a very low variation. These data allowed the production and the good preservation of *kivuguto* milk at 4°c on 36 days.

Keywords: Manufacturing, stability, *kivuguto* milk, *lactococcus*, *leuconostoc*

Introduction

Fermented milk was first produced around 10000 years ago (tamime, 2002) and approximately 400 generic names are applied to traditional and industrialized products (Campbell-Patt, 1987; Kurmann *et al.*, 1987).

Nowadays, progress in the area of the development of defined starter cultures has been driven by an increased awareness of commercial food safety and the search for novel flavors, textures or potential health benefits (Wullschleger, 2013). In the dairy industries, the production of fermented milk results from the introduction in sterile milk of starter culture. Thereafter, the ph values of milk decreased during the manufacturing process, from the time it was inoculated with bacterial cultures to the time when it was manufactured (O'neil *et al.,* 1979; Sokolinska *et al.,* 2004). Once coagulated at acid ph, fermented milks are cooled and kept cold until they are sold to consumers. During the cold storage, the biochemical composition of this fermented milk must stay stable for quality and consumer acceptance. These include the acidity, the rheological properties as well as the organoleptic features. All these characteristics depend on the viable cells and their balance in the fermented milk. According to codex alimentarius standards (Fao & Who, 2011), these starter microorganisms shall be viable, active and abundant in the product to the date of minimum durability. For instance, it is generally accepted that yogurt contains 10^7cfuof viable bacteria (*streptococcus thermophilus*and *lactobacillus bulgaricus*) per ml (Chougrani *et al.,* 2009).

In dairy industries, it is well known that a slow acidification of fermented milk goes on even under refrigeration (Accolas *et al.,* 1977)for some microorganisms and a high rate of post-acidification affects the cells viability in storage. Studying the use of probiotic bacteria as starter culture, Demerdash and Otiabi (2008) found how an increase in the acidity of the product during storage adversely affects viability. Damin *et al.*(2008) showed that the fermented milk prepared with a coculture of *streptococcus* and *bifidobacteriumlactis*gave the most constant rheological behavior and the best cell viability during cold storage. An important factor affecting the rheological behavior of the casein coagulum is the production of exopolysaccharides (eps) by the starter culture. These polymers confer firmness and texture to the final product (Moreira *et al.,* 2000). According to Purohit *et al.* (2009), the molecular characteristics of exopolysaccharides and their interaction with milk proteins determine the rheological and sensorial characteristics of fermented milk.along with the post-acidification, the starter cultures in fermented milk during storage bring about some changes in the proteolytic system. This system is involved in the aroma generation, but also in the bitter peptides occurring during milk acidification and post-acidification. According to law and Kolstad (1983), the proteolytic system of starter bacteria consists of proteinases, which catalyze the hydrolysis of native or denatured protein molecules, and peptidasewhich catalyze the degradation of the smaller peptides produced by proteinases action.

The present paper deals with the production and the behavior of the *kivuguto* formulated milk in storage under refrigeration. Three strains were previously selected from samples collected in *kivuguto*, a traditional fermented milk of rwanda (Karenzi *et al.,* 2012). The evolution of the acidification, viable cells, proteolysis, rheology and volatile compounds analyzed after the fermentation is quantified using instruments for the estimation of the first alteration indicators. The work is a part of sequential analyses with the objective of industrial production of *kivuguto* milk.

Materials and methods

Fermentations

The production of the three samples was made as following:

- *Kivuguto* milk sample: the pre-culture was inoculated at 10^6 cells.ml^{-1}of milkas a total initial concentration. This inoculum was used to culture at 4% (vol/vol) a 20 liters fermentor with 16 liters of joyvalle sterile milk as a working volume(it means that we prepared 640 ml of pre-culture for 16 l). This initial inoculum was composed by the three selected strains as previously reported: cwbi-b1466 *lactococcuslactis*, cwbi-b1465 *leuconostocmesenteroides* and cwbi-b1470 *leuconostocpseudomesenteroides* at a ratio respectively of 40%, 35% and 25%.
- For the two fermentative *kivuguto* strains, cwbi-b1466 *lactococcuslactis* and cwbi-b1470 *leuconostocpseudomesenteroides*, samples were made by two cultures for each strain. The fermentations were performedin monoculture at 10^6 cells.g^{-1} for the pre-culture used to inoculate at 4% (vol/vol) a 20 liters fermentor with 16 liters of joyvalle sterile milk as a working volume.

The pre-cultures of joyvalle sterile milk were prepared using 10^6 freeze-dried cells for monocultures (*lactococcus* milk or *leuconostoc* milk). For *kivuguto* milk (mixed strains), the pre-culture was also inoculated by a total of 10^6 cells (40% of *lactococcus*, 35% of *leuconostocmesenteroides* and 25% of *leuconostocpseudomesenteroides*).indeed, after the freeze-drying process, 1 g of each powder concentration was estimated by plating on solid medium and the corresponding quantity for pre-culture preparation was weighed.

The cultures were carried out at 19°c which is the average fermentation temperature in rwanda. The incubation time was determined by the end of the acidification, meaning at the ph ≈4.5-4.6. The experiment was conducted in triplicate.

Post-acidification

Milk samples were stored at 4°c for 36 daysin head-space (hs) vial (filter service, eupen, belgium) sealed hermetically with a polytetrafluoroethylene-coated rubber septum and an aluminum cap (filter service, eupen, belgium. The ph (phmeter wtw ph351i, weilheim, Germany) was measured and the titratable acidity (°d) was measured after incubation time (tf) by titrating a 10 ml sample with naoh (1/9n) using phenolphthalein as an indicator (afnor, 1980). Measurements were also made after 12, 24 and 36 days.

Viability

After suitable dilutions, the enumeration of *kivuguto*milk strains was performed on mrs agar incubated at 30°c for 48 h. Viable cells counts were expressed as colony forming units per gram ($cfu.g^{-1}$) of milk. Four counts were carried out to determine the number of bacteria during storage: just after fermentation (at tf), after 12, 24 and 36 days.

Rheological data evolution

The rheological parameters complex viscosity, elastic modulus (g') and loss modulus (g") of the milk samples were estimated at 10°c using a high resolution bohlin cvo 120 rotational rheometer (malvern instruments, worcestershire, uk). The measuring geometry employed was a rotating upper cone and a fixed lower plate (α=4°, ø=40 mm). The oscillation frequency was 1.0 hz, and the shear stress was 1pa, which was found to be within the linear viscoelastic region of fermented milk samples according to Stern *et al.* (2008). Three replicates were applied.

Proteolysis

The proteolytic activity of the tested strains in the three milk samples stored at 4°c was determined using the o-phthaldialdehyde (opa) method (Church *et al.*, 1987). This method is based on the reaction of opa and β-mercaptoethanol with the α-amino groups released during hydrolysis of milk proteins. The results were calculated from a standard curve obtained from dilution of leucine in distilled water and expressed in leucine equivalent ($mg.l^{-1}$) (sigma aldrich, diegem, belgium) of milk. Analyses were made in triplicate four times during the storage: day 1, day 12, day 24 and day 36.

Flavor stability

Flavor compounds were studied on 24 days by static headspace sampling and gc/ms analysis.

Headspace sample preparation

Headspace (hs) samples were prepared manually. A milk sample (10 g) was introduced in a 20-ml hs vial (filter service, eupen, belgium)sealed hermetically with a polytetrafluoroethylene-coated rubber septum and an aluminum cap (filter service, eupen, belgium). The samples were kept at 4°c before analysis for a short time and those stored longer were put at −20°c and put at 4°c the day before analysis. Samples were equilibrated for 65 min at 70°c prior to analysis, and the volatile compounds trapped in the headspace region of the vial (2000 µl) were taken with a micro syringe (filter service, eupen, belgium)and analyzed by gc using direct gas injection.

Gas chromatography

Milk samples volatiles (2000 µl) were injected into an agilent technologies 7890a gc system (agilent technologies, santa clara, ca, usa) equipped with a flame ionization detector (fid) and a 30-m x 250-µm x 0.25-µm vf-wax polar column (agilent technologies, santa clara, ca, usa) was used for the study. Helium was used as the carrier gas, at a flow rate of 1.5 ml min^{-1}, and the splitless mode was used. The following temperature program was used: 50°c for 6 min, increased to 180°c for 5 min at a rate of 8°c min^{-1}, and held for 10 min at 15°c min^{-1} from 180 to 250°c. The injector and detector temperatures were 220 and 250°c, respectively.

Mass spectrometry analysis

The volatile compounds were identified by mass spectrometry using an agilent technologies 5875c with triple-axis detector coupled to 6890 gc system (agilent technologies, santa clara, ca, usa). The ms was carried out in ei mode, with an ionization potential of 70 ev, an ionization current of 2 a, an ion source temperature of 200°c, a resolution of 1000 and a mass range 30 to 450 m/z.

Chemical identification

Compounds were identified by comparing recorded mass spectra with the willey 275l mass spectra library (scientific instrument service, ringoes, nj, usa), the nist ms library (nist, gaithersburg, md, usa), the pal 600k mass spectral library (palisade corporation, ithaca, ny, usa), and those in literature, as well as comparison of their retention times with authentic standards of saturated *n*-alcanes standard solution (c_7-c_{30} alkanes) (sigma aldrich, diegem, belgium), as external references under the same chromatographic conditions, allowing calculation of kovats index (Kovats, 1987)of the separated volatile compounds (Harris, 1987).

Standard solutions and quantification

Aqueous solutions of acetic acid, pentan-1-ol, and methyl benzoate as an external standards were prepared from high purity chemicals (>99%) purchased from sigma-aldrich (Diegem, Belgium). The mass of 40 µl of each standard was accurately measured and diluted in 100 ml in double-distilled waterand thereafter mixed at a ratio of 1:1. The prepared solution was hermetically sealed in 20-ml headspace vials at -20°c until they were used. The compounds were quantified by external standard technique as previously described.

Statistical analyses

Results were analyzed by using analysis of variance to determine significant differences ($p \leq 0.05$) between mean values from three independent experiments.

Results

Post-acidification

Fermented milks were made in three types: with mixed *kivuguto* strains, with single cultures of cwbi-b1466 lactococcus and with single culture of cwbi-b1470 *leuconostoc*. The results of *kivuguto* milk titra table acidityare presented in **fig.1**

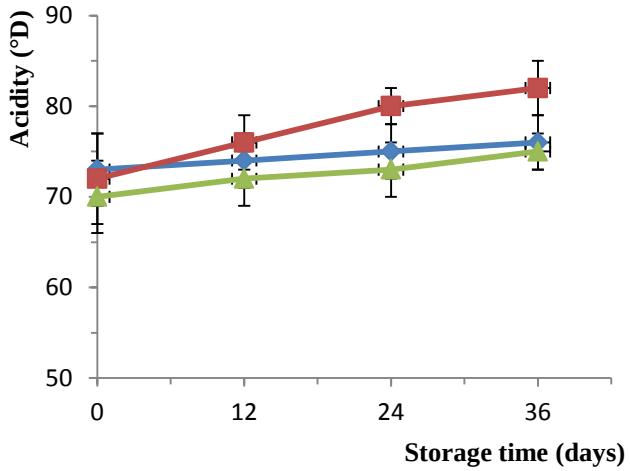

Figure 1. Evolution of titratable acidity in storage (at 4°c) of milk fermented by kivuguto selected strains (♦kivuguto milk, ■lactococcus cultured milk, ▲leuconostoc cultured milk)

For *kivuguto* milk, the acidity varied in storage from 73 to 76 °d. For *lactococcus* milk, the titrable acidity evolution in storage was in the range of 72 to 82°d, whereas for *leuconostoc*milk, the acidity changed from 70°d to 75°d. During the storage time, the ph of *kivuguto* milk changed slightly from

4.54 to 4.45 as illustrated on **fig. 2.** *Lactoccus* milk ph varied from 4.56 to 4.38, whilst the *leuconostoc* milk ph decreased from 4.58 to 4.46.)

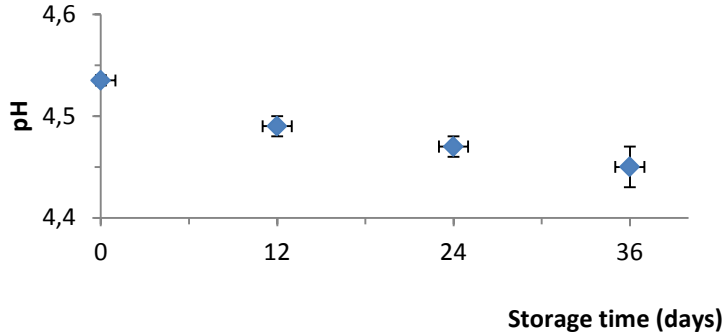

Figure 2. Evolution of ph of *kivuguto* milk during 36 days in cold storage (4°c)

Viability

During 36 days of cold storage, the viable cells in *kivuguto* milk decreased from 38.5 10^9 cfu.g^{-1} to 0.59 10^8 cfu.g^{-1} as shown in **table 1**.

Table 1. Evolution of cells viability of **kivugu to* fermented milk storage at 4°c

	Cells concentration (10^8 cfu.g^{-1})			
	Before storage (at tf)**	**Day 12**	**Day 24**	**Day 36**
***Kivuguto* milk**	38.5	35.0	2.7	0.6

*milk with mixed strains, **tf: end of fermentation

Rheological data evolution

The evolution of rheological properties complex viscosity, elastic modulus (g') and viscous modulus (g") as a function of time at 10°c during storage of *kivuguto* milk were followed on 36 days using a high-resolution bohlin cvo 120 rotational rheometer (malvern instruments, worcestershire, uk). Results are presented in **fig. 3, 4, 5 and 6** respectively at the end of fermentation (time tf), at day 12, at day 24 and at day 36.

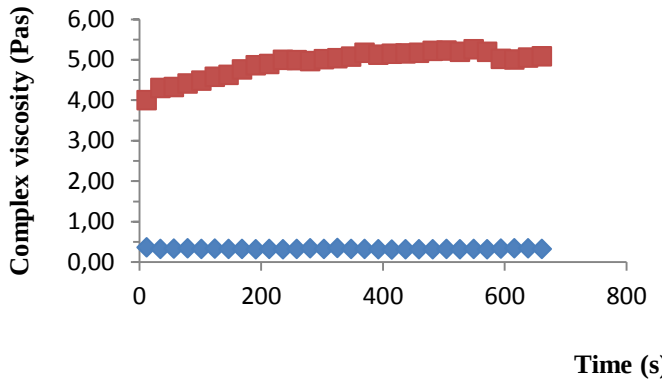

Figure 3. Viscosity of *kivuguto* after fermentation (■ complex viscosity in pas;♦ tan δ as the ratio g"/g')

At tf, the ratio g"/g′ expressed as tan δ varies from 0.3-0.4 and the complex viscosity changes from 4 to 5.3 pas, as shown on **fig. 3**

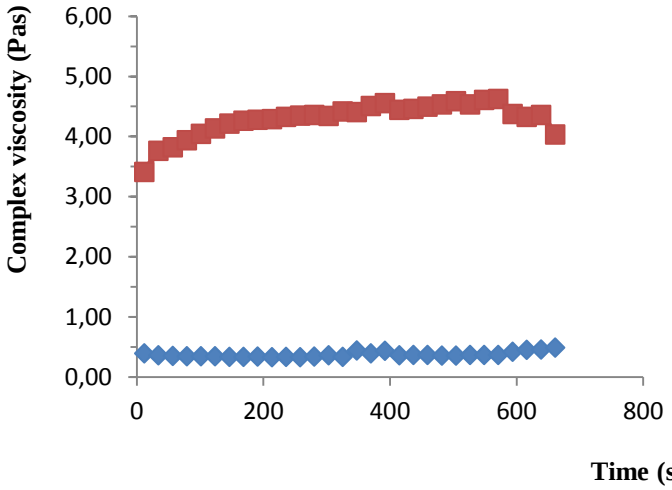

Figure 4. Viscosity of *kivuguto* after 12 days in storage (4°c) (■ complex viscosity in pas;♦ tan δ as the ratio g"/g')

After 12 days in storage, tan δ varies from 0.3-0.5 and the complex viscosity changes from3.4 to 4.6 pas, as shown on **fig. 4**

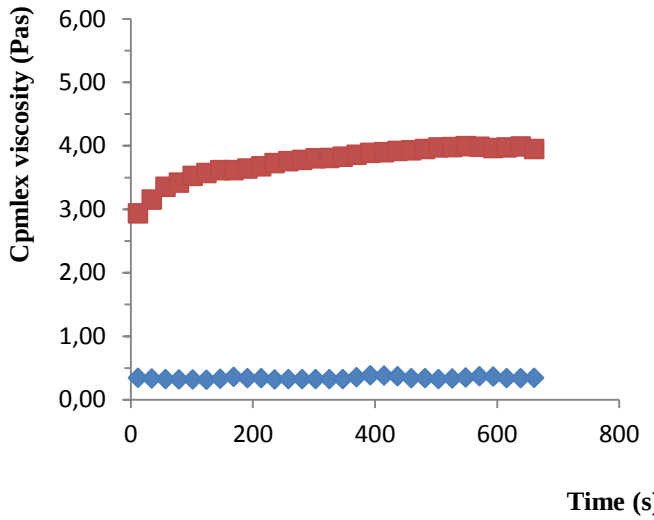

Figure 5. Viscosity of *kivuguto* after 24 days in storage (4°c) (■ complex viscosity in pas;♦ tan δ as the ratio g"/g')

After 24 days in storage, tan δ varies from 0.3-0.4 and the complex viscosity changes from 2.9 to 4.0 pas, as shown on **fig. 5**.

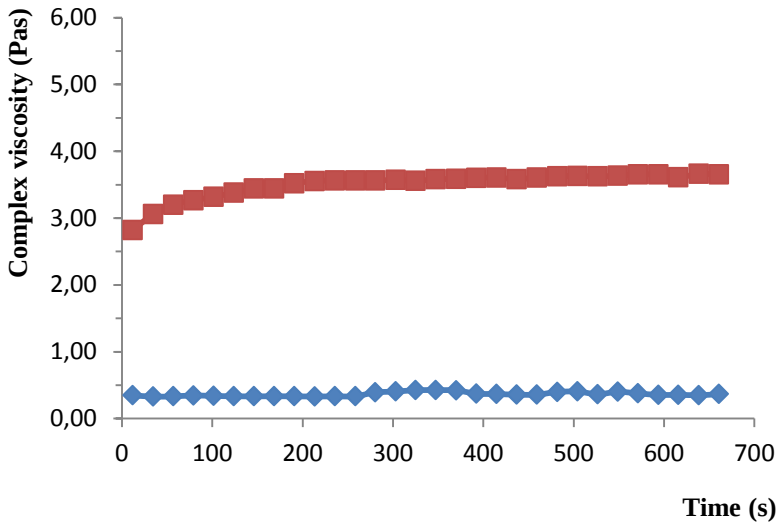

Figure 6. Viscosity of *kivuguto* after 36 days in storage (4°c) (■ complex viscosity in pas;♦ tan δ as the ratio g''/g').

After 36 days in storage, tan δ varies from 0.3-0.4 and the complex viscosity changes from 2.9 to 4.0 pas, as shown on **fig.6**.

Proteolysis

The proteolytic activity of *kivuguto* strains in the 3 samples showed an increase more in single cultured milks than in mixed cultured milk as summarized in **table 2.** The increase of proteolysis in *kivuguto* milk was 2 fold after 36 days, 13 fold in *lactococcus* cultured milk and varied from 5.5 to 42.8 in leuconostoc cultured milk.

Table 2. Proteolysis evolution of *kivuguto* milk & *kivuguto* strains milks in storage at 4°c

Milk	**Concentration of peptides/free amine acids in storage (mg/l)**			
	Before storage (at tf)*	**Day 12**	**Day 24**	**Day 36**
Kivuguto	3.0 ± 0.7	4.9 ± 0.4	5.6 ± 1.0	7.0 ± 1.0
Lactococcus milk	3.1 ± 0.3	4.6 ±1.1	20.0 ± 0.1	39.4 ± 6.2
Leuconostoc milk	5.5 ± 0.2	21.3 ± 3.5	31.9 ± 3.4	42.8 ± 3.5
Sterile joyvalle**	< 0	< 0	< 0	< 0

*tf end of fermentation, **sterile joyvalle is the milk used for fermentation. Here, it was not inoculated.

Aroma compounds stability

The volatile compounds (vcs) profile of *kivuguto* milk were extracted by headspace and identified by mass spectrometry using an agilent

technologies 5875c coupled to 6890 gc system. It was composed of five main molecules of: 3-methylbutan-1-ol, pentan-1-ol, acetic acid, 3-methylbutan-1-ol and furan-2(5h)-one. The evolution of the 5 volatile compounds previously characterized was studied on 24 days for stability assessment. As illustrated in **table 3**, furan-2(5h)-one, acetic acidand 3-methylbutan-1-ol stayed stable on the period of storage, whereas pentan-1-ol carried out an increase of 18.6 % on 24 days. The vc furanmethan-2-ol content grew quickly up to 14.9% after 12 days and stays stable until 24 days.

Table 3. Evolution of vcs in *kivuguto* milk in storage on 24 days

T_r [(1)]	Cas [(2)] Number	Iupac name	Identification [(3)]	Vcs in *kivuguto* milk (%) [(4)]			Sample Ri [(5)]	Reference Ri [(6)]
				0	Day 12	Day 24		
8.46	**123-51-3**	**3-methylbutan-1-ol**	**Ms, std, ri**	**11.5± 0.6**	**13.58±1.4**	**12.95± 0.4**	**1202**	**1204** [a]
8.92	**71-41-0**	**Pentan-1-ol**	**Ms, std, ri**	**18.7± 0.3**	**21.98±0.3**	**22.21± 0.1**	**1226**	**1244** [b]
14.26	**64-19-7**	**Acetic acid**	**Ms, std, ri**	**4.4±0.4**	**4.13±0.4**	**5.0±0.6**	**1473**	**1477** [c]
16.61	**93-58-3**	**Methyl benzoate**	**Ms, std, ri**	**4.0±0.0**	**-** [(7)]	**-** [(7)]	**1613**	**1635** [d]
17.14	**98-00-0**	**Furanmethan-2-ol**	**Ms, std, ri**	**39.9± 1.6**	**45.9±0.6**	**44.6±0.3**	**1650**	**1661** [e]
17.91	**92618-89-8**	**1,7,7-trimethylbicyclo [2.2.1]hept-2yl acetate**	**Ms, std, ri**	**5.8±0.0**	**-** [(7)]	**-** [(7)]	**1699**	**1584** [f]
18.49	**497-23-4**	**Furan-2(5h)-one**	**Ms, std**	**15.7± 1.0**	**14.5±0.1**	**15.2±0.4**	**1739**	**-** [(8)]

[(1)]Retention time; [(2)]CAS number of compounds listed in order of elution from a VF-Wax. Source: CAS Scifinder® (Chemical Abstracts Service, Colombus, USA); [(3)]Identification methods: MS, comparison of mass spectra with those in Nist 08, Wiley275 and PAL 600K libraries; RI, comparison of retention indices with those in literature; STD, comparison of retention time and mass spectra of available standards; [(4)]*kivuguto* milk relative contents (%): milk with 3 *kivuguto*'s strains; [(5)]Retention indices on VF-Wax column experimentally determined using a saturated C7-C30 alcanes standard solution; [(6)]Kovats indices taken from literatures: [a]Fukami*et al.* (2002) (measured with a TC-Wax column); [b]Umano*et al.* (2002) (measured with a DB-Wax column); [c]Cullere*et al.* (2004) (measured on a DB-Wax column);[d]Ferreira *et al.* (2001) (measured on a DB-Wax column);[e]Wong& Bernhard(1988)(measured on a DB-Wax column); [f]Davies (1990)(measured on a Carbowax column); [(7)]not calculated as it do not come from starters used: [(8)]not found.

Discussion

In this study, using three strains selected in traditional *kivuguto*[18], three fermented milks were processed: one with mixed strains (*kivuguto* formulated milk), two with two *kivuguto* fermentative strains in monoculture. The investigation was followed with 5 parameters sustaining the quality of fermented milk. By monitoring the change of these parameters in our samples in storage under refrigeration, our objective was to predict the level at which they can be unacceptable for consumption. These parameters are:

(i) the acidification as its evolution bring about many changes both in taste and in viable starters in milk, and we measured along the storage time the ph and the titratable acidity (°d);

(ii) the viability as living biomass is very important for evaluating the quality of a dairy product, for instance, the idf (1992) suggests for the minimum level for bacteria in probiotic milk a range of 10^6 to10^7 cfu.ml^{-1} in order to produce therapeutic benefits (Moayednia *et al.,* 2009);

(iii) the proteolysis as a beneficial property at a certain level, unless, it is responsible of flavor defects;

(iv) the rheology as consistence and firmness variations leads to negative properties such as syneresis and atypical texture. It is obvious that the rheological propertiesinfluence the texture which in turn affects the sensory perception and ultimately the acceptance of a product by the consumer (Aichinger *et al.*, 2003)and;

(v) the aroma and flavor attributes whose stability in milk guides a favorable effect on customer's decision until the final day of storage. And this was complemented by a casual sensory evaluation made for detection of any appearance of noticeable bitterness or off-flavor at each stage of storage.

The post-acidification was estimated by ph and titratable acidity. The evolution of acidity of *kivuguto* milkshowed insignificant changes in ph as presented on **fig. 2**. The decrease from 4.54 to 4.45 is almost tolerated by the majority of local *kivuguto* customers. Even fresh fermented milks can reach these values or more during manufacturing (Sokolinska *et al.,* 2004; O'neil *et al.,* 1979). At the same time, the titratable acidity increases slightly up to 79°d in some samples, which represents 8.2 %. This value is very low compared with other data in literature. For instance, titratable acidity increased on average by 22.3% in the yogurts and by 14.9% in the yogurt-related products during storage (Kneifel *et al.,* 1993). for the *lactococcus* cultured milk, it reached 85°d. This is in agreement with its acidifying capacity, as the best acidifier among the three strains of *kivuguto* starter. But the titratable acidity of *kivuguto* strains is very low compared to those of yogurt. Indeed, the titratable acidity of yogurt reaches 101.5°d (chougrani*et al.,*2009; salvador and fiszman, 2004; gueimonde*et al.,* 2003). And it is in

the range of wild *streptococcus thermophilus* and *lactobacillusdelbrueckii*subsp.*bulgaricus* isolated by soomro and masud(2008) in *dahi*. Indeed, they found values ranging from 69°d to 77°d in milk fermented by each strain.

The explanation is only based on the capacity of *lactococcus* to grow in milk. It needs only 7 amino acids to grow, when *leuconosto*c needs 9 amino acids. Generally, *lactoccus* is a good acidifier. Specifically, it acidifies milk more than *leuconostoc*. This is due to its capacity to hydrolyze milk caseins using its extracellular proteases. *Lactococcus* also has endocellularproteinases which allow it the degradation of the peptides formed during caseins catabolism. The growth rate of *lactococcus*is very important in dairy technology as it reduces quickly the lactosis in lactic acid, and subsequently reduces the ph with the formation of the curd.

The counts of bacteria in the fresh *kivuguto* milk were about 3.9 10^9 $cfu.ml^{-1}$, and the count at the end of storage was 5.9 10^7 $cfu.ml^{-1}$. After 24 days, viable counts were 2.7 10^8 $cfu.ml^{-1}$, which is far from the recommended level of 10^6 cells during shelf life in storage.akalin*et al.* (2004) found the highest viable number of bifidobacteria of 3.6-2.3 10^7 $cfu.g^{-1}$ in the milk containing *b. Animalis* and fructooligosaccharides and viability of *b. Longum* in yogurt containing fructooligosaccharides remained above 10^6 $cfu.g^{-1}$ for up to 21 days.

The rheological parameters evolution in storage were followed using a high-resolution bohlin cvo 120 rotational rheometer (malvern instruments, worcestershire, uk) coupled to a computer, allowing a real time recording of data during analyses: the time, temperature, frequency, phase angle, complex modulus, elastic modulus g', viscous modulus g", complex viscosity, shear stress, strain. The visco-elastic data of the samples were characterized on 600 s (10 min) using a couple of two parameters: tan δ and the complex viscosity. From the data of elastic modulus g' and viscous modulus g", the ratio g"/g' or tan δ was calculated and plotted with the complex viscosity against time. The initial complex viscosity was between 4 And 5.3 pas, whilst tan δ was about 0.3. At day 12, the complex viscosity varies from 3.7 to 4.6 pas and tan δ increases about 0.3 to 0.5. The decrease of viscosity was also observed at day 24 at a range of 2.9 to 4.0 pas, corresponding to tan δ of 0.3 to 0.4. Only on 24 days, a correlation with the ph decline from 4.54 to 4.45 can explain the observed decrease of complex viscosity. Correspondingly, the live biomass began a big drop from 35.0 10^8 $cfu.g^{-1}$ at day 12 to 2.7 10^8 $cfu.g^{-1}$ at day 24, until to reach, 0.6 10^8 $cfu.g^{-1}$ at day 36. At this level, the cell lysis didn't bring bitter peptides in milkas confirmed by routine tastes along the storage period and confirmed by instrumental analyses. Note that the general flavor is a result of the equilibrium of small peptides and other molecules released in the milk during the acidification

and the storage as well. Interestingly, tan δ at day 36 was about 0.3 to 0.40. This value shows a stability comparatively to the variation at day 24. The reduction of tan δ is subsequent to the increase of g' and this make the milk more firm according to Kristo *et al.*(2003).this fact is in agreement with the equilibrium of the three strains involved in *kivuguto* fermentation in storage. At low ph, cwbi-b1470 *leuconostocpseudomesenteroides* and cwbi-b1466 *lactococcus* begin to decline, whilst cwbi-b1465 *leuconostocmesenteroides* resists well. This strain also produceexopolysaccharides, bearing the firmness up to day 36 in the milk. It seems that its activity is not fully stopped during storage.however, the low variation of ph, the titratable acidity and the viable cells in *kivuguto* milk is in agreement with the usual behavior of the strains found in *kivuguto*milk and their association in milk. Extending the correlation to proteolysis and aroma compounds, the equilibrium encountered in *kivuguto* milk versus *lactococcus* cultured milk and *leuconostoc* cultured milk revealed the importance of the strain cwbi-b1465 *leuconostocmesenteroides.* In *kivuguto* milk, the proteolysis (**table 2**) varies from 3.0 to 7.0mg.l^{-1}, which is very low comparatively to *lactococcus* cultured milk and *leuconostoc* cultured milk, with high values reaching about 40 mg.l^{-1}. It seems that the third strain of *kivuguto* milk, the strain cwbi-b1465 *leuconostocmesenteroides,* is a good stabilizer throughout the storage time. This is also highlighted by its capacity in aroma compounds stability in *kivuguto* milk. Over three weeks' storage, the variability in aroma compounds (**table 3**) was also very low for the 5 volatile compounds (vcs) identified. In a previous study, we have shown that this strain produce the majority of *kivuguto* vcs.

Conclusion

This work showed the behavior of *kivuguto* milk in storage with regard to post-acidification, viability, rheology, proteolysis and flavor profile stability. The interaction of the three strains forming the *kivuguto* starter culture revealed the activity of each strain. However, it is paramount to pursue a distinctive work focusing only to the interaction of these bacteria in milk fermentation process. The presence of *leuconostoc* strains in the *kivuguto* starter culture revealed the importance of this genus in dairy industry. On overall basis, the present work has shown the importance of a good association during the selection of starter cultures, which allowed a stable milk upon the storage period.

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