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Antibacterial activity of different formulations of cheese and whey produced with kefir grains[1]

Atividade antibacteriana de diferentes formulações de queijo e soro produzidos com grãos de kefir

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ABSTRACT - The development of different products that confer health benefits on the population is a challenge for those who work with food. The aim of this study was to elaborate two formulations of kefir cheese (C1 and C2) and whey (W1, W2), and to evaluate their *in situ* antibacterial activity against microorganisms of interest in food. Pasteurized milk, powdered milk and kefir grains were used in preparing the products and their percentage composition was determined. C1, C2, W1 and W2 were contaminated with five different logarithmic fractions (A = 8log to E = 4log CFU/ml) of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 11229), with antibacterial activity assessed over 0, 24, 48 and 72 hours of exposure. The results demonstrated the antibacterial activity of kefir cheese and whey, especially after 24 hours. *Escherichia coli* was the most sensitive of the bacteria, with maximum antibacterial activity seen in the cheese at population densities D and E, and in the whey at densities B, C, D and E after 48 and 72 h, showing that the *in situ* antibacterial activity of foods produced with kefir grains tends to be lower when compared with studies *in vitro*. The greater the nutrient content of the food, the lower the antibacterial activity seen, probably due to the protective action that the nutrients confer on the microorganisms against bacteriocins and the metabolites from fermentation.

Key words: Kefir grains. Dairy products. Antibacterial potential.

RESUMO - O desenvolvimento de produtos diferenciados e que confiram benefícios à saúde da população é um desafio para quem trabalha com alimentos. O objetivo do trabalho foi elaborar duas formulações de queijo (C1 e C2) e soro (W1, W2) de kefir e avaliar a atividade antibacteriana *in loco* dos mesmos, frente a microrganismos de interesse em alimentos. Leite pasteurizado, leite em pó e grãos de kefir foram utilizados para a elaboração dos produtos, sendo determinada a composição centesimal. C1, C2, W1 e W2 foram contaminados com cinco diferentes frações logarítmicas (A = 8 log a E = 4 log UFC/ml) de *Staphylococcus aureus* (ATCC 25923) e *Escherichia coli* (ATCC 11229) e a atividade antibacteriana avaliada ao longo de 0; 24; 48 e 72 h de exposição. Os resultados evidenciaram atividade antibacteriana do queijo e do soro de kefir, principalmente após 24 h. *Escherichia coli* foi a bactéria mais sensível, e a atividade antibacteriana máxima foi observada no queijo, nas densidades populacionais "D" e "E" e no soro nas densidades "B", "C", "D" e "E" após 48 e 72 h, indicando que *in loco* a atividade antibacteriana de alimentos produzidos com grãos de kefir tende a ser menor quando comparada com estudos que a avaliaram *in vitro*. Quanto maior o aporte de nutrientes no alimento, menor é a atividade antibacteriana observada, provavelmente em função da ação protetora que os nutrientes conferem aos microrganismos frente às bacteriocinas e aos metabólitos oriundos da fermentação.

Palavras-chave: Grãos de kefir. Derivados lácteos. Potencial antibacteriano.

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INTRODUCTION

Various foods have made considerable progress in the market, and in the area of dairy products, cheese is one of the most versatile. Produced throughout the world, it has a diversity of flavors, textures and shapes, is pleasing to the palate of many people and suitable for any age group. Whey, a byproduct of cheesemaking, has also been gaining prominence, given the volume produced and its nutritional composition; it is widely used for the manufacture of products such as dairy beverages and ricotta (MAGALHÃES *et al*., 2011; PAGNO *et al*., 2009; PERRY, 2004).

The cheese production process requires the coagulation of milk, which can be achieved through the physical action of rennet, specific enzymes, specific bacteria or organic acid (singly or combined), all of a suitable quality to be used in food. The microbial coagulation of milk has been replacing rennet in many processes, and confers physicochemical and sensory characteristics that are peculiar to the prepared cheeses (BRASIL, 1996; PERRY, 2004; SAAD; CRUZ; FARIA, 2011).

Kefir grains consist of a symbiotic association of yeasts, lactic acid bacteria and acetic bacteria wrapped in a gelatinous matrix, referred to as 'kefiran', which serves as sustenance for the different constituents of the grains. These grains multiply and double in weight when frequently transferred to milk and, even when handled under artisanal conditions, maintain their structural characteristics and appearance (MAGALHÃES *et al*., 2010; RIMADA; ABRAHAM, 2006; WESCHENFELDER *et al*., 2011).

Numerous microorganisms have been identified in kefir grains, including probiotic microorganisms. These microorganisms have beneficial effects on health, such as improved intestinal flora balance and mucosal defense, relief of the symptoms related to lactose intolerance, stimulation of the immune system, relief of constipation, antioxidant potential and antibacterial activity (DIAS *et al*., 2016; MAGALHÃES *et al.*, 2011; MOREIRA *et al.*, 2008; PLESSAS *et al*., 2007; SHAH, 2007; WESCHENFELDER; WIEST; CARVALHO, 2009).

The development of different products prepared from kefir grains is of great importance, since the relationship between food and health is increasingly discussed in a context where consumers seek healthier foods that do not present health risks; this is seen as a challenge to the food industry. Fresh and high-moisture cheeses produced from kefir grains, with high water activity, pH range close to neutral, low salt concentration, lack of preservatives and measured lipid concentration, can be an excellent environment for the growth and multiplication of microorganisms with probiotic potential (BURITI; CARDARELLI; SAAD, 2007; DIAS; LOBATO; VERRUMA-BERNARDI, 2009; LONDERO *et al.*, 2012; SAAD; CRUZ; FARIA, 2011; SANTOS *et al.*, 2012; SOARES *et al*., 2011; WESCHENFELDER *et al*., 2011). In view of the above, the aim of this study was to prepare two formulations of cheese and whey through microbial coagulation of milk using kefir grains, and to evaluate the *in situ* antibacterial activity of the cheese and whey against *Staphylococcus aureus* and *Escherichia coli*.

MATERIAL AND METHODS

The experiments were developed in the laboratories for bromatology and food hygiene of the Institute of Food Science and Technology (ICTA) of the Federal University of Rio Grande do Sul (UFRGS), Brazil.

I - Production and evaluation of the percentage composition of the cheese and whey

Two cheese formulations (referred to as C1 and C2) were prepared from the microbial coagulation of milk using kefir grains (obtained from the Food Hygiene Laboratory of UFRGS). C1 was produced with kefir grains and standard commercial pasteurized milk (purchased at the supermarket). Fifty grams of kefir grains were weighed and added to 500 g of pasteurized milk in a sterilized glass container (ratio of 1:10), incubated in an aerobic medium in a BOD Incubation Chamber (model SP-500, SPLABOR) for 24 hours at 25 °C ± 2 °C and subsequently maintained at 5 °C ± 2 °C for a further 24 hours to obtain the coagulated mass (SANTOS *et al*., 2012; WESCHENFELDER *et al.*, 2011). Formulation C2 was produced under the same conditions and with the same ingredients as formulation C1, except that 12% powdered skimmed-milk (purchased at the supermarket) was added to the substrate (pasteurized milk) prior to fermentation, increasing the nutrient content of the raw material to be fermented.

After coagulation, the curd was broken and the kefir grains removed with the aid of a sterilized 12-mesh stainless steel sieve. The kefir grains retained in the sieve were again inoculated into another aliquot of the substrate (milk), repeating the above steps of the experiment. The whey was left to drain for 24 hours at 5 °C ± 2 °C using a glass funnel and paper filter (Brigitta). The cheese (C1 and C2) and whey (W1 and W2) formulations were stored in glass containers, identified, maintained in a BOD Incubation Chamber (model SP-500, SPLABOR) at 5 ºC ± 2 ºC and then sent for physicochemical and microbiological analysis.

The percentage composition was determined for the pasteurized and powdered milk used as raw material in the two formulations of kefir cheese (C1 and C2) and whey (W1 and W2). Evaluation of the lipid fraction was by ether extraction in a Soxhlet extractor, crude protein by the Kjeldahl method, moisture by vacuum oven desiccation (85 ºC), fixed mineral residue or ashes by incineration in a muffle furnace at 550 ºC, and the value for total carbohydrates determined by difference. The pH of the dairy products was also evaluated (BRAZIL, 2006).

II - Evaluation of the antibacterial activity of cheese and whey produced with kefir grains against *Staphylococcus aureus* and *Escherichia coli*

The kefir cheese and whey were analyzed for the presence of *Staphylococcus aureus* and *Escherichia coli* to verify the initial microbiological quality of the product, as per the technique in Normative Instruction No. 62 of August 2003 (BRAZIL, 2003). Standard strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 11229) were used to evaluate the *in situ* antibacterial activity of kefir cheese and whey. The bacteria were reactivated in BHI (Brain Heart Infusion, OXOID) at 37 ºC for 24 hours in an aerobic medium (AVANCINI; WIEST, 2008).

Different population densities of *Staphylococcus aureus* (ATCC 25923) referred to in the study as A, B, C, D and E (where A> B> C> D> E) were incorporated into the formulations of kefir cheese and whey at the proportion of 20:180, where A = 8log to E = 4log CFU/g or mL. The dairy products were kept in a BOD incubation chamber at 5 °C ± 2 °C throughout the experiment, and analyzed after 0 (initial population density), 24, 48 and 72 hours confrontation. Evaluation of *in situ* antibacterial activity was based on a count of typical colonies in a Baird-Parker Agar selective culture medium, as per the technique adapted from Normative Instruction No. 62 of August 2003 (BRAZIL, 2003). The above steps were also carried out on *Escherichia coli* (ATCC 11229), and differed only in relation to the selective culture medium used (Chromocult Agar).

The results were submitted to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) using the SAS 9.0 software. The data were used to differentiate the formulations of kefir cheese and whey, and to verify *in situ* antibacterial activity against the microorganisms of interest in tested foods.

RESULTS AND DISCUSSION

I - Percentage composition of the two formulations of cheese and whey

The percentage composition of the raw material used to make the cheese and whey (Table 1) showed that the values agreed with the reference literature (ORDONEZ, 2005).

The addition of powdered skimmed milk to the C2 formulation gave an increase in the levels of protein, carbohydrates and ash (Table 1). The pH of C1 and C2 was 4.8 and 5.0 respectively. A study developed by Weschenfelder *et al*. (2011) found similar values in leban cheese produced with kefir, the main difference being that leban cheese had no carbohydrates in its composition and was more acidic. These differences can be explained by the length of time the kefir grains were in contact with the milk, which was greater (168 hours) in the above study.

When considering the legislation of cheeses, C1 and C2 can be classified as 'fresh cheese', since they are ready for consumption soon after manufacture, and ripening is not necessary. C1 is a semi-fat cheese and C2 is a low-fat cheese, with 40% and 21.46% lipids in the dried extract respectively. For moisture, C1 and C2 receive a classification of 'very high moisture' cheeses, since the values found were higher than 55% (BRAZIL, 1996).

Table 1 - Percentage composition of the pasteurized milk, powdered milk and two formulations of kefir cheese and whey

	Moisture	Proteins	Lipids	Carbohydrates	Ash
Milk (pasteurized)	87.10 ± 0.05 a	3.75 ± 0.08 a	3.00 ± 0.15 a	5.20 ± 0.11 a	0.95 ± 0.31 a
Milk (powdered)	1.95 ± 0.31 b	32.95 ± 0.49 b	0.71 ± 0.10 b	55.77 ± 0.82 b	8.62 ± 0.49 b
Kefir cheese (C1)	74.13 ± 0.13 b	9.10 ± 0.08 b	10.35 ± 0.59 a	5.58 ± 0.77 a	0.59 ± 0.04 b
Kefir cheese (C2)	77.73 ± 0.64 a	9.69 ± 0.32 a	4.78 ± 0.06 b	6.48 ± 0.99 a	1.32 ± 0.05 a
Kefir whey (W1)	93.7 ± 0.04 a	0.62 ± 0.02 b	0.3 ± 0.01 a	4.88 ± 0.04 b	0.5 ± 0.07 b
Kefir whey (W2)	87.35 ± 0.18 b	1.15 ± 0.04 a	0.31 ±0.02 a	9.87 ± 0.05 a	1.31 ± 0.1 a

Similar lower case letters in the same column (considering each type of food separately) indicate that there was no statistically significant difference ($p<0.05$) by Tukey's test

The percentage composition of the whey (Table 1) showed that the whey from the kefir cheese (W1 and W2) is similar to the whey from the manufacture of other types of cheeses, and basically consists of water (more than 85%), with 20% milk protein, lactose and mineral elements (SOARES *et al.*, 2011). The pH of 4.0 and 4.2 found in W1 and W2 respectively, classifies the whey as 'acid whey' (BRAZIL, 1996). The study developed by Magalhães *et al*. (2011) pointed out that cheese whey could be used as raw material for the manufacture of kefir derivatives, since similarities are found when comparing milk kefir with whey kefir. Thus, the kefir whey obtained in the present study could be fermented by kefir grains, resulting in a new product.

Determining the percentage composition of the produced kefir cheese and whey is fundamental to their inclusion in the diet of the population and in analysing *in situ* antibacterial activity, since the behavior of the microorganisms in the food is directly related to the intrinsic characteristics of the product.

II - Evaluation of the antibacterial activity of cheese and whey produced with kefir grains against *Staphylococcus aureus* and *Escherichia coli*

Neither the kefir cheese nor the whey showed any contamination with *Staphylococcus aureus* or *Escherichia coli*, proving the initial quality of the prepared foods.

The results for *in situ* antibacterial activity against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 11229) evaluated in the kefir cheese, showed that the kefir cheese, irrespective of formulation (C1 or C2), contains in its composition substances with antibacterial potential, since the concentration of confronted microorganisms decreases, irrespective of the concentration being tested (Tables 2 and 3). In relation to time, greater antibacterial activity was seen after 24 hours confrontation. Rodrigues, Carvalho and Schneedorf (2005) obtained a similar result in a study of anti-inflammatory action, where they found a broad effect against *Staphylococcus aureus* from kefir produced with milk.

It can be seen that *Escherichia coli* (ATCC 11229) was more sensitive than *Staphylococcus aureus* (ATCC 25923), as maximum antibacterial activity or total pathogen destruction was obtained at population densities D and E after 48 and 72 hours confrontation (Table 3). *Staphylococcus aureus* can easily be isolated from dairy products due to bovine mastitis, whereas *Escherichia coli* is more tied to hygienic and sanitary aspects, which may explain the greater resistance of the former in the cheese (ACHA; SZYFRES, 2003).

Results of the antibacterial activity of the whey are shown in Tables 4 and 5. The more acidic pH of the W1 formulation may have influenced the greater antibacterial activity seen when compared to W2 (Table 5), however it was not responsible for inhibiting the bacteria. Weschenfelder, Wiest and Carvalho (2009) found the maximum antibacterial activity in kefir whey (pH 5.8) against *Escherichia coli* in an *in vitro* study. Santos *et al*. (2013) also found antibacterial activity in kefir with a pH of 6.05 against different pathogens, showing that substances with antibacterial potential originating from kefir grains and resulting from the fermentation process, such as bacteriocins, were present in the foods under evaluation.

The percentage composition of the C2 and W2 formulations may also have influenced antibacterial activity, since there are more nutrients in these formulations when compared to C1 and W1 respectively (Table 1). The composition of the food may have had a 'protective action' for the microorganisms under study.

Table 2 - Antibacterial activity of kefir cheese against *Staphylococcus aureus* (ATCC 25923), expressed as log (CFU concentration/g)

Kefir cheese	Time (hours)	Population density of *Staphylococcus aureus* (ATCC 25923) (A > B > C > D > E)				
		A	B	C	D	E
C1	0	8.59 a	7.59 a	6.59 a	5.59 a	4.59 a
	24	7.18 b	5.66 b	4.71 b	4.29 b	3.25 c
	48	6.51 c	5.70 b	4.66 b	3.62 c	3.65 b
	72	6.35 c	5.17 c	4.30 c	3.33 c	3.12 c
C2	0	8.59 a	7.59 a	6.59 a	5.59 a	4.59 a
	24	6.24 b	5.66 b	4.38 b	3.61 b	2.58 b
	48	6.39 b	5.65 b	4.54 b	3.73 b	2.75 b
	72	6.06 b	5.42 b	4.49 b	3.67 b	2.06 c

Similar lower case letters in the same column (considering each kefir cheese separately) indicate that there was no statistically significant difference ($p<0.05$) by Tukey's test

Table 3 - Antibacterial activity of kefir cheese against *Escherichia coli* (ATCC 11229), expressed as log (CFU concentration/g)

Kefir cheese	Time (hours)	Population density of *Escherichia coli* (ATCC 11229) (A > B > C > D > E)				
		A	B	C	D	E
C1	0	8.62 a	7.62 a	6.62 a	5.62 a	4.62 a
	24	5.30 b	4.23 b	4.21 b	3.40 b	2.30 b
	48	5.40 b	4.31 b	3.81 bc	2.50 c	*
	72	5.20 b	4.24 b	3.59 c	*	*
C2	0	8.62 a	7.62 a	6.62 a	5.62 a	4.62 a
	24	6.45 b	5.31 b	4.27 b	3.54 b	2.55 b
	48	5.63 c	5.40 b	3.64 c	2.46 c	2.03 c
	72	5.62 c	4.58 c	3.20 c	2.34 c	*

*Maximum antibacterial activity/no bacterial growth; Similar lower case letters in the same column (considering each kefir cheese separately) indicate that there was no significant statistical difference ($p<0.05$) by Tukey's test

Table 4 - Antibacterial activity of kefir whey against *Staphylococcus aureus* (ATCC 25923), expressed as log (CFU concentration/mL)

Kefir whey	Time (hours)	Population density of *Staphylococcus aureus* (ATCC 25923) (A > B > C > D > E)				
		A	B	C	D	E
W1	0	8.59 a	7.59 a	6.59 a	5.59 a	4.59 a
	24	6.82 b	5.58 b	4.74 b	3.61 b	3.26 b
	48	5.65 c	4.84 c	3.83 c	3.04 c	2.03 c
	72	5.49 c	4.64 c	2.33 d	2.65 c	2.05 c
W2	0	8.59 a	7.59 a	6.59 a	5.59 a	4.59 a
	24	6.28 b	5.62 b	4.66 b	3.47 b	2.42 b
	48	5.69 c	4.73 c	4.35 c	3.42 b	2.54 b
	72	5.68 c	4.70 c	3.24 d	3.18 b	2.04 c

Similar lower case letters in the same column (considering each whey separately) indicate that there was no statistically significant difference ($p<0.05$) by Tukey's test

Table 5 - Antibacterial activity of kefir whey against *Escherichia coli* (ATCC 11229), expressed as log (CFU concentration/mL)

Kefir whey	Time (hours)	Different population densities of *Escherichia coli* (ATCC 11229) (A > B > C > D > E)				
		A	B	C	D	E
W1	0	8.62 a	7.62 a	6.62 a	5.62 a	4.62 a
	24	4.35 b	3.44 b	2.45 b	2.13 b	2.20 b
	48	3.30 c	2.34 c	*	*	*
	72	2.18 d	*	*	*	*
W2	0	8.62 a	7.62 a	6.62 a	5.62 a	4.62 a
	24	6.60 b	5.43 b	4.50 b	3.29 b	2.09 b
	48	5.12 c	4.70 c	3.11 c	2.18 c	*
	72	4.57 c	4.03 d	2.09 d	2.03 c	*

* Maximum antibacterial activity/no bacterial growth; Similar lower case letters in the same column (considering each whey separately) indicate that there was no statistically significant difference ($p<0.05$) by Tukey's test

As in the cheese, the antibacterial activity of the whey was higher for *Escherichia coli*, with maximum antibacterial activity seen at different tested population densities (B, C, D, E), especially for the W1 formulation. However, it is worth mentioning that these results were reached only after 48 hours confrontation, which does not necessarily guarantee food safety, especially in cases where the microorganism is capable of producing toxins. Dias *et al*. (2012), in an *in situ* study with kefir produced from contaminated milk, also found that microorganisms such as *Escherichia coli* and *Staphylococcus aureus*, survive in fermented milk for up to 72 hours confrontation.

The results demonstrate that dairy products produced with kefir grains show antibacterial activity and, compared with other studies, it can be seen that the intensity of this activity is associated with several elements, such as the microbial constitution of the kefir grains (which harbor different types of microorganisms, including probiotics), the quality and the percentage composition of the raw material, the chemical processes involved in fermentation, the handling, packaging and storage conditions of the food, as well as the characteristics of the microorganism under evaluation. It is also worth pointing out that the microorganisms tested in this study showed resistance to the low temperatures used in storing the cheese and whey (5 ºC ± 2 ºC), and to the acidic pH of the whey, demonstrating the ability to adapt to the medium when in high concentrations.

CONCLUSION

When tested *in situ* the kefir derivatives demonstrated antibacterial activity, the greatest activity being against *Escherichia coli* (ATCC 11229). However, the antibacterial activity only reached maximum after 48 and 72 hours confrontation of C1, C2, W1 and W2 with smaller population densities of the microorganism, reinforcing the importance of hygiene when preparing these foods, and highlighting the capacity of *Escherichia coli* and *Staphylococcus aureus* to survive in the product. The results from determining the percentage composition serve as the basis for the development of kefir derivatives and their inclusion in diets, and show that the greater the nutrient content of the food, the lower the antibacterial activity seen *in situ*.

REFERENCES

ACHA, P. N.; SZYFRES, B. **Zoonosis and communicable diseases common to men and animals**: bacteriosis and mycosis. 3. ed. Washington: World Health Organization. Cientifical and Technical Publication, 2003. 398 p.

AVANCINI, C. A. M.; WIEST, J. M. Atividade desinfectante do decocto de Hypericum caprifoliatum Cham. Eshclecht. Guttiferae ("escadinha/sinapismo") frente a diferentes doses infectantes de Staphylococcus aureus (agente infeccioso em mastite bovina). **Revista Brasileira de Plantas Medicinais**, v. 10, p. 64-69, 2008.

BURITI, F. C. A.; CARDARELLI, H. R.; SAAD, S. M. I. Biopreservation by *Lactobacillus paracasei* in coculture with *Streptococcus thermophilus* in potentially probiotic and symbiotic fresh cream cheeses. **Journal of Food Protection**, v. 70, p. 228-235, 2007.

BRASIL. Ministério da Agricultura, do Abastecimento e da Reforma Agrária. Portaria nº 146, de 7 de março de 1996. Regulamento Técnico de Identidade e Qualidade de Queijos, Regulamento Técnico de Identidade e Qualidade de Leite em Pó, Regulamento Técnico de Identidade e Qualidade de leite UAT (UHT). **Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, 11 mar. 1996.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução normativa nº 62, de 26 de agosto de 2003. Oficializa os métodos analíticos oficiais para análises microbiológicas para controle de produtos de origem animal e água. **Diário Oficial da União**, 26 ago. 2003.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução normativa nº 68, de 12 de dezembro de 2006. Métodos analíticos oficiais físico-químicos para controle de leite e produtos lácteos. **Diário Oficial [da] República Federativa do Brasil**, 14 dez. 2006.

DIAS, P. A. *et al*. Survival of pathogenic microorganisms in kefir. **Revista do Instituto Adolfo Lutz**, v. 71, p. 182-186, 2012.

DIAS, S. S; LOBATO, V; VERRUMA-BERNARDI, M. R. Metodologias para identificar adulteração em queijos produzidos com leite de diferentes espécies de animais. **Revista Instituto Adolfo Lutz**, v. 68, p. 327-333, 2009.

DIAS, P. A. *et al*. *Antimicrobial properties of kefir*. **Arquivos do Instituto Biológico**, v. 83, p. 1-5, 2016.

LONDERO, A. *et al*. Kefir grains as a starter for whey fermentation at different temperatures: chemical and microbiological characterization. **Journal of Dairy Research**, v. 79, p. 262-271, 2012.

MAGALHÃES, K. T. *et al*. Production of fermented cheese whey-based beverage using kefir grains as starter culture: evaluation of morphological and microbial variations. **Bioresource Technology**, v. 101, p. 8843-8850, 2010.

MAGALHÃES, K. T. *et al*. Comparative study of the biochemical changes and volatile compounds during the production of novel whey-based kefir beverages and traditional milk kefir. **Food Chemistry**, v. 126, p. 249-253, 2011.

MOREIRA, M. E. C. *et al*. Atividade antiinflamatória de carboidrato produzido por fermentação aquosa de grãos de quefir. **Química Nova**, v. 31, p. 1738-1742, 2008.

ORDÓNEZ, J. A. **Tecnologia de alimentos de origem animal**. Porto Alegre: Artmed, 2005. v. 2, 280 p.

PAGNO, C. H. *et al*. Obtenção de concentrados protéicos de soro de leite e caracterização de suas propriedades funcionais tecnológicas. **Alimentos e Nutrição**, v. 20, p. 231-239, 2009.

PERRY, K. S. P. Queijos: aspectos químicos, bioquímicos e microbiológicos. **Química Nova**, v. 27, p. 293-300, 2004.

PLESSAS, S. *et al*. Immobilization of *kefir* and *Lactobacillus casei* on brewery spent grains for use in sourdough wheat bread making. **Food Chemistry**, v. 105, p. 187-194, 2007.

RIMADA, P. S.; ABRAHAM, A. G. Kefiran improves rheological properties of glucono-ô-lactone induced skim ilk gels. **International Dairy Journal**, v. 16, p. 33-39, 2006.

RODRIGUES, K. L.; CARVALHO, J. C. T.; SCHNEEDORF, J. M. Anti-inflammatory properties of kefir and its polysaccharide extract. **Inflammopharmacology**, v. 13, p. 485-492, 2005.

SAAD, S. M. I; CRUZ, A. G.; FARIA, J. A. F. **Probióticos e prebióticos em alimentos**: fundamentos e aplicações tecnológicas. 1. ed. São Paulo: Livraria Varela, 2011. 669 p.

SANTOS, J. P. V. *et al*. Evaluation of antagonistic activity of milk fermented with kefir grains of different origins. **Brazilian Archives of Biology and Technology**, v. 56, p. 823-827, 2013.

SANTOS, T. S. S. *et al*. "Petit suisse" cheese from *kefir*: an alternative dessert with microorganisms of probiotic activity. **Ciência e Tecnologia de Alimentos**, v. 32, p. 485-491, 2012.

SHAH, N. P. Functional cultures and health benefits. **International Dairy Journal**, v. 17, p. 1262-1277, 2007.

SOARES, D. S. *et al*. Aproveitamento de soro de queijo para produção de iogurte probiótico. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 63, p. 996-1002, 2011.

WESCHENFELDER, S.; WIEST, J. M.; CARVALHO, H. H. C. Atividade anti-*Escherichia coli* em kefir e soro de kefir tradicionais. **Revista Instituto de Laticínios Cândido Tostes**, v. 64, p. 48-55, 2009.

WESCHENFELDER, S. *et al*. Caracterização físico-química e sensorial de kefir tradicional e derivados. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 63, p.473-480, 2011.