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Full Length Research Paper

Assessment of the biological activity of kefir grains by biospeckle laser technique

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This work presents the biospeckle laser technique as a potential tool to analyse the kefir grains activity. 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 beverage production. The monitoring of the activity of the kefir is a key factor to guarantee the efficiency of the production of beverages, however the routine ways it is done compromise its use in the online production. The kefir grains were illuminated by a laser HeNe 17 mW 632 nm and analysed by the numerical biospeckle laser method. The results presented the statistical separation of the kefir in distinct levels of activity as expected. This can be an innovative technique to be used in the beverage industries for kefir grains inoculum control.

Key words: Kefir, speckle activity, inoculum control.

INTRODUCTION

Kefir is a culture employed to produce beverages, for example, the traditional Russian beverage also named "kefir" which is produced from milk, and has low alcohol content (Güzel-Seydim et al., 2005; Irigoyen et al., 2005; Magalhães et al., 2010; Puerari et al., 2012). The kefir is a mixed culture of various yeast species of the genus *Kluyveromyces, Candida*, *Saccharomyces* and it has lactic acid from bacteria of the genus *Lactobacillus*, and they are combined in a matrix of proteins and polysaccharide 'kefiran', which are formed during cell growth under aerobic conditions (Güzel-Seydim et al., 2005). The grains of kefir are irregularly shaped, with yellowish-white colour, and hard granules which resemble miniature cauliflower blossoms (Magalhães et al., 2011; Hamet et al., 2013).

In Brazil, the grains of kefir are used in private household for fermentation of milk (Magalhães et al., 2011), and they are added to different types of milk, such

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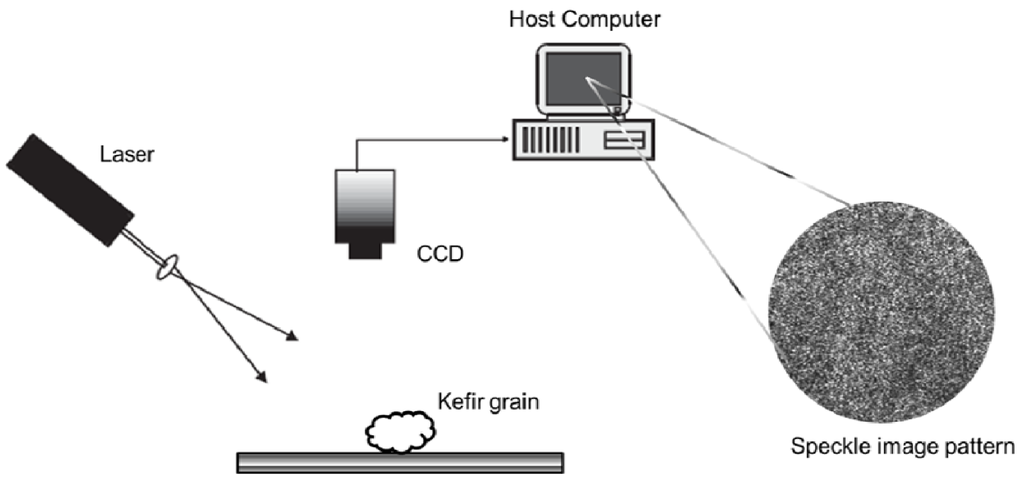

Figure 1. Experimental setup used to illuminate the kefir grain sample.

as the milk from a cow, a goat or a sheep, and as well from coconut, rice and soy. The grains are responsible for the fermentation that results in the production of numerous components in the kefir, including lactic acid, acetic acid, CO_2, alcohol (ethyl 2 alcohol) and aromatic compounds. This provides kefir's unique sensory characteristics: fizzy, acid taste, tart and refreshing flavor (Güzel-Seydim et al., 2005). The beverage contains vitamins, minerals and essential amino acids that help the body with healing and maintenance functions and also contains easily digestible complete proteins (Irigoyen et al., 2005). In accordance with Medrano et al. (2008), the benefits of consuming kefir in the diet are numerous, for instance, the antitumoral activity (Vinderola et al., 2005), the antimicrobial activity (Rodrigues et al., 2005), the antiinflammatory and the antiallergical activity (Lee et al., 2007).

The study of the biological activity of kefir grains is necessary to control the microbial stability of fermenting microorganisms. The microorganisms associated with Brazilian kefir grains in fermentative process are investigated using a combination of phenotypic and genotypic methods. Phenotypic identification of microorganisms is by Bac-Tray Kits I, II and III (Difco, S/P, Brazil) and API 50 CHL (BioMerieux, S/P, Brazil) according to the manufacturer instructions. Genotypic identification of microorganisms is by sequencing of portions of the 16S rRNA gene and Internal Tanscribed Spacer region (ITS). In addition, the visual evaluation by analysis using scanning electron microscopy (SEM) is also used to identify the microbiota of kefir grains for a fermentation process, however all these methods are time consuming and expensive (Magalhães et al., 2011).

An optical technique with potential use in biological metrology, particularly in biological activity, is the biospeckle laser (Zdunek et al., 2014). When a laser beam is scattered by a biological sample, 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. The intensity of the bright dots differentiates the microbial activity during fermentation process.

Many efforts have been devoted to characterise quantitatively the activity of biological material, such as in the activity of botanical specimens (Ansari and Nirala, 2013), in the evaluation of blood flow (Zakharov et al., 2009), in the viability of seeds (Braga Jr. et al., 2003) and in the maturation of meat (Amaral et al., 2013). Therefore, this work aimed to evaluate the feasibility to access the biological activity of the kefir grains fermentation in milk and its expected behaviour along the period from the substrate.

MATERIALS AND METHODS

The grains of kefir (250 g) were washed with distilled water and inoculated in 2.250 mL of milk substrate (ultra high temperature milk - UHT) and were statically incubated in a closed recipient during 24 h at room temperature. After 24 ours, that is considered as the fermentation process time, the samples of the kefir grains were taken aseptically from the milk and were evaluated by the biospeckle laser over a period of 24 h at every 3 h to assess the activity of the kefir out of the substrate (milk).

The grains of kefir were illuminated by a HeNe laser, wavelength of 632 nm, and 17mW power, enlarged by a plane concave lens in order to cover the entire sample (Figure 1). The interference patterns formed on them were captured by a CCD camera 640 × 486 pixels, with a shutter speed of 1/60 s and anacquisition rate between images of 0.08 s, creating a collection of 128 images. The analysis of the speckle images, from the laser illumination were performed by monitoring the temporal history of the speckle pattern (THSP) (Arizaga et al., 1999) and its numerical output, the absolute

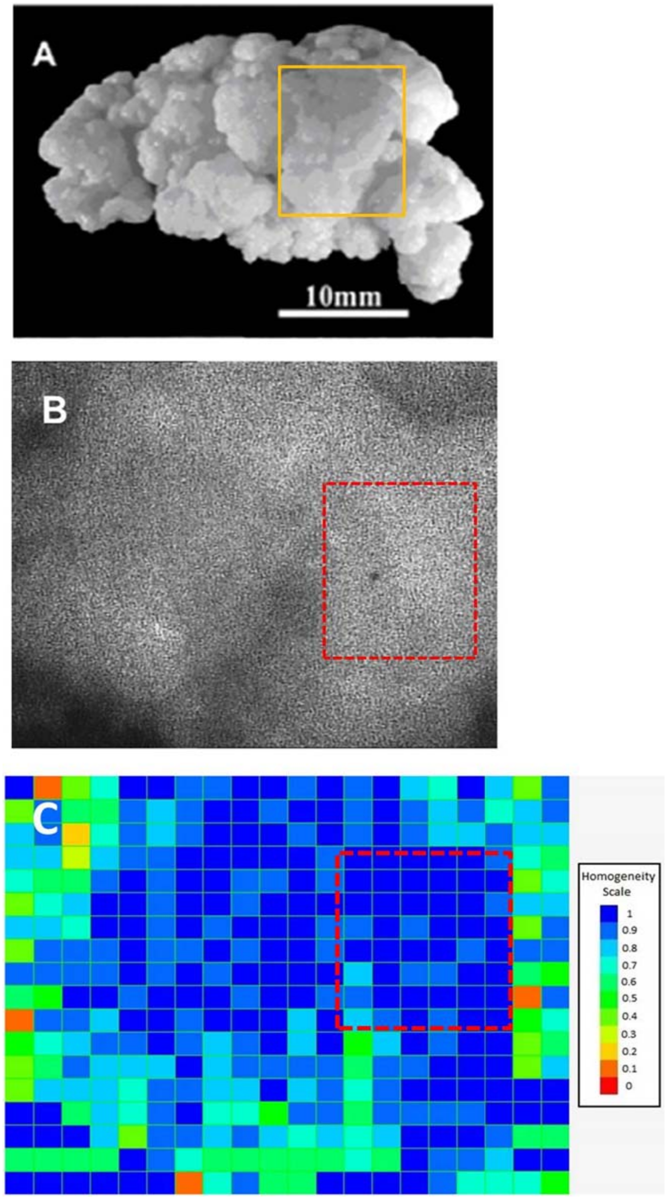

Figure 2. Analysis of kefir grains by biospeckle laser. A- Kefir grains; B- Image of kefir grains generated by biospeckle laser; C - Result of the homogeneity test and the cropped area used to process the AVD technique.

values of the differences (AVD) according to the Equation (1) described below (Braga et al., 2011):

$$AVD = \sum_{ij} \frac{OCM.|i-j|}{Normalization} \quad (1)$$

where the OCM is the occurrence matrix of the successive values in the THSP, and the i and j variables are the dimensions of the OCM matrix. The normalization provides the relation between the AVD values and the summation of all the occurrences. We performed eight sessions of illuminations in four grains of kefir with three replications at every three hours during 24 h.

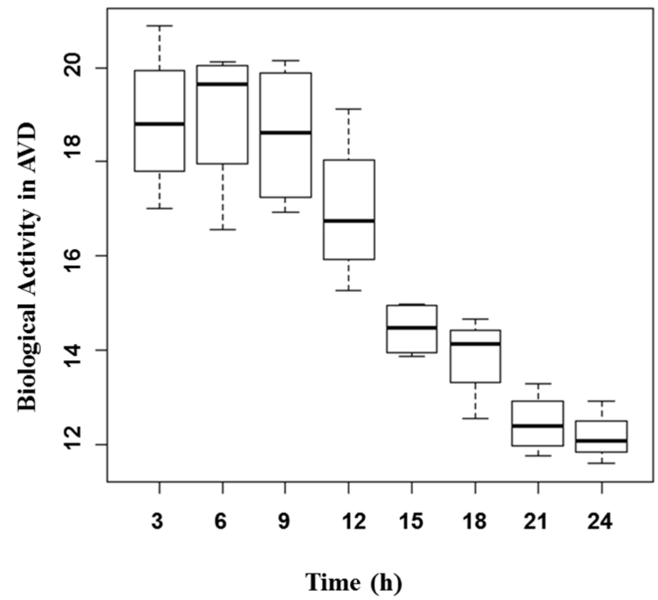

Figure 3. Evolution of the AVD values from kefir grains in time.

Each set of 128 frames was tested regarding its homogeneity (Braga et al., 2012) and the cropped area was processed by the AVD method.

RESULTS AND DISCUSSION

The test of homogeneity of the grains of kefir is shown in Figure 2, in the Figure 2a, the image of the grain is presented with an illustration of a window where the sample was illuminated and the images assembled (Figure 2b). The result of the homogeneity test is presented in Figure 2c where it is possible to see the areas with the highest homogeneity, which means the area where there is no changesof the activity, and in accordance with Braga et al. (2012), the area with high homogeneity can be processed using numerical approaches.

Therefore, the illustrated test was conducted in all the collection of images, and the numerical approach, particularly, the AVD was carried out. The results presenting the activity of the grains of kefir in time were reduced as presented in Figure 3, where it is possible to observe the sensitivity of the technique to follow the reduction of the expected activity. The kefir from the milk does not have any form to develop, therefore, reducing its activity.

An additional observation of the phenomenon presented the reduction of the dispersion of the values along the time, with a clear change of phase, which can be a useful information on the viability of the grain of kefir. The experimental results show it is possible, however, to distinguish between viable and non-viable tissue by quantitative method. Amaral et al. (2013) also used the technique of laser bioespckle to quantify biological activity in pork meat. They describe the laser biospeckle technique combined with analysis of inertia moment to show an efficient tool for monitoring and quantifying biological activity of meat during aging process, which demonstrates the technique potential for evaluating and predicting beef quality.

The next steps to be followed are related to the research of fermentative process, by adapting kefir grains for new experiments in substrates new, and to extend the metabolic activity of the kefir grains during the fermentative process. The use of different wavelengths for illumination and alternative algorithms for the processing of the data is also to be considered. These experiments are an indication that the biospeckle technique can be used as a methodology to evaluate the kefir grains during the beverages production. The proposed technique is simple, relatively cheap and fast, easy to implement, and requires only a laser and standard digital imaging processing hardware components. The application of biospeckle methods for the kefir viability detection was the main objective of this work, but the optical technique could also be applied to characterise the kefir grains in other types of processes.

Conclusions

Laser biospeckle technique show an efficient tool for monitoring and quantifying biological activity of kefir grains, showing the viability time of these grains after a fermentation process, which demonstrates the technique potential for evaluating and monitoring of kefir grains in production of fruits beverages and fermented/distilled beverages, in addition to beer production.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Amaral IC, Braga JrRA, Ramos EM, Ramos ALR, Roxael, EAR (2013). Application of biospeckle laser technique for determining biological phenomena related to beef aging. J. Food Eng. 119:135-139.

Ansari MDZ, Nirala AK (2013).Biospeckle activity measurement of Indian fruits using the methods of cross-correlation and inertia moments. Opt. Int. J. Light Electron Opt. 124:2180-2186.

Arizaga R, Trivi M, Rabal H (1999). Speckle time evolution characterization by the co-occurrence matrix analysis. Opt. Lasers Eng.31:163-169.

Braga JrRA, Dal Fabbro IM, Borem FM, Rabelo G, Arizaga R, Rabal HJ, Trivi M(2003). Assessment of seed viability by laser speckle techniques. Biosyst. Eng. 86:287-294.

Braga RA, Cardoso RR, Bezerra PS, Wouters F, Sampaio GR, Varaschin MS (2012). Biospeckle numerical values over spectral image maps of activity. Opt. Commun.285:553-561.

Braga RA, Nobre CMB, Costa AG, Sáfadi T, Costa FM (2011). Evaluation of activity through dynamic laser speckle using the absolute value of the differences. Opt. Commun. 284:646-650.

Güzel-Seydim Z, Wyffels JT, Seydim AC, Greene AK(2005).Turkish kefir and kefir grains: microbial enumeration and electron microscobic observation.Int. J. Dairy Technol. 58:25-29.

Hamet MF, Londero A, Medrano M, Vercammen E, Hoorde KV, Garrote GL, Huys G, Vandamme P, Abraham AG (2013). Application of culture-dependent and culture-independent methodsfor the identification of *Lactobacillus kefiranofaciens* in microbialconsortia present in kefir grains. Food Microbiol. 36:327-334.

Irigoyen A, Arana I, Castiella M, Torre P, Ibáñez FC (2005). Microbiological, physicochemical and sensory characteristics of kefir during storage. Food Chem. 90:613-620.

Lee MY, Ahn KS, Kwon OK, Kim MJ, Kim MK, Lee IY, Oh SR, Lee HK (2007). Anti-inflammatory and anti-allergic effects of kefir in a mouse asthma model. Immunobiology 212:647-654.

Magalhães KT, Pereira GVM, Campos CR, Dragone G, Schwan RF (2011).Brazilian kefir: structure, microbial communities and chemical composition. Braz. J. Microbiol. 42:693-702.

Magalhães KT, Pereira GVM, Dias DR, Schwan RF (2010).Microbial communities and chemical changes during fermentation of sugary Brazilian kefir. World J. Microbiol. Biotechnol. 33:1-10..

Medrano M, Pérez PF, Abraham AG (2008). Kefiran antagonizes cytopathic effects of *Bacillus cereus* extracellular factors. Int. J. Food Microbiol. 122:1-7.

Puerari C, Magalhães KT, Schwan RF (2012). New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis. Food Res. Int. 48:634-640.

Rodrigues KL, Caputo LRG, Carvalho JCT, Evangelista J, Schneedorf JM (2005). Antimicrobial and healing activity of kefir and kefiran extract. Int. J. Antimicrob. Agents 5:404-408.

Vinderola CG, Duarte J, Thangavel D, Perdigón G, Farnworth E, Matar C (2005). Immunomodulating capacity of kefir. J. Dairy Res. 72:195-202.

Zakharov P, Völker AC, Wyss MT,Haiss F, Calcinaghi N, Zunzunegui C, Buck A (2009). Dynamic laser speckle imaging of cerebral blood flow. Opt. Express 17:13904-13917.

Zdunek A, Adamiak A, Pieczywek PM, Kurenda A (2014). The biospeckle method for the investigation of agricultural crops: A review. Opt. Lasers Eng. 52:276-285.