QUARK CHEESE PRODUCED WITH KEFIR AND AGAVE INULIN

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ABSTRACT: Quark cheese is fresh cheese obtained by the coagulation of milk through the action mainly of lactic bacteria, resulting in fresh-flavor cheese with high acidity. A specific starter culture is used for its commercial production. However, in this work, this culture was replaced by kefir, a symbiotic system of lactic acid bacteria and yeasts, which is considered a probiotic product. Agave inulin is a soluble fiber regarded as prebiotic with the ability to improve the balance of the intestinal flora. The purpose of this work was to develop Quark cheese using kefir as a starter culture, with supplementation by agave inulin. Cheese was produced using 24-h fermentation at 25 ºC followed by drainage of the whey, with the addition of 3% inulin to the supplemented cheese. After whey drainage, the cheese was kept in plastic pots under refrigeration until analysis. Microbiological, pH and acidity parameters were analysed at 1, 7 and 14 days after manufacture. The results of the physical-chemical analyses remained close to those found in literature. The resulting cheese presented high counts of *Lactobacilli* indicating their probiotic potential. The addition of agave inulin decreased the amount of those microorganisms; however, they remained in high counts. It can be verified that the use of kefir in the production of Quark cheese is feasible, mainly in artisanal productions since it is usually inexpensive and easy to maintain, and the *Lactobacilli* remained stable during the storage period.
KEY WORDS: Fibers. Functional food. Prebiotics. Probiotics. Symbiotic foods.

QUEIJO QUARK PRODUZIDO COM KEFIR E INULINA DE AGAVE

RESUMO: O queijo Quark é um queijo de massa fresca obtida por coagulação do leite pela ação principalmente de bactérias láticasobtendo-se um queijo de sabor refrescante, em sua produção comercial é empregada uma cultura starter específica para a produção de queijos, no presente trabalho essa cultura foi substituída pelo kefir, um sistema simbiótico de bactérias láticas e leveduras e que é considerado um produto probiótico. A inulina de agave é uma fibra solúvel considerada prebiótica com capacidade de melhorar o equilíbrio da flora intestinal. O objetivo desse trabalho foi desenvolver queijo Quark empregando kefir como cultura *starter* e suplementado com inulina de agave. Os queijos foram produzidos por fermentação durante 24h/25ºC seguido por drenagem do soro, no queijo suplementado com inulina foi adicionada 3%, após a dessoragem, os queijos foram mantidos em potes plásticos sob temperatura de refrigeração até o momento das análises, sendo que as microbiológicas, acidez e pH foram realizadas em 1, 7 e 14 dias após a fabricação. Os resultados das análises físico-químicas mantiveram-se próximos aqueles encontrados na literatura. Os queijos obtidos apresentaram altas contagens de *Lactobacilli* indicando potencial probiótico, a adição de inulina de agave diminuiu o número destes microrganismos, mesmo assim mantiveram-se em altas contagens. Pode-se constatar que o uso de kefir na produção de queijo Quark é viável, principalmente em produções artesanais uma vez que ele normalmente não possui custo e é de fácil manutenção e os *Lactobacilli*mantiveram-se estáveis durante o período de armazenamento.
PALAVRAS-CHAVE: Alimentos funcionais. Alimentos simbióticos. Fibras. Prebióticos. Probióticos.

QUESO QUARK PRODUCIDO CON KEFIR E INULINA DE AGAVE

RESUMEN: El queso Quark es un queso de masa fresca obtenido por coagulación de la leche y acción principalmente de bacterias lácticas, obteniendo un queso de sabor refrescante. En su producción comercial se utiliza un cultivo iniciador específico para producción de quesos, en el presente trabajo esa cultura fue reemplazada por kéfir, un sistema simbiótico de bacterias del ácido láctico y levaduras, que se considera un producto probiótico. La inulina de agave es una fibra soluble considerada prebiótica con la capacidad de mejorar el equilibrio de la flora intestinal. El objetivo de esa investigación ha sido desarrollar queso Quark, usando kéfir como cultivo inicial y complementado con inulina de agave. Los quesos se produjeron por fermentación durante 24h / 25ºC seguido de drenaje del suero, en el queso suplementado con inulina se añadió 3%, después del drenaje del suero y se mantuvieron en macetas de plástico a temperatura de refrigeración hasta el momento del análisis, siendo que las microbiológicas, el pH y la acidez se realizaron a los 1, 7 y 14 días después de la fabricación. Los resultados de los análisis fisicoquímicos se mantuvieron cerca de los encontrados en la literatura. Los quesos mostraron altos conteos de *Lactobacilli* indicando su potencial probiótico, la adición de inulina de agave disminuyó el número de estos mi-

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croorganismos, sin embargo, permanecieron en conteos altos. Se puede verificar que el uso de kéfir en la producción de queso Quark es factible, principalmente en las producciones artesanales, ya que por lo general es económico y de fácil manutención. Los lactobacilos se han mantenido estables durante el período de almacenamiento.
PALABRAS CLAVE: Alimentos funcionales. Alimentos simbióticos. Fibras. Prebióticos. Probióticos.

Introduction

The current internationally accepted definition for probiotics is that they are living microorganisms when administered in adequate quantities give health benefits to the host (SAAD, 2006; HAULY et al., 2005), theBrazilian Resolution RDC no. 2 /2002 defines probiotics as living microorganisms capable of improving the intestinal microbial balance producing beneficial effects on the health of the individual (BRASIL, 2002).

Many benefits of probioticshave been described among them: reestablishment of the balanced intestinal microbiota; resistance to colonization and / or prevention of diarrhea; systemic reduction of serum cholesterol, reduction of fecal enzymes and mutagenic compounds that can induce tumors; improvement in lactose metabolism and reduction of lactose intolerance; improvement in immune system response; better absorption of calcium and vitamin synthesis and pre-digestion of proteins (ZIEMER; GIBSON, 1998).

Kefir is a product with probiotic action that contains *Lactobacillus acidophillus* and *Bifidumbacterium bifidus* and lactic acid bacteria and yeasts, the term is also used to designate a fermented drink, milk-based or water-based both produced from the inoculation of grains of kefir, which it is believed to be originate in the Caucasus region, obtaining a viscous drink with a creamy consistency and a gentle refreshing yeast aroma and a natural effervescence due to the formation of carbon dioxide gas and may have between 0.08 and 2.0 % alcohol (ARSLAN, 2014; MONTANUCCI et al., 2012; POGACIC et al., 2013; ROCHA et al., 2014).

Pogacic et al. (2013) stated that independently of the processing method and different cultures the Codex Alimentary Standard describes that the typical microbiological composition of Kefir must contain *L. kefiri* as well as the species *Leuconostoc, Lactococcus* and *Acetobacter* as well as yeasts that ferment lactose, as *Kluyveromyces marxianus* and those that do not ferment lactose such as *Saccharomyces cerevisae* and *Saccharomyces exiguos.*

Kefir has hypocholesterolemic effects, provides modulation of the intestinal microbiota which improves the immune system (immunomodulation), antimicrobial effect, reduction of cancer risk, control of diarrhea, glycemic control in type II diabetics and anti-allergenic effects (BOURRIE; WILLING; COTTER, 2016; SOLDATI, 2006; YONG et al., 2006;).

Inkefir grains can be found an exopolysaccharide known as kefiran, which is a carbohydrate that has bioactivity and it is produced by *Lactobacillus faciens* which acts as an encapsulating agent of the microbial strains present in the grains. It is partially soluble in water, it has equal amounts of galactose and glucose and its several beneficial effects to the health are being reported (BOURRIE; WILLING; COTTER, 2016; MOREIRA et al., 2009; PRADO et al., 2015). John and Deeseenthum (2015) furthermore stated that the produced kefiran has prebiotic activity.

Prebiotics are nondigestible carbohydrates that benefit the host by selectively stimulating the growth and / or activity of one or a limited number of bacterial species in the colon, the prebiotic effects related to increasing the number of *bifidobacteria* lead to stimulation of the immune system, production of B-complex vitamins, inhibition of pathogens, reduction of blood cholesterol levels and restoration of the normal microbiota, the term symbiotic is used designate products containing association of probiotics and prebiotics (HAULY, 2005; SAAD, 2006).

Agave inulin has health benefits and is considered a prebiotic with high solubility and capacity of improving the balance of the intestinal flora and stimulating the growth of beneficial *bifidobacteria*, as well as it has the ability to control of body weight and improve the absorption of calcium, it is obtained by thermal or enzymatic hydrolysis of the agave juice, the products obtained are fructans, called commercially by inulin and it can be sold in liquid or white powder forms (BOUAZIZ; RASSAOUI; BESBE, 2014; VASQUÉZ; HERRERA; MENDONZA, 2015).

In the *Agave americana*, inulin has been identified as the main carbohydrate reserve, in the stems of *Agave veracruz* and *Agave tequilana* are stored a complex blend of fructooligosaccharides, inulin and branched fructose and fructans which are substances associated with a number of benefits in health care, including their beneficial effect as prebiotic, strengthening of defense mechanisms, improvement of lipid metabolism and prevention of certain diseases (HANNAH et al., 2014; ULLOA et al., 2010).

Quark type cheese is the product obtained from the fermentation of whole or skim milk followed by whey drainage. It is classified as a fresh cheese, acidified, not ripened, it not have a long shelf life due its high water content then it needs be kept under refrigeration, it is a cheese originated in Germany and can be used as cream cheese or in general cooking, it can also be consumed with sugar and fruits or jams adition, when it is known as Petit Suisse (SCHULZ--COLLINS; SENGE, 2004).

In Europe where it has great commercial importance it is processed with advanced technology. The consumption of Quark cheese and its similar Petit-Suisse occurs with or without the addition of others ingredients as fruits, jams or balanced blends of condiments. It may be aerated by mixing whipped cream, sugar, flavorings and stabilizers. The growth or incorporation of probiotic cultures into the Quark cheese seems an interesting alternative for producing probiotic cheese. (YUHARA et al., 2014).

The purpose of this work was elaborate a symbiotic Quark type cheese employing kefir as culture starter and agave inulin as prebiotic and also to determine its physicochemical and microbiological characteristics.

Material and Methods

Starter culture of kefir

The kefir starter culture was obtained from the Laboratory of Food Microbiology of the Department of Food Science and Technology the Londrina State University (UEL) and was cultivated in integral UHT milk at 20ºC / 48h.

Manufacturing of cheeses

Non-standard whole pasteurized milk (HTST) was used for cheese productions. The milk was heated to 35 °C followed by the addition 5% of the kefir starter culture and 10% of the coagulant dose normally used in cheese production (Chr. Hansen).This mixture was maintained at 25º C/48h for fermentation and milk coagulation. After this period the obtained curd was drained for 12 h under refrigeration in previously sterilized fabric bags, the mass was took out of the bags and 1.0% salt was added. The cheese was divided in 2 treatments, the first one without addition of agave inulin (T1) and in the second one with addition of 3% of agave inulin (T2). Cheeses were packed in plastic pots and kept at 8-10ºC until realization of the analysis.

Chemical analysis

Total solids (TS), proteins, fat, titratable acidity and pH according to BRASIL (2003). The pH and titratable acidity of the cheeses were determined on the day of manufacture and after 7 and 14 days under refrigeration temperature storage.

The fat content in the total solids (FTS) and solids--not-fat (SNF) were obtained by calculating the percentage considering the butterfat contents and the total solids obtained in the cheeses.

The yield of cheese processing was calculated by the ratio between the mass of cheese obtained by the volume of milk used in processing and expressed in kg/L.

Microbiological analysis

In cheeses were analysed molds and yeasts in Potato Dextrose Agar acidified with tartaric acid (10%), positive coagulase *Staphylococcus* in Baird Parker agar added of yolk emulsion, termotolerant coliforms in EC Broth and *Lactobacilli* in MRS agar according to BRASIL (2003).

Statistical Analysis

Samples characterizationwereexpressed by mean ± standard deviation and the t-student test was used to compare the statistically significant differences between the means of the samples, with the significance level of 5%. The software BioEstat was used to statistical analysis (AYRES et al., 2007).

Results and Discussion

No standardized milk was used consequently it gavea high value of butterfat in cheeses as shown in table 1. According Brazilian legislation, this milk is classified as whole pasteurized milk that must have at least 3.0 % of butterfat, in addition the milk must have values between 0.14 to 0.18% of lactic acid and a minimum of 8.4% of solids-not-fat (SNF), therefore the values found for the milk used follow current legislation. (BRASIL, 2006).

Table 1: Chemical properties of the milk used in manufacturing of the cheeses

Component	
Proteins (Nx6,38)	2.51% ±0.393
Fat	3.70% ±0.058
TS	12.16% ±0.174
FNS	8.46%±0.175
Ashes	0.43% ±0.047
Carbohydrates*	5.45% ±0.463
Titratable acidity (in lactic acid)	0.16% ±0.006
pH	6.90±0.002

*Calculated by difference of 100%. TS - Total solids. SNF- solids--not-fat

The yield of the cheeses production was 5.30 L/kg, which represents loss of 80.5% of the whey mass. Rocha et al. (2014) found losses of 75.0% in the manufacture of Labneh with kefir, a concentrated yogurt with the same technology of Quark cheese production, but in industrial modern scale techniques of centrifugation and ultrafiltration are employed which minimize losses of proteins of the product.

There is notlegislation cheese in Brazil for Quark cheese, on the other hand,there is for *Petit Suisse*, a cheese obtained from acid curd and manufactured with the same Quark cheese technology. *Petit Suisse* cheese is defined as fresh cheese obtained by milk coagulation with or without rennet and / or enzymes addition, it may be added from other food substances, as it is a very high-water content cheese it must be consumed fresh, it must have no less of 55% of water content e no less than 6% of dairy proteins. (BRASIL, 2001).

The kefir culture has gained researchers'attention withregarding to cheese manufacturing due to its potential effect onquality, health, and safety properties of the product, kefir grainsor kefir has been used as a starter in many types of cheese (GONKU; ALPKENT, 2005; MEI; GAO; LI, 2016).

Table 2: Chemical properties of the cheeses obtained in the treatments without and with the addition of agave inulin.

	T1	T2
Protein (N x 6,38)	14.26 ±0.469[a]	13.99 ±0.208[a]
Fat	11.46 ±0.361[a]	11.12 ±0.794[a]
TS	34.08 ±0.289[a]	36.95 ±0.626[b]
FTS	33.63 ±1.014[a]	30.09 ±1.557[b]
Ashes	2.48 ±0.759[a]	2.09 ±0.065[a]
Carbohydrates*	5.88 ± 1.967[a]	9.75 ±2.132[b]

* Calculated by difference of 100%. T1 - without addition of agave inulin ; T2 - with addition of 3.0% of agave inulin . TS - Total solids. FTS - Fat content in the total solids.
Values with different letters in the same line are significantly different ($p < 0.05$).

Significant differences were found between the treatments for TS, FTS and carbohydrate parameters (Table 1), this result was already expected due to the addition of agave inulin to T2, which it raised carbohydrate levels and altered the other parameters. The fat content in the total solids (FTS) is a parameter for cheeses classification as a result cheeses

found in this work can be classified with semi-fat cheeses (25.0% <FTS<44.9%) (BRASIL, 1996).

The agave inulin concentration used was defined based in Brasil (2012) which describes that a product is considered a source of dietary fibers when it presents at least 3% and with a high content of at least 6% of fibers.

The chemical properties of the cheeses are shown in Table 1, they were close to those found by Yuhara et al. (2014), they produced Quark cheese using the probiotics *L. acidophillus* and *L. casei* however they used skim milk in cheese making, so the butterfat content in cheeses was much lower (2.1%) comparing with the present work. Buriti, Cardarelli, Saad (2008) found in probiotic Quark cheese with *Lactobacillus paracasei* and chicory inulin (Raftiline®) values for fat of 7.7% and FTS of 21.3%.

Gonçalves (2009) produced three different types of symbiotic Quarks cheeses with addition of inulin (Raftiline GR) and *Lactobacillus acidophilus* (LA5), *Bifidumbacterium animalis* ssp *lactis* (BB12) and *Lactobacillus delbrueckii* UFV H2b20, in these cheeses were found values of proteins that ranged from 6.01 to 6.22%; fat from 0.60 to 0.78%; TS of 18.89 to 20.16%, these results differ from those found in this study due they were produced with skim milk.

Abd el-Aziz e Darwish (2014) produced cheeses like Quark with different kinds of milk, cow's milk, buffalo and goat and for cow's milk and its cheese the compositions were like those found in this work, these authors explained that make cheese with kefir is easy and contains all the beneficial probiotic microorganisms which kefir is famous for.

Since the first established use, 100s of years ago, the propagation of kefir has been performed by transferring kefir grains from one batch to fresh milk and incubating at ambient temperature. Over this period there has been substantial opportunity for the microbial component of kefir grains to evolve and diverge, resulting in the addition or loss of bacteria and yeasts as well as the addition and loss of genes (BOURRIE; WILLING; COTTER, 2016).

Palatinik et al. (2016) described that introduction of agave fructans into cheeses is important because they are considered soluble fibers from a natural and abundant source, besides being classified with prebiotics then they become an alternative to produce foods with functional properties.

The presence of thermotolerant coliforms, coagulase positive staphylococci and filamentous fungi were not found in cheeses. As shown in Table 3, it might be observed that the *lactobacilli* were able to grow in the cheese in large quantity. This demonstrates the cheese matrix is a suitable medium for the growth of *lactobacilli*, it is likely a part of them is of kefir origin and another part is from the pasteurized milk.

There were not differences in the *lactobacilli* counts during the 14 days of storage, but the addition of agave inulin significantly decreased their counts, but itstill remained high indicating a probable probiotic capacity of the cheeses.

Table 3: *Lactobacilli* and yeasts counts in cheeses without and with the addition of agave inulin (Expressed in log CFU/g).

Days of storage	*Lactobacilli* (log CFU/g)		Yeasts (log CFU/g)	
	T1	T2	T1	T2
1	12.15±0.159aA	11.51±0.102aB	5.54±0.053aA	5.44±0.095aA
7	13.10±0.044aA	11.02±1.646aB	5.63±0.116 aA	4.95±0.161bB
14	12.95±0.850aA	11.05±0.250aB	5.86±0.252 aA	5.87±0.252aA

T1- Without addition of agave inulin; T2 - With the addition of agave inulin.
Values with different lowercase letters in the same column are significantly different ($p < 0.05$).Values with different upper-case letters in the different treatments for the same counts and in the same line are significantly different ($p < 0.05$).

Maruyama et al (2006) found values for probiotic counts lower than that found in this work, which may be due to the use of isolated cultures of *L. acidophillus* and *B. longum*, while in kefir up to 40 different lactic bacteria can be found (DINIZ et al., 2003).

Yeast counts remained almost constant during storage time in both treatments. Awaished et al. (2016) found values like the present work for yeasts and lower values for *Lactobacilli*. The higher values found in the present work might be explained by the 24 hours of fermentation which allowed a greater growth of the lactic bacteria.

Fermentation characteristics of the cheeses are shown in Table 4. The initial pH of the milk was 6.80, the fermentation process produces lactic acid, causing the pH decrease with increasing of titratable acidity, as it can be seen in the table. During storage there were variations in pH and acidity, indicating that there was metabolic activity of the microorganisms present in the kefir even under refrigeration conditions even though their counts remained without significative differences.

Table 4: Changes in pH values and titratable acidity in Quark cheese without or with agave inulin upon storage under refrigeration for 14 days.

Days of storage	pH		Titratable acidity (% latic acid)	
	T1	T2	T1	T2
1	5.32±0.006 aA	4.84±0.140 aB	1.20±0.137 aA	1.3±0.081 aA
7	4.56±0.00bA	4.41±0.040bB	1.37±0.029 aA	1.40±0.000 bA
14	4.96±0.079 cA	4.62±0.080bB	1.30±0.081aA	1.41±0.052bcA

T1- Without addition of agave inulin; T2 - With the addition of agave inulin
Values with different lowercase letters in the same column are significantly different ($p < 0.05$).
Values with different upper-case letters in the different treatments for the same count and in the same line are significantly different ($p < 0.05$).

The pH values found were close to those found by Maruyama et al. (2006), they found values close of 4.70 with cultures isolated from *L. acidophillus* and *B. longum.*

Gonçalves (2004) found pH values for probiotic Quark cheeses ranging from 4.38 to 4.50. Having a pH close to 4.5 is one of the main characteristics of Quark cheese independent of the type of starter culture employed.

Conclusion

According to the results found in this work it was possible to develop Quark cheeses with the addition of kefir as starter culture and the agave inulin prebiotic. It was produced cheeses with chemical composition, pH and acidity compared to cheeses using other microorganisms as starter cultures. Cheeses matrixes produced by both treatments showed to be adequate for the development of the Lactobacilli present in kefir, it was produced cheeses with high counts of them, this have shown that the cheeses had significative probiotic potential.

Although the addition of agave inulin had decreased Lactobacilli counts, its values were still high. The manufacture of cheeses using kefir as starter culture has potential for employment in the industrial manufacture of this type of cheese.

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