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KEFIR - GRAINS AND BEVERAGES: A REVIEW

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ABSTRACT - Kefir is a fermented milk beverage produced by the action of bacteria and yeasts that exist in symbiotic association in kefir grains. The large number of microorganisms present in kefir and their microbial interactions, the possible bioactive compounds resulting of microbial metabolism, and the benefits associated with the use this beverage confers kefir the status of a natural probiotic, designated as the 21th century yoghurt. The importance of probiotics in food industry is growing nowadays and further research should be performed on the symbiotic relations between different microorganisms and how these interactions can result in nutritional and therapeutic benefits as curing and preventing human diseases and other disorders. This review includes a thorough and detailed discussion on the structure, microbiological and chemical composition, and the production and utilization of kefir will be presented covering different aspects.

Key words: bacteria, probiotic, symbiotic, yeast.

Kefir – grãos e bebidas: uma revisão

RESUMO - Kefir é uma bebida de leite fermentado produzido pela ação de bactérias e leveduras que existem em associação simbiótica em grãos de kefir. O grande número de microrganismos presentes no kefir, suas interações microbianas, os possíveis compostos bioativos resultantes do metabolismo microbiano e os benefícios associados ao uso de bebidas kefir, conferem o status de um probiótico natural, designado como o iogurte século XXI. A importância dos probióticos na indústria de alimentos está crescendo hoje em dia e mais pesquisas devem ser realizadas sobre as relações simbióticas entre diferentes microrganismos e como essas interações podem resultar em benefícios nutricionais e terapêuticas, como a cura e prevenção de doenças humanas e outras desordens. Esta revisão inclui uma discussão aprofundada e detalhada sobre a composição estrutural, microbiológica e química do kefir. E a produção e utilização de kefir serão também apresentados.

Palavras-chave: bactéria, levedura, probiótico, simbiótico.

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INTRODUCTION

Kefir is a fermented milk beverage. The milk fermentation is achieved by the use of kefir grains, a cluster of microorganisms held together by a polysaccharide matrix named kefiran. Kefir grains are small, irregularly shaped, yellowish-white, hard granules that resemble miniature cauliflower blossoms (LORETAN et al., 2003; CHEN et al., 2008).

Kefir grains are an example of symbiosis between yeast and bacteria. They have been used over years to produce kefir, a fermented milk beverage that is consumed all over the world, although its origin is Caucasian. A vast variety of different species of organisms forming the kefir grains, comprising yeast and bacteria, have been isolated and identified. The *Lactobacillus* genus is the most frequent throughout the fermentation period. Other lactic bacteria in addition to the *Lactobacillus*, including species of the *Leuconostoc, Lactococcus* and *Streptococcus* genera are commonly detected. The Gram negative bacteria are represented by species of the *Acetobacter* genera. The yeast isolates belonging to species of the *Kluyveromyces, Candida, Torulaspora, Pichia, Cryptococcus, Debaromyces, Kazachstania, Lachancea, Torulaspora, Zygosaccharomyces, Trichosporon* and *Saccharomyces* genera (GARROTE et al., 2001; WITTHUHN et al., 2005; CHEN et al., 2008; JIANZHONG et al., 2009; MIGUEL et al., 2010; MAGALHÃES et al., 2011c; PUERARI et al., 2012).

The microorganism groups present in kefir carried out three types of fermentation during the process: lactic, alcoholic and acetic. The flavour compounds responsible for the typical kefir aroma flavour can be divided in two groups: major and minor end-products (secondary metabolites). The former group is only composed of lactic acid and the latter of flavour compounds produced especially during the stationary growth phase. Carbonyl compounds (acetaldehyde, ethanol, diacetyl, acetoin, 2-butanone and ethyl acetate), volatile organic acids (formic, acetic, propionic, butyric) and non-volatile acids (lactic, pyruvic, oxalic and succinic) are secondary metabolites and classified as flavour-forming compounds of the kefir (OTLES; CAGINDI, 2003; AGHLARA et al., 2009; MAGALHÃES et al., 2011c). Kefir is a probiotic food (OTLES; CAGINDI, 2003). Probiotics have shown to be beneficial to health, being presently of great interest to the food industry.

In this review, a thorough and detailed discussion on the structure, microbiological and chemical composition, and the production and utilization of kefir will be presented covering different aspects.

DEVELOPMENT

Structure and Microbial composition of kefir grains

Kefir grains are small, irregularly shaped, yellowish-white, hard granules that resemble miniature cauliflower blossoms. The scanning electron microscopy (SEM) of kefir grains revealed a complex and tightly packed biofilm could be observed around the kefir grains, while the interior was comprised mainly of unstructured material. Figure 1 A and B show the association of the kefir microbiota through SEM (MAGALHÃES et al., 2011c). The kefir grains showed a smooth surface covered by an agglomerate of microorganisms (bacteria and yeast) (Figure 1 A) The microbial cells on the inner portion were less than that on the outer portion (Figure 1 B). Fibrillar material (polysaccharide kefiran) was observed in the inner portion of the kefir grains (Figure 1 B). SEM inspection of the kefir grains revealed the structure and relative proportion of microbiota and polysaccharides in the kefir grains.

Kefir grains are microbially-rich, normally consisting of three groups of microorganisms living as part of a symbiotic association. These include lactic acid bacteria (LAB), yeasts and acetic acid bacteria (AAB) (LORETAN et al., 2003; CHEN et al., 2008; MAGALHÃES et al., 2010; MAGALHÃES et al., 2011c; PUERARI et al., 2012). Marsh et al. (2013) shows that the bacterial populations in Irish kefir are dominated by 2 phyla, the Firmicutes and the Proteobacteria.

LAB: They include lactobacilli, lactococci, (GARROTE et al., 2001; WITTHUHN et al., 2005; CHEN et al., 2008; MIGUEL et al., 2010; MAGALHÃES et al., 2011c) and leuconostocs (GARROTE et al., 2001; JIANZHONG et al., 2009). The major LAB population may be either homofermentative or heterofermentative (Lin et al. 1999) comprising 65 – 80% of the total microbial population (Wouters et al. 2002). In a study by Angulo et al. (1993), the heterofermentative lactobacilli counts were found to be higher than the homofermentative counts (74.5 and 25.7% respectively). The same distribution pattern was report by Garrote et al. (2001) where 20 isolates of heterofermentative lactobacilli were found versus 16 homofermentative isolates.

AAB: They were reported to represent only 20% of the total microbial population and are usually present in lower counts ($<10^5$ CFU g^{-1}) (GARROTE et al., 2001; MAGALHÃES et al., 2011c; MIGUEL et al., 2010). However, counts as high as 10^8 CFU g^{-1} were found by Abraham and De Antoni (1999). In the other studies AAB were not found (WITTHUHN et al., 2005) and sometimes they were just considered to be contaminants (ANGULO et al., 1993). According to Rea et al. (1996) AAB may stimulate the growth of other organisms since they are vitamin B_{12} producers. Koroleva (1988) report that the consistency of kefir can be improved by using a starter containing AAB, but not at a level higher than 10^6 CFU g^{-1}. This fact implies that the presence of AAB may be important a good kefir consistency and therefore a quality product.

Yeasts: The yeasts present in kefir grains have been reported to be either lactose fermenting and/or non-lactose fermenting (SIMOVA et al., 2002; LORETAN et al., 2003; LATORRE-GARCIA et al., 2007). Their number is usually lower than that of the LAB, and specifically around 10^4 – 10^5 CFU g^{-1} (LATORRE-GARCIA et al., 2007) but in some grains higher yeast counts than LAB counts have been reported (50 and 31.2%

respectively) (Angulo et al., 1993; ZAJSEK & GORSEK, 2010).

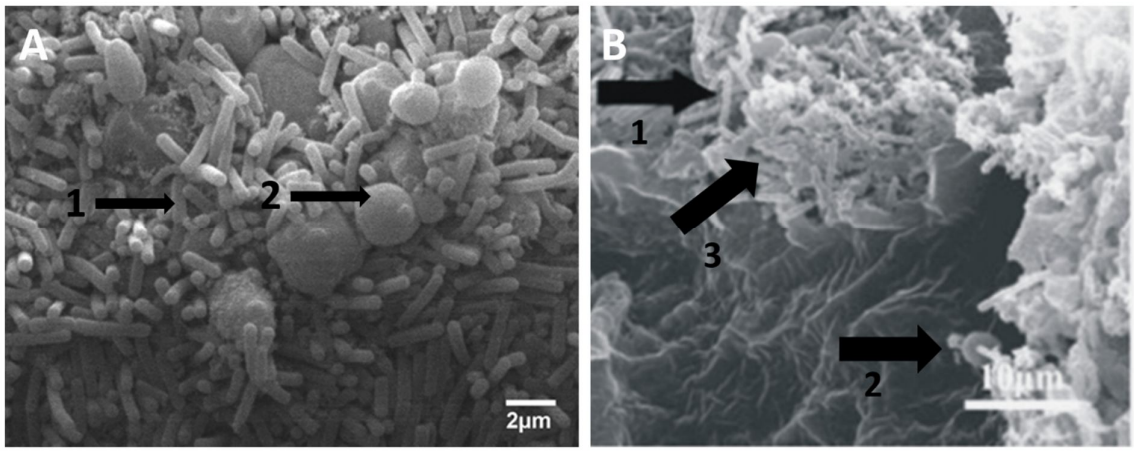

FIGURE 1 - A. External surface of kefir grain. Arrow 1 – Bacteria. Arrow 2 – Yeast. **B.** Internal surface of kefir grain. Arrow 1 – Bacteria. Arrow 2 – Yeast. Arrow 3 – Kefiran polysaccharide. Figure created by the authors.

Microbial composition of kefir beverages

Kefir beverages owe its microbial composition to the presence of kefir grains. Once in milk, kefir grain microorganisms are released and continue to multiply by using the available nutrients in the milk, and especially lactose that serves as the carbon and energy source. It is therefore expected that both the kefir grains and kefir beverages should have a very similar composition. Even though the microbial profile of kefir grains and kefir beverages is very similar, it is not advised to use kefir beverage as inoculum to make a new batch of kefir since the grains are essential to obtain the traditional kefir (SIMOVA et al., 2002). In the same way, it is preferable to use kefir grains as a starter rather than a mixture of pure cultures (ASSADI et al., 2000). According to Marshall (1984), the integrity of the grains is necessary to have the effervescent character and the typical yeast flavor of kefir associated with a creamy texture (SIMOVA et al., 2002).

LAB: The LAB population of beverage has been reported to be higher than the yeast population (WITTHUHN et al., 2005; ERTEKIN; GUZEL-SEYDIM, 2010), especially the lactobacilli population (10^8 – 10^9 CFU mL^{-1}). In contrast, other researchers (REA et al., 1996; BESHKOVA et al., 2002) found a higher population of lactococci (10^9 CFU mL^{-1}) and a lower population of lactobacilli (10^6 CFU mL^{-1}). Leuconostocs were the second major group of microorganisms isolated from an Irish kefir beverage with a count of 10^8 CFU.mL^{-1}. Leuconostocs naturally grow poorly in milk and are usually found in association with lactococci (REA et al., 1996).

AAB: They are also found in the kefir beverage at levels between 10^8 – 10^9 CFU mL^{-1} (REA et al., 1996; LORETAN et al., 2003; MAGALHÃES et al., 2011c). AAB are not always present in kefir beverage and sometimes considered as contaminants by Angulo et al. (1993).

Yeasts: They have been reported to either be lactose or non-lactose fermenting yeasts at levels of 10^4 – 10^5 CFU mL^{-1} (LORETAN et al., 2003; JIANZHONG et al., 2009; MIGUEL et al., 2010). Yeasts are generally present in kefir beverage (SIMOVA et al., 2002; ZAJSEK; GORSEK, 2010; MAGALHÃES et al., 2010; MAGALHÃES et al., 2011c; PUERARI et al., 2012).

The overall microbial composition of kefir is complex and is known to vary from region to region. The environment (cultivation, preservation and storage conditions) is the principal factor leading to the microbial diversity of kefir grains (LATORRE-GARCIA et al., 2007; MIGUEL et al., 2010; MAGALHÃES et al., 2011c).

Chemical composition of kefir

The data summarised in this section show the microbiological and chemical characteristics of some LAB involved in kefir fermentations. The ratio and type of flavour compounds produced by these microorganisms differ according to species and/or strains present. This variation in the composition of the LAB can greatly affect the final product quality (MAURELLIO et al., 2001; PUERARI et al., 2012; MARSH et al., 2013).

The major / secondary end-products formed by LAB some is characterized as lactic acid, acetaldehyde, diacetyl, acetoin, acetone, ethanol, CO_2 and acetic acid (Table 1). O lactic acid is a no-volatile, odourless compound, responsible for the characteristics acidity of fermented products. The total lactic acid content of kefir varies from 0.80 to 1.15% (m v^{-1}) (GARROTE et al., 2001) and originates from the degradation of lactose by the homofermentative and heterofermentative LAB present in kefir grains. The homofermentation produces only two moles of lactic acid and two adenosine-5'-triphosphate (ATPs) per mole glucose consumed whereas the heterofermentation produces one mole each of lactic acid, ethanol, CO_2, and 1 ATP per glucose. In presence of oxygen, Nicotinamide adenine dinucleotide (NAD^+) can be

regenerated by NADH oxidases and peroxidases, leaving acetyl-P available for conversion to acetic acid (GARROTE et al., 2001). The LABs responsible for lactic acid synthesis are either homofermentative or heterofermentative. The former groups are better acid producers than the latter (REA et al., 1996). The LAB belonging to the genus *Lactococcus* are homofermentative. Species of this genus are generally used in dairy fermentations for their acidification capacity, lowering the pH to about 4.5. *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* belong to this genus and are the principal species used as dairy atarter cultures (CHEN et al., 2008). Lactobacilli as *Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus delbrueckii* ssp. *lactis, Lactobacillus helveticus* and *Lactobacillus acidophilus* are mostly added into dairy foods (OTLES; CAGINDI, 2003).

Acetaldehyde is considered to be the compound responsible for the characteristics aroma of yogurt as it is responsible for the "fresh-fruit" note (OTT et al., 2002). Acetaldehyde is one of the principal aroma compounds found in kefir with concentrations ranging from 0.5 to 10 $mg.L^{-1}$ in kefir beverages (GUZEL-SEYDIM et al., 2000).

Diacetyl is also a desirable constituent of many dairy products which at very low concentrations up to 5 $mg.L^{-1}$ is responsible for the buttery aroma of milk products (GUZEL-SEYDIM et al., 2000). In contrast, diacetyl is seen as undesirable in the brewery and wine industries as it causes off-flavours (BELIN et al., 1992). Diacetyl is considered to be an important aroma compound of kefir (BESHKOVA et al., 2003). It has been found at different concentrations (0.30 $mg.L^{-1}$ to 1.85 $mg.L^{-1}$) in kefir beverages (BESHKOVA et al., 2003; AGHLARA et al., 2009).

Acetoin has been reported to be present in good quality kefir beverage at a concentration of 9 $mg.L^{-1}$ (GUZEL-SEYDIM et al., 2000). Acetoin is formed through citrate metabolism via acetolactate decarboxylase or by reduction of diacetyl by diacetyl reductase. At concentrations encountered in cultured product acetoin is usually flavourless and odourless so they may under certain circumstances be of little flavour value (GUZEL-SEYDIM et al., 2000).

Acetone is a normal constituent of milk and cheese and it was found in kefir beverage at different concentrations (0.6 - 4.91 mg L^{-1}) (BESHKOVA et al., 2003; AGHLARA et al., 2009). Acetone plays only a minor role in kefir's organoleptic characteristics and it is believed that concentrations of acetone below 1 $mg.L^{-1}$ are unlikely to have a great effect on flavour (AGHLARA et al., 2009). Acetone originates from citrate and lactose metabolisms and its production appears to be strain related. Some lactobacilli strains such as *Lactobacillus bulgaricus, Lactobacillus helveticus*, as well as streptococci cultures such as *Streptococcus lactis, Streptococcus cremoris* and *Streptococcus diacetylactis* are able to synthesize it in small amounts (BESHKOVA et al., 2003).

The ethanol concentration of kefir has been reported to vary 0.01 to 2.5% (m v^{-1}) depending on the starter and the method used to prepare the kefir (BESHKOVA et al., 2003; MAGALHÃES et al., 2011c; PUERARI et al., 2012). The formation of ethanol is essentially obtained by the conversion of acetaldehyde to ethanol by alcohol dehydrogenase, an enzyme present in both yeasts and LAB (GUZEL-SEYDIM et al., 2000). Yeasts and *Leuconostoc* are considered the principal producers of ethanol. But since no ethanol is produced during co-metabolism of lactose and citrate by *Leuconostoc,* yeasts can be considered to be the main ethanol producers (GUZEL-SEYDIM et al., 2000). Two types of yeasts may be present in kefir: non-lactose and lactose fermenting yeasts. It was demonstrated that the lactose fermenting yeasts do not have sufficient alcohol dehydrogenase activity and the final beverage obtained only had a weak yeast flavour compared to beverages prepared with non-lactose fermenting yeasts (SIMOVA et al., 2002; BESHKOVA et al., 2003). Carbon dioxide originating from the alcoholic fermentation and from the heterofermentation, gives kefir its subtle effervescence (LIU et al., 2002).

Acetic acid is a short chain volatile fatty acid which has been identified in kefir at concentrations between 200 and 850 mg L^{-1} (GARROTE et al., 2001). However, Guzel-Seydim et al. (2000) did not find any acetic acid in kefir produced in their study. Moreover Magalhães et al. (2011c) found acetic acid low concentrations (0.273 mg L^{-1}) in milk kefir beverages and this not affect the organoleptically beverage. Acetic acid gives a vinegar-like flavor but in kefir this flavour is not predominant. It is unlikely for acetic acid to be a product of lipolysis since natural lipase in milk is destroyed during pasteurization (KONDYLI et al., 2002). Biosynthesis of acetic acid may be from various amino acids, e.g. *Streptococcus diacetylactis* is able to form acetic acid from glycine, alanine and leucine (LIU et al., 2002). Acetate may also be formed from pyruvate is absence or presence of oxygen. In the former case and under substrate limitation, pyruvate is cleaved into formate and acetyl-CoA by pyruvate-formate lyase. Acetyl-CoA is phosphorylates to yield acetyl-P which is then converted to acetic acid by acetate kinase. In the latter case, NAD^+ can be regenerated by NADH oxidases and peroxidases, leaving acetyl-P available for conversion to acetic acid (GARROTE et al., 2001).

Variations in the storage conditions, the growth medium and the environment, will lead to the development of a community of microorganisms that will be characteristics of the grains (OTLES; CAGINDI, 2003; SCHOEVERS; BRITZ, 2003). Therefore, the organoleptic features of kefir will be directly linked to the microorganisms present in the grains and can be influenced by changes in the intrinsic factors of the grains (grain activity, grain to milk ratio, starter) or by one or several environment factors which include: incubation temperature, effect of pH and storage conditions.

Grains activity: Kefir grains kept as dried, freeze-dried or frozen must be considered as inactive grain forms due to the fact that the microorganisms are in lag phase. Therefore, they need to be activated to their exponential

growth phase before use. Physically, this can be seen when the grains float to the surface of milk or when the milk has clotted. No standard method of activation exists; however a recommended activation process is activating in pasteurized full cream or even skimmed milk incubation at room temperature ranging from 20 ºC to 25 ºC for 18 h to 24 h (SCHOEVERS & BRITZ, 2003). The activation process can last up to one week when frozen grains are used (WITTHUHN et al., 2005) and up to one month for lyophilized grains (SIMOVA et al., 2002). The grains are transferred daily (SCHOEVERS; BRITZ, 2003; MAGALHÃES et al., 2011c) into a new batch of milk or thrice a week (ANGULO et al., 1993; GUZEL-SEYDIM et al., 2000).

Grain to milk ratio: The impact of the inoculum size on the characteristics of kefir beverage, especially pH, lactococci concentration, apparent viscosity and CO_2 content, were studied by Garrote et al. (2001). They demonstrated that there were significant differences in the characteristics of the kefir obtained with an inoculum size of 1% and 10%. The former inoculum size gave a highly viscous and low acid beverage whereas the latter inoculum size gave a low viscosity, highly acidic and effervescent product. Some authors (KUO; LIN, 1999) agree that an inoculum size of 5% ($m.v^{-1}$) is suitable to make the traditional high-quality refreshing kefir beverage with a prickling and slight yeasty taste associated with a clear acid taste without bitterness, a smooth texture and a pleasant flavour (ASSADI et al., 2000).

Starter: The strains present in the starter, whether as pure cultures or as kefir grains, can affect the quality of kefir. Indeed, it has been shown that the amount of aroma compounds vary according to the strains present (LIU et al., 2002). Burrow et al. (1970) showed that the amount of diacetyl produced by *Lactococcus lactis subsp. lactis biovar. diacetylactis* strains varies from 0.07 to 3.72 mg L^{-1} whereas none of the other lactococci strains isolated from kefir produced diacetyl (YUKSEKDAG et al., 2004). The irreversible conversion of diacetyl to acetoin which is further reduced to 2.3 butanediol and volatilization, are responsible for the low level of diacetyl and acetoin in cultured products especially during long incubation periods (OSTLIE et al., 2003). In the case of acetaldehyde, which is toxic to the organism, it may be reduced to ethanol by alcohol dehydrogenase rather than excreted. Thus, accumulation of acetaldehyde in the growth medium will depend on the level of alcohol dehydrogenase activity (OSTLIE et al., 2003).

Incubation temperature: The incubation temperature is an important parameter in the manufacture of the final kefir since it may enhance or inhibit the activity of a specific group of microorganisms (ZAJSEK; GORSEK, 2010). The result is that specific desirable or oven undesirable flavour's may develop. A good kefir is obtained with an inoculum size of 5% ($m\ v^{-1}$) and incubation at 25 ºC (ZAJSEK; GORSEK, 2010). But according to Koroleva (1988), fermentation at 25 ºC – 27 ºC leads to an atypical product whereas fermentation at lower temperature (20 ºC – 22 ºC) permits the development of all the characteristics microorganisms. Consequently, the cycles of manufacture should last 24 h and consist of two steps: the first is fermentation at 20 ºC – 22 ºC for 10 h – 12 h and the second step is maturation at 8 ºC – 10 ºC for the remaining 12 h.

Effect of pH: Citrate permease (Cit-P) is the key enzyme of the citrate metabolic pathway because it is the means by which citrate is transported into the cell (SAMARZIJA et al., 2001). Thus, possibly due to pH constraints, citrate uptake may limit the rate of citrate utilization and may therefore directly affect the yield of aroma compounds. Studies have demonstrated that Cit-P optimum activity lies between pH 4.5 and 5.5 in *Lactococcus lactis* ssp. *diacetylactis* and between pH 5.0 to 6.0 in some other species. Under these conditions, citrate is metabolized and converted to flavor compounds. Thus, lack of flavour in kefir may be attributed to inadequate pH due to a short fermentation time or the absence of a diacetyl producer such as *Lactococcus lactis* ssp. *diacetylactis* among kefir grains microbiota (SAMARZIJA et al., 2001).

Storage conditions: The absence of diacetyl in dairy products such as cultured buttermilk and sour cream is mainly due to the irreversible conversion of diacetyl into acetoin by diacetyl reductase which is widely spread among LAB, but its activity varies among species and among strains within species. This enzyme has been found in several species including strains of *Streptococcus diacetylactis*, *Lactobacillus lactis* sp. *cremoris* and *Lactobacillus mesenteroides* ssp. *dextranicum*. Reduction of diacetyl proceeds rapidly at high temperatures and decreases with decreasing temperatures. Therefore, to stabilize and even increase diacetyl content of cultured products including kefir it is recommended that they be kept at refrigerated temperature (4 ºC – 5 ºC). However, it is interesting to note that of *Streptococcus diacetylactis* 16 ºC - 18ºC possessing 100 units of diacetyl reductase per milligram of enzyme protein was able to reduce 9 mg L^{-1} of diacetyl in 10 min. This highlights the importance of choosing the right combination of species in a mixed culture. Diacetyl reductase was also found in coliforms (*Escherichia coli*) and psychrophilic bacteria (*Pseudomonas putrefaciens, Pseudomonas fragi*). While diacetyl reductase activity is generally low in *Leuconostoc* and *Lactococcus* species, the opposite is true for coliforms and psychrophilic species that exhibit activities ranging from 3 to 345 $units.mg^{-1}$ of enzyme protein. Thus, defects in refrigerated cultured products where diacetyl is present may then be attributed to contamination by spoilage psychrophilic bacteria (BASSIT et al., 1995).

Production and utilities kefir

The "traditional" way of producing kefir is using raw unpasteurized, pasteurized, or ultra high temperature (UHT) treated milk (Figure 2).

The milk is poured into a clean suitable container with the addition of kefir grains. The content is left to stand at room temperature for approximately 24 h. The cultured-milk is filtered in order to separate and retrieve the kefir grains from the liquid-kefir. This fermented milk is appropriate for consumption. The grains are added to

more fresh milk, and the process is simply repeated. This simple process can be performed on an indefinite basis, since kefir grains are a living ecosystem complex that can be preserved forever as long as it is feeder. As active kefir grains are continually cultured in fresh milk to prepare kefir, the grains increase in volume or in biological mass (MAGALHÃES et al., 2011c).

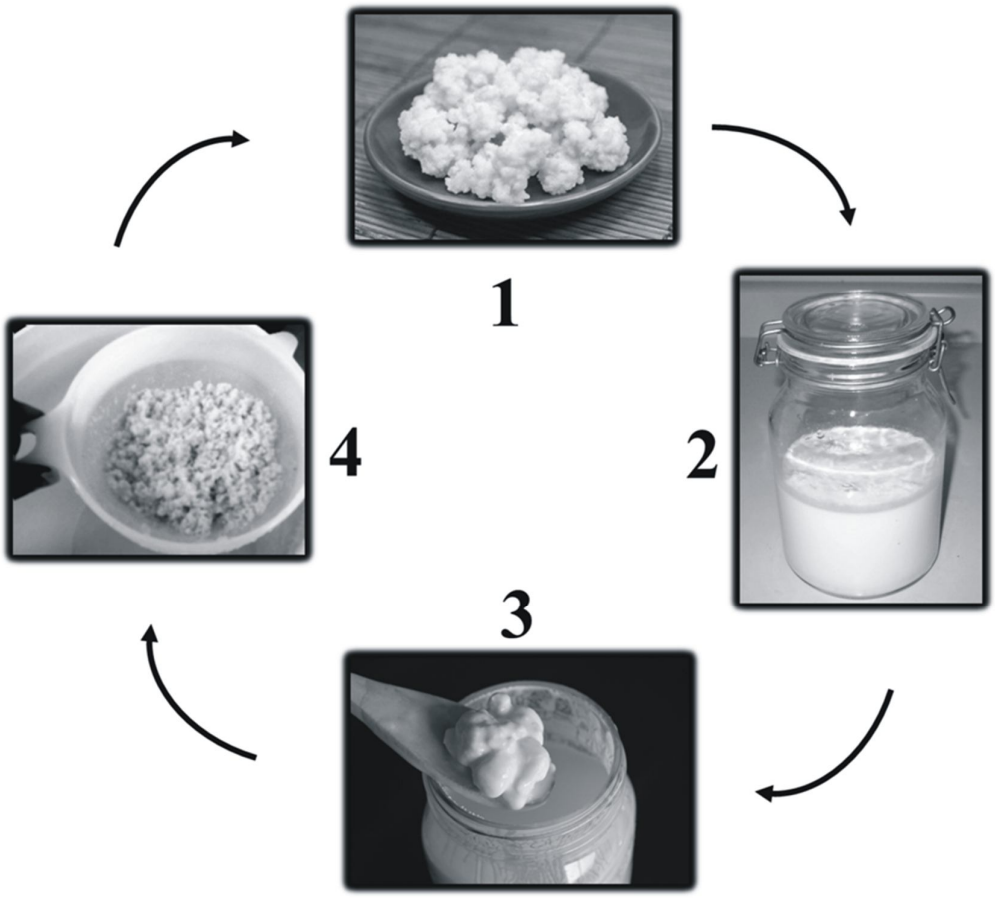

FIGURE 2 - Milk kefir beverage production. Kefir grains (1) are added to milk and are left to stand at room temperature for fermentation 18-24 h (2), the milk is then fermented forming the kefir beverage (3), after which they are filtered, (4) and ready to start another cycle. The fermented milk that results from step 4 is appropriate for consumption. Figure created by the authors.

Milk kefir beverage production is not the only industrial application being investigated. The conversion of agricultural and industrial waste materials into commercially valuable products is of great interest, especially when the final product can be a nutritive food source. Recent studies show new nutritive beverages technologies kefir as cheese whey-based kefir beverages (MAGALHÃES et al., 2011ab), cocoa pulp-based kefir beverages (PUERARI et al., 2012), and walnut milk kefir beverage (CUI et al., 2013).

Historically, kefir has been recommended for the treatment of several clinical conditions such as gastrointestinal problems, hypertension, allergies, and ischemic heart disease (LEITE et al., 2013). It has been demonstrated that some kefir grains show β-galactosidase enzyme activity, which stays active when consumed, and that kefir contains less lactose than milk (LEITE et al., 2013).

New technologies for kefir microbial analysis

The major proportion of earth's biological diversity is inhabited by microorganisms and they play a useful role in diversified environments. However, taxonomy of microorganisms is progressing at a snail's pace, thus less than 1% of the microbial population has been identified so far. The major problem associated with this is due to a lack of uniform, reliable, advanced, and common to all practices for microbial identification and systematic studies. However, recent advances have developed useful techniques in the microbial identification. Pyrosequencing, an automated high-

throughput sequencing technique that involves the synthesis of single-stranded deoxyribonucleic acid and the detection of the light generated by pyrophosphate released in a coupled reaction with luciferase (DOBSON et al., 2011). This technique allows the rapid and accurate sequencing of nucleotide sequences that can then be used to analyze the population structure, gene content and metabolic potential of the microbial communities in an ecosystem.

Pyrosequencing has recently been used to study the diversity and dynamics of the bacterial populations of an Irish kefir grain and its corresponding fermented product (DOBSON et al., 2011). The aim of the present work was to characterize the microbial diversity of three different kefir grains collected in different regions of Brazil. Several studies of Brazilian kefir have already been undertaken (MAGALHÃES et al., 2010; MAGALHÃES et al., 2011abc; MIGUEL et al., 2010) but most of these focused on the microbial composition of the kefir beverage during fermentation (MAGALHÃES et al., 2010). The present work catalogues the microbial species identified in three kefir grains using pyrosequencing. The pyrosequencing analysis allowed the identification of minor bacterial components.

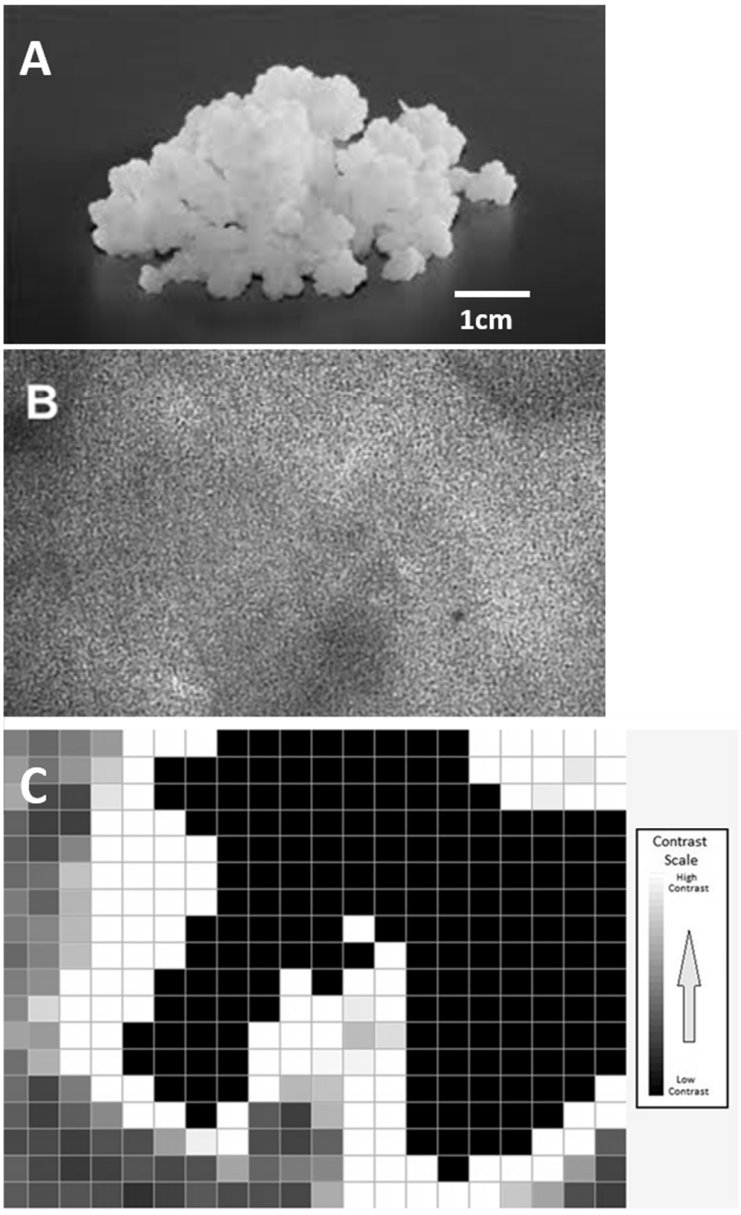

FIGURE 3 - Analysis of kefir grains by biospeckle laser. A - Kefir grains; B - Image of kefir grains generated by biospeckle laser; C - Result of the homogeneity test. Figure created by the authors.

Another technique is the biological activity of kefir grains. The study of the biological activity of kefir grains is necessary to control the microbial stability of fermenting microorganisms. An optical technique with potential use in biological metrology, particularly in biological activity, is the biospeckle laser (GUEDES et al., 2014). When a laser beam is scattered by a biological sample (Figure 3A), the scattered waves generated in the illuminated sample create the speckle pattern that changes its image in accordance with the changes in the monitored material. Thus, the surface appears to be covered with tiny bright dots that fluctuate in a seemingly random way as for a boiling liquid. (Figure 3B). The Figure 3C shows the area of the kefir grain analyzed. The intensity of the bright dots differentiates the microbial activity during fermentation process. The intensity of bright dots is estimated and calculated.

The work of Guedes et al. (2014) presents the biospeckle laser technique as a potential tool to analyze the microbial activity of kefir grains. In the present work, the kefir grains biological activity was measured from quantitative measurements by means of their speckle activity. The aim was to show that the biospeckle laser is a potential methodology to assess kefir grains viability, monitoring the kefir grains during the beverages production. This can be an innovative technique to be used in the beverage industries to kefir grains inoculum control.

CONCLUSIONS

Kefir is an example of coexistence of bacteria and yeast in the same environment, in equilibrium being beneficial for one another. The importance of the symbiotic relation in kefir between yeast and bacteria seems clear since they are both necessary in order to produce the components that are beneficial to health. The quality of probiotic kefir beverages is mainly influenced by the microorganisms present in kefir grains and kefir processing conditions. Currently, the application of probiotics in the food industry is in expansion and understanding the symbiotic relationships between different microorganisms present in food, as well as their interactions, could assist in the improvement of technological processes. However, from an industrial point of view, research involving kefir are welcome, given the lack of standardization in the production and marketing of kefir products.

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