UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

PERFIL DE ÁCIDOS GRAXOS DO QUEIJO MATURADO PRODUZIDO COM *LACTOBACILLUS HELVETICUS* E UTILIZAÇÃO DE COBERTURA COMESTÍVEL

Autora: Paula Martins Olivo
Orientadora: Prof.ª Dr.ª Magali Soares dos Santos Pozza

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TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

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“A imaginação é mais importante que a ciência, porque a ciência é limitada, ao passo que a imaginação abrange o mundo inteiro”

(Albert Einstein)

A Deus pai, que é digno de receber toda a honra, toda a glória,
À minha família, Olivo, Goreti e Carla, pelo apoio incondicional e por estarem sempre comigo;
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BIOGRAFIA

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RESUMO

O efeito sobre o perfil dos ácidos graxos com utilização dos microrganismos *Streptococcus thermophilus* e *Lactobacillus helveticus* foram analisados na fabricação do queijo maturado. Avaliaram-se os tempos de maturação (0, 10, 20 e 30 dias) e a qualidade do produto final, por meio de análises de ácidos graxos, químicas e físicas, de antioxidantes e microbiológicas. O delineamento experimental foi inteiramente ao acaso em esquema fatorial com os dados analisados por meio do ProcMixed do SAS 9.3. No primeiro experimento os queijos apresentaram qualidade microbiológica e físico-química dentro dos padrões estabelecidos pela legislação brasileira pertinente (*Listeria monocytogenes* e *Staphylococcus aureus* ausentes, coliformes 2,69 log). Houve redução nos valores de coliformes para ambos os tratamentos. Com relação às contagens de bactérias ácido láticas (BAL), estas mantiveram-se viáveis até o 30º dia de maturação e as bactérias proteolíticas diminuíram durante a maturação (5,51log e 5,23log respectivamente). Não houve diferença entre os tratamentos com relação a cor instrumental das amostras. Os valores de textura dos queijos não apresentaram diferença entre os parâmetros. Os ácidos graxos quantificados em maior proporção foram ácido esteárico, oleico, linoleico e linolênico. Aumentaram-se os níveis de ácidos graxos monoinsaturados e houve diminuição dos ácidos graxos saturados no queijo contendo *L. helveticus*, portanto a inclusão de tal bactéria mostrou-se efetiva por promover o desenvolvimento de produto com características desejáveis ao consumidor. No segundo experimento avaliou-se o uso do revestimento comestível de Açafrão-da-terra 1% e alginato de sódio aplicado ao queijo maturado com *Lactobacillus helveticus* por 30 dias, suas características físico-químicas e microbiológicas e assim como as características intrínsecas ao revestimento. O delineamento experimental foi inteiramente ao acaso com

dois tratamentos, sendo um com aplicação de cobertura de alginato de sódio e Açafrão-da-terra 1% e o outro sem a cobertura comestível, com os dados analisados por meio do programa SAS 9.3. Houve redução nos valores de coliformes para ambos os tratamentos e os valores de bactérias ácido láticas, apresentaram- se até o 30º dia de maturação (BAL: 6,17 log e 6,06 log). Para cor, não houve diferença entre os tratamentos para os parâmetros L*, a* e b*, somente em relação ao tempo de armazenamento, tornando os queijos mais escuros (L*:64,38). Os valores obtidos para textura apresentaram diferenças significativas para tratamento, na dureza, gomosidade, mastigabilidade e para coesividade, para os tempos avaliados o parâmetro elasticidade não apresentou diferença significativa ($p<0.05$). As propriedades mecânicas obtidas das coberturas não apresentaram diferenças significativas para tensão na ruptura, elongação, Módulo de Young, Permeabilidade ao vapor de água (PVA) e espessura da cobertura de alginato de sódio (Controle) e alginato de sódio com 1% Açafrão-da-terra. A utilização de cobertura de alginato de sódio e Açafrão da terra 1% para queijos maturados não melhorou efetivamente a qualidade microbiológica, entretanto apresentou aumento no número de bactérias ácido lácticas, aumento na atividade de água e melhoria na textura dos queijos tornando-os mais macios, com diminuição da gomosidade, coesividade e mastigabilidade. No terceiro experimento avaliou-se a cobertura comestível como veículo para bactérias ácido lácticas, por meio da adição de revestimento de alginato e microrganismos (*L. acidophilus* e *L. helveticus*) em queijos maturados. As coberturas foram avaliadas com relação a características químicas, estabilidade microbiológica, viabilidade e resistência a passagem trato gastrointestinal e a microestrutura em microscópio eletrônico de varredura (MEV). Avaliaram-se as propriedades intrínsecas do revestimento como características da cobertura comestível do queijo através das propriedades mecânicas, Permeabilidade ao vapor térmico (PVA) e isoterma de adsorção. O delineamento experimental foi inteiramente ao acaso com quatro tratamentos (queijo sem revestimento comestível (SEM), queijo com revestimento de alginato de sódio (AG), queijo com revestimento de alginato de sódio e *L. acidophilus* (AGLA) e queijo com revestimento de alginato de sódio e *L. helveticus* (AGLH)) em quatro tempos de armazenamento (0,5, 10 e 15 dias) com os dados analisados por meio do programa SAS 9.3. Houve redução nos valores de coliformes para todos os tratamentos com o tempo de armazenamento aos 15 dias de armazenamento (5.82 log). Com relação às contagens de bactérias ácido lácticas, estas mantiveram-se viáveis até o 15º dia de maturação (SEM: 7.15 $\log_{10}$, AG: 6.39 $\log_{10}$, AGLA: 7.01 $\log_{10}$ e AGLH: 7.09 $\log_{10}$ respectivamente). Para a identificação

dos microrganismos presentes no queijo pela técnica de RAPD- PCR foram isolados clones do *L. helveticus* comprovando a migração do revestimento para o interior do queijo. A análise de MEV mostrou que as BAL se apresentaram distribuídas por todas a superfície dos filmes (AGLA, AGLH), sendo uma alternativa para veicular estes microrganismos no queijo. Para caracterização das coberturas os parâmetros foram significativos (P<0,05) para a permeabilidade ao vapor de água, espessura e modulo de young e não para tensão na ruptura e elongação. Em relação a simulação gastrointestinal das amostras de queijo com cobertura, estas não apresentaram diferenças significativas para os tratamentos e para sua interação com o tempo de armazenamento, porém apresentaram diferenças para os tempos avaliados. A utilização de revestimentos comestíveis de alginato de sódio e microrganismos para queijos maturados não melhorou efetivamente a qualidade microbiológica do produto em relação a presença de coliformes totais. A migração de células de microrganismo (*L. helveticus*), acrescentado na cobertura, para o interior do queijo, mostrou que a cobertura pode ser um veículo para as BAL. As bactérias lácticas permaneceram viáveis durante os 10 dias de armazenamento e sobreviveram ao transitar pelo trato gastrointestinal.

Palavras-chave: açafrão-da-terra, ácido linoleico conjugado, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, perfil de ácidos graxos, embalagens ativas

ABSTRACT

The effect on fatty acid profile using *Streptococcus thermophilus* and *Lactobacillus helveticus* microorganisms were analyzed in the maturated cheese production. The rippened times (0, 10, 20 and 30 days) and final product quality were evaluated by fatty acids profile, chemical and physical, antioxidants and microbiological analysis. The experimental design was completely randomized in a factorial scheme with data analyzed through ProcMixed of SAS 9.3. In the first experiment the cheeses presented microbiological and physic-chemical quality within the standards established by pertinent Brazilian legislation (*Listeria monocytogenes* and *Staphylococcus aureus* absent, coliforms 2.69 log). There was reduction in coliforms values for both treatments. In relation to lactic acid bacteria (LAB) counts, they remained viable until the 30th maturation day and proteolytic bacteria decreased during maturation (5.51log and 5.23log respectively). There was no difference between the treatments in relation to the sample's instrumental colors. The cheeses texture values did not present differences between the parameters. The most quantified fatty acids were stearic, oleic, linoleic and linolenic acid. The monounsaturated fatty acids levels were increased and there was a decrease of saturated fatty acids in cheese containing *L. helveticus*, so such bacteria inclusion was effective for promoting a product with desirable characteristics to the consumer. The second experiment evaluated the use of turmeric 1% and sodium alginate edible coating applied to cheese matured with *Lactobacillus helveticus* for 30 days, its physicochemical and microbiological characteristics as well as the intrinsic coating characteristics. The experimental design was completely randomized with two treatments (with and without sodium alginate and 1% turmeric application) and data were analyzed through the SAS 9.3 program. There was reduction in coliform values for both treatments and the lactic acid bacteria counts remained viable until the 30th maturation day (LAB: 6.17 log and 6.06log). For color, there was no difference between the treatments for L *, a * and b *

parameters, only in relation to the storage time, making the cheeses darker (L*:64.38). The values obtained for texture were significant for treatment, in the hardness, gum, chewability and for cohesiveness. For evaluated times the elasticity parameter did not present significant difference ($p < 0.05$). The mechanical properties of coatings did not present significant differences in rupture tension, elongation, Young's modulus, Steam permeability (SP) and sodium alginate cover (control) and sodium alginate with 1% of turmeric thickness. The use of sodium alginate and turmeric 1% for ripened cheeses did not improve effectively the microbiological quality, however, it increased lactic acid bacteria, water activity and cheeses texture, making them softer, decreasing gum, cohesiveness and chewability. In the third experiment there was evaluated the edible coating as a transport for acid lactic bacteria, by adding sodium alginate coating and microorganisms (*L. acidophilus* and *L. helveticus*) in matured cheeses. The covers were evaluated regarding chemical characteristics, microbiological stability, viability and resistance to the gastrointestinal tract passage and microstructure in scanning electron microscope (SEM). It was evaluated the coating intrinsic properties as the edible coating characteristics of the cheese through mechanical properties, stream permeability (SP) and isotherm adsorption. The experimental design was completely randomized with four treatments (SEMC: cheese without edible coating, AG: cheese with sodium alginate coating, AGLA: cheese with sodium alginate + *Lactobacillus acidophilus* coating and AGLH: cheese with sodium alginate + *Lactobacillus helveticus* coating) in four storage times (0, 5, 10 and 15 days) with data analyzed through the SAS 9.3 program. There was reduction in coliform values for both treatments with storage time of 15 days (5.82 log). In relation to lactic acid bacteria counts, they remained viable until the 15th maturation day (SEMC: 7.15 log, AG: 6.39 log, AGLA: 7.01 e AGLH:7.09 log respectively). To identify the microorganisms in cheese by the RAPD-PCR technique, *L. helveticus* clones were isolated proving the coating migration inside the cheese. The SEM analysis showed that the LABs were distributed throughout all the edible coating surface (AGLA, AGLH), being an alternative to transport these microorganisms in the cheese. For coating characterization, the parameters were significant ($P < 0.05$) for steam water permeability, thickness and Young's modulus, and not for rupture tension and elongation. Regarding the gastrointestinal simulation of the cheese samples with cover, these presented no differences for treatments and their interaction with storage time, however they presented differences for evaluated times. The use of sodium alginate edible coatings and microorganisms for matured cheeses did not improve effectively the product

microbiological quality in relation to the total coliforms' presence. The microorganism (*L. helveticus*) cells migration, added in the cover to inside the cheese, showed that the cover can be a transport for LAB. Lactic bacteria remained viable during the 10 storage days and survived to the gastrointestinal tract.

Keywords: turmeric, conjugated linoleic acid, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, fatty acid profile, intelligent packaging.

REVISÃO DE LITERATURA

1.O leite e seus derivados

Nas últimas décadas o termo “alimento funcional” é tema de estudo e discussão por diversos autores, pode ser considerado funcional se for demonstrado que o mesmo pode trazer benefícios para uma ou mais funções alvo no organismo, além de possuir efeitos nutricionais adequados, de forma que seja tanto relevante para o bem-estar e saúde, quanto para a redução do risco de uma doença (Zeraik et al., 2010).

A legislação vigente, aprovada pela Agência Nacional de Vigilância Sanitária (Anvisa), em 1999, não define o termo “alimentos funcionais”, mas sim “alegação de propriedade funcional”, que é “aquela relativa ao papel metabólico ou fisiológico que o nutriente ou não nutriente tem no crescimento, desenvolvimento, manutenção e outras funções normais do organismo humano”. A alegação de saúde é “aquela que afirma, sugere ou implica a existência da relação entre o alimento ou ingrediente com doença ou condição relacionada à saúde” (BRASIL, 1999). O alimento detentor da alegação de propriedade funcional precisa ser avaliado pela Gerência Geral de Alimentos (GGALI) da Anvisa e comprovado sua segurança de uso e eficácia e então pode ser disponibilizado no mercado para consumo. As alegações podem ser veiculadas em alimentos e ingredientes para consumo humano, em rótulos e propagandas de produtos elaborados, embalados e prontos para a comercialização e oferta ao consumidor (Pinhati et al. 2014).

O leite e seus derivados, apresentam um perfil de ácidos graxos em que sua maior parte é caracterizada pela presença de gordura saturada na sua composição, e assim consequentemente tem sua ingestão relacionada ao aumento dos distúrbios metabólicos e doenças relacionadas a alimentação (Fernandes et al., 2011). Em contrapartida alguns

estudos têm comprovado o efeito benéfico de algumas moléculas encontradas no leite e derivados, pelas modificações realizadas nas dietas dos ruminantes ou modificações realizadas nos produtos (Oliveira et al., 2008; Mourão et al., 2005).

Os queijos são considerados como alimento comum na dieta humana que compõe a alimentação de todas as classes sociais (Silva, 2011), suas modificações ou inclusões de ingredientes podem torná-lo com alegação de funcional. A modificação no perfil de ácidos graxos de queijos por meio da fermentação microbiana pode transformá-lo em alimento mais saudável e benéfico ao consumidor.

1.1 Composição do leite de vaca

O leite de vaca é composto em média de água (87,5%), lactose (4,7%), gordura (3,5%), proteínas (3,5%), minerais e vitaminas (0,8%), sua composição é importante para determinar qualidade nutricional, capacidade para processamento, fabricação de derivados e consumo humano. Na composição de sua gordura (3,5%) é encontrado cerca de 66,9% de ácidos graxos saturados e 33,1% de ácidos graxos insaturados (Santos, 2001).

A água é um dos principais componentes e representa cerca de 87% a 90% do total do leite. Para que ocorra a secreção do leite pela glândula mamária, é necessário apresentar água em quantidade adequada (Lagger et al., 2000) sendo que o leite também é dependente da síntese de lactose, pois a lactose atrai a água para as células epiteliais mamárias (González e Campos, 2003), devido ao efeito osmótico. A lactose, é o açúcar característico do leite e representa o componente sólido predominante com menor variação no leite de vaca, de 4,4% a 5,2%, possui importante função, pois é um fator limitante para produção de leite (Rodriguez, 2013).

Para produção de queijos e derivados, as proteínas se apresentam como um dos principais fatores a serem observados na composição do leite. São divididas em fração nitrogenada proteica (95%) e não proteica (5%) (Silva, 1997). Os compostos nitrogenados são divididos em dois grupos: proteínas do soro e caseínas.

As caseínas são sintetizadas pelas glândulas epiteliais na glândula mamária, compõe cerca de 80% do total de proteínas do leite (Farrell et al., 2004). Quando agregadas, formam grânulos denominados micelas, que contêm também água e minerais, principalmente cálcio e fósforo (González e Campos, 2003). As proteínas do soro apresentam excelente perfil de aminoácidos, conferindo alto valor biológico, relacionadas à formação, crescimento e manutenção de músculos, ossos, órgãos e produção de

anticorpos e hormônios, e seu teor no leite tem sido importante indicador de qualidade para indústrias de derivados lácteos (Rodriguez, 2013).

Os principais minerais presentes no leite são cálcio e fósforo encontram-se associados com a estrutura das micelas de caseína (González e Campos, 2003), outros minerais também são presentes no leite como o cloro, potássio, sódio e magnésio. Em contrapartida, o leite possui baixos teores de ferro, alumínio, bromo, zinco e manganês. O poder de associação entre os minerais e as proteínas do leite é determinante para a estabilidade das caseínas frente a diferentes agentes desnaturadores, os utilizados para produzir os derivados lácteos, os coalhos (Silva, 1997).

As vitaminas mais conhecidas estão presentes, A,D,E e K, no leite bovino (Silva, 1997), porém, a glândula mamária não tem capacidade de síntese das mesmas, nos ruminantes pode ocorrer por meio de bactérias ruminais ou por conversão de provitaminas para a forma ativa, no fígado, no intestino delgado e na pele (González e Campos, 2003). As vitaminas lipossolúveis (A, D, E e K) são associadas aos glóbulos de gordura e as hidrossolúveis à fase aquosa do leite. O teor das vitaminas A, D e E no leite são provenientes do alimento consumido pelos animais. A vitamina K e as vitaminas hidrossolúveis, são sintetizadas no sistema digestivo dos ruminantes (Silva, 1997). A vitamina B12 geralmente está presente em alimentos de origem animal, especialmente no leite e na carne, podendo causar diversos transtornos hematológicos, neurológicos e cardiovasculares (Paniz et al., 2012).

A gordura é o componente que apresenta maior variação na composição do leite, de 3,2% a 6,0% (Rodriguez, 2013), também está relacionada com o rendimento de derivados lácteos, em teor reduzido tem aspecto negativo para a indústria de laticínios (Machado, 2012) e em quantidades adequadas pode ser relacionada à obtenção de derivados com melhor cor, aroma e sabor. Aproximadamente 98% da fração lipídica do leite de vaca é composta por três ácidos graxos, cada um em ligação éster com uma mesma molécula de glicerol (Rodriguez, 2013). O leite bovino está relacionado à presença de ácidos graxos saturados (Eifert et al., 2006), que são associados ao aumento do risco de doenças e outros distúrbios metabólicos em humanos (Santos et al., 2013). Com foco na redução dos problemas metabólicos tem-se buscado a diminuição dos ácidos graxos saturados (AGS) de cadeia média, como láurico (C12:0), mirístico (C14:0) e palmítico (C16:0) (Lopes et al., 2009) e aumento dos ácidos graxos insaturados (AGI).

Em relação ao mercado consumidor atual este apresenta-se com forte apelo por um consumo consciente de derivados lácteos ricos em gorduras com melhor perfil de

ácidos graxos, buscando alimentos saudáveis e funcionais, com efeito de gerar efeitos benéficos para quem os consome (Machado, 2012).

2. Biohidrogenação nos ruminantes

Os suplementos lipídicos fornecidos aos animais são incluídos na dieta de ruminantes para aumentar sua densidade energética, melhorar a utilização de nutrientes, incrementar a produção de leite e possibilitar a manipulação da composição em ácidos graxos dos produtos finais, carne e leite (Palmquist et al., 1993; Vilanova et al. 2012).

Os lipídeos são caracterizados como compostos solúveis em solventes orgânicos como éter e clorofórmio e insolúveis em água; sua estrutura básica é um grupo glicerol e três ácidos graxos (triacilgliceróis). Alguns lipídeos são encontrados na natureza principalmente nas folhas (galactolipídeos) e sementes dos vegetais (triglicerídeos), apresentando pequenas diferenças quanto ao radical ao qual estão ligados os ácidos graxos. Os galactolipídeos apresentam estrutura similar aos triglicerídeos, exceto por uma diferença, um dos seus ácidos graxos é substituído por um açúcar, a galactose. No entanto, quando ocorre a substituição de um ácido graxo por um fosfato, os lipídeos são chamados fosfolipídeos, estes são mais encontrados nas bactérias presentes no rúmen do animal, do que nos alimentos (Berchielli et al., 2006).

Os triglicerídeos (TG) são a principal forma de armazenamento de gordura no tecido animal, são sintetizados principalmente no fígado, tecido adiposo, glândula mamária e intestino delgado, porém a maioria das células possuem a capacidade de realizar sua síntese (Bruss, 2008).

No ambiente ruminal ocorre uma extensiva hidrólise dos lipídeos esterificados da dieta, em que triglicerídeos, galactolipídeos e fosfolipídios pela ação de lipases dos microrganismos, liberam ácidos graxos livres permitindo que a galactose e o glicerol sejam fermentados a ácidos graxos voláteis. A lipólise corresponde ao início do processo de metabolismo dos lipídeos no rúmen, sendo imprescindível para que ocorra a biohidrogenação (Harfoot e Hazlewood, 1988), pois a presença de ácidos graxos poli-insaturados, é tóxico para as bactérias ruminais, sendo as mais susceptíveis as Gram positivas, metanogênicas e protozoários (Palmiquist & Mattos, 2011). Assim a toxicidade dos ácidos graxos poli-insaturados está relacionada à natureza anfipática dos ácidos graxos, ou seja, aqueles que são solúveis, tanto em solventes orgânicos como em água sendo os que causam mais toxicidade (Jenkis et al., 2008).

Para que ocorra a biohidrogenação, o passo inicial é uma reação de isomerização que converte a dupla ligação cis-12 no ácido graxo insaturado para o seu isômero trans-11. A isomerase não é funcional a menos que o ácido graxo tenha um grupo carboxila livre, o que ocorre no caso de ácidos graxos poli-insaturados assim como C18:2. A extensão na qual trans-11 C18:1 é hidrogenado a C18:0 (ácido esteárico) depende das condições do rúmen (Jenkins, 1993; Demeyer e Doreau, 1999).

O metabolismo dos ácidos graxos insaturados no rúmen resulta como principal produto o ácido esteárico que passará ao abomaso e ao intestino em que será absorvido. O processo normal da biohidrogenação dos ácidos oleico, linoleico e linolênico formará ácido esteárico que será absorvido posteriormente no intestino, porém em algumas situações, devido à incompleta biohidrogenação dos ácidos graxos, ocorrem alterações que levam a formação final de ácidos graxos trans (Berchielli et al., 2006).

Na glândula mamária somente os ácidos graxos de cadeia curta e média são sintetizados. Dos ácidos graxos de cadeia média presentes no leite, 50% são sintetizados pela vaca e o restante é proveniente de ácidos graxos pré-formados, os ácidos graxos de cadeia longa e os outros 50% restantes de cadeia média são oriundos da corrente sanguínea, que os transporta para a glândula mamária (Martinez, 2009), e ainda podem sofrer transformações por ação enzimática. Uma enzima importante nesse metabolismo é a estearoil-CoA dessaturase (Δ9 -dessaturase) que tem seu papel na modificação da composição de ácidos graxos do leite de ruminantes e é responsável pela conversão de ácido esteárico (C18:0) em ácido oleico (C18:1 cis-9), palmítico (C16:0) em palmitoleico (C16:1 cis-9) e vacênico em ácido linoleico conjugado (C18:2 cis-9 trans11 CLA) (Palmquist e Mattos, 2011).

3. Definição de queijo

Definição de queijo segundo a portaria do MAPA Decreto Nº 1812 de 08 de fevereiro de 1996:

> Entende-se por queijo o produto fresco ou maturado que se obtém por separação parcial do soro do leite ou leite reconstituído (integral, parcial ou totalmente desnatado), ou de soros lácteos, coagulados pela ação física do coalho, de enzimas específicas, de bactérias específicas, de ácidos orgânicos, isolados ou combinados, todos com grau alimentício, com ou sem agregação de substâncias alimentícias e/ou especiarias e/ou condimentos,

> aditivos especificamente indicados, substâncias aromatizantes e matérias corantes. A denominação QUEIJO está reservada aos produtos em que a base láctea não contenha gordura e/ou proteínas de origem não láctea."
>
> "Entende-se por queijo fresco o que está pronto para o consumo logo após sua fabricação."
>
> "Entende-se por queijo maturado o que sofreu as trocas bioquímicas e físicas necessárias e características da variedade do queijo.

Embora os queijos se diferenciem quanto a forma e estrutura, todos apresentam basicamente quatro principais ingredientes: leite, coalho, microrganismos e sal; e são caracterizados por suas principais etapas, a produção de ácido, formação do gel, expulsão do soro e tempo de maturação (Beresford et al., 2001).

3.1 Queijos maturados

3.1.1. Maturação

A maturação envolve processos bioquímicos, que promovem modificações na textura e no sabor, o tempo varia de acordo com o tipo do queijo, de semanas a anos. As enzimas bacterianas atuam na massa do queijo, através de atividades proteolítica e lipolítica, promovendo modificação nas características físico-química e influenciando na textura, aroma e sabor (Moreno, 2013). A maturação pode ser dividida em três eventos principais: glicólise, proteólise e lipólise.

A glicólise é a primeira etapa e consiste na conversão da lactose em ácido lático e demais ácidos orgânicos, em seguida ocorre a proteólise, que consiste na hidrólise das proteínas do leite em peptídeos de médio peso molecular, atingindo níveis de aminoácidos, modificando a textura e contribuindo para o aroma e sabor dos queijos. A lipólise ocorre originalmente pela ação das lipases naturais presentes no leite ou pela adição de culturas láticas lipolíticas, que hidrolisam os lipídeos em ácidos graxos e contribuem fortemente no aparecimento do aroma nos queijos (Fox et al.,2000).

No processo de glicólise, ocorre a conversão da molécula de lactose em ácido láctico pela ação das bactérias lácteas, o principal composto intermediário formado durante a conversão da lactose em ácido láctico é o piruvato. Esse processo é responsável por vários outros compostos que podem ser convertidos em substâncias voláteis nos

queijos como diacetil, acetona, acetaldeído, etanol, acetato e ácido acético (Voigt et al., 2010; Moreira, 2011).

Neste contexto os principais gêneros de microrganismos lácticos são: *Enterococcus, Lactobacillus, Lactococcus, Leutonostoc, Pediococcus e Streptococcus* (Wourters et al., 2002). Entre os microrganismos responsáveis pela microbiota láctica compreendem as culturas iniciadoras e as não iniciadoras. As culturas iniciadoras ou "starters", são responsáveis por produzir os ácidos orgânicos que promovem a redução do pH do leite (pH=5,3) em período de seis horas, na temperatura de 30-37º graus. Podem ser adicionadas no início do processo ou serem provenientes do próprio leite, flora microbiana intrínseca dos animais (Beresford et al., 2001).

Sucessivamente, a proteólise consiste em três etapas, primeiramente ocorre a hidrólise da caseína em longas cadeias peptídicas por ação das enzimas proteases, afetando o queijo em sua consistência. Na segunda etapa acontece a hidrólise desses peptídeos menores, formando aminoácidos livres contribuindo no sabor e com pouca influência no aroma. Na terceira etapa ocorre as transformações dos aminoácidos livres por meio das enzimas que dependem da cultura lática, formando compostos aromáticos, o pH é o principal fator que influência (Alais, 1985; Moreno, 2013). As enzimas que atuam no processo de proteólise são as endoproteínases, que são responsáveis por hidrolisar as ligações peptídicas específicas do interior da cadeia polipeptídica e as exopeptidases, em ambas as extremidades N-terminal (aminopeptidases) e C-terminal (carboxipepetidases) (Hayaloglu e al., 2013; Steele et al.,2013).

Ao final da maturação ocorre a última etapa, a lipólise, que é caracterizada pela hidrólise dos triglicerídeos, com liberação de glicerol e ácidos graxos, devido a ação de enzimas lipases. Os ácidos graxos de cadeia curta (C4- C12) são liberados na hidrólise e conferem as características aromáticas aos queijos (Bontinis et al., 2012; Medeiros et al., 2014), podendo reagir e produzir outros compostos aromáticos que conferem sabores típicos em determinados queijos (Delgado et al., 2010). Ocorre principalmente pela ação dos agentes lipolíticos, microrganismos presentes no leite ou no queijo, enzimas nativas do leite (lipoproteína lipase, LPL) no caso de queijos produzidos com leite cru; por coagulante (renina), ou através de enzimas (lipases) adicionadas especificamente para esse fim (McSweeney, 2004; Perry, 2004), podendo ser divididas em duas principais categorias: lipase animal e lipase microbiana (Birschbach, 1994).

A lipólise pode ser influenciada pelo sistema metabólico de bactérias ácido lácticas "starters", bactérias propiônicas, assim como, pelas leveduras ou bolores

presentes nos queijos (principalmente os *Penicillium spp.*) (McSweeney e Souza, 2000). Além das alterações quanto ao aspecto nutricional nos queijos, pode ocorrer a liberação de ácidos graxos poli-insaturados e diminuir os triglicerídeos hepáticos. Com a liberação de ácido linoleico, pode ocorrer ação de isomerases e produção de isômeros do ácido linoleico (CLA-ácido linoleico conjugado), já existem pesquisas associando o potencial bioativo a presença de algumas bactérias lácteas (Prandini et al., 2011).

4. Coberturas comestíveis

A demanda do mercado consumidor por produtos com características e qualidades superiores vêm de encontro com o desenvolvimento de novos produtos que agreguem inovações tecnológicas, neste cenário as embalagens aparecem sob destaque.

As embalagens podem ser denominadas em ativas ou inteligentes, as ativas são aquelas que interagem de maneira intencional com o alimento, visando melhorar ou conservar algumas de suas características. Enquanto as embalagens inteligentes podem ser definidas como aquelas que monitoram as condições do alimento acondicionado ou do ambiente externo à embalagem, comunicando-se com o consumidor (Han, 2005; Yam et al.,2005).

Em comparação às embalagens já existentes, passivas (tradicionais) que são limitadas a proteger os alimentos de condições externas, as embalagens ativas têm várias funções adicionais como alterar as condições do produto aumentando sua vida de prateleira, segurança e qualidade e, ou melhorando suas características sensoriais (Vermeiren e al., 2002). Consistem em incorporar e/ou imobilizar aditivos à embalagem ao invés de incorporar diretamente no produto (Kerry et al., 2006).

As coberturas comestíveis são considerados como alternativa para as embalagens tradicionais, quando aplicadas diretamente sob a superfície do alimento, são responsáveis por redução na perda de vapor de água, contato com o oxigênio, migração de lipídios e aroma ou para estabilização dos gradientes de atividade de água e consequentemente mantêm as diferentes propriedades de textura (Giancone et al., 2008).

Existem diferentes substâncias que podem ser utilizadas na formulação de coberturas comestíveis em geral, polissacarídeos, proteínas e lipídeos, podendo ser empregadas isoladas ou em combinações (Chiumarelli e Hubinger, 2012).

Apresentam-se como características das coberturas comestíveis o espalhamento uniforme, boa aderência, secagem rápida e não formação de espumas. Quando aplicado,

não deve quebrar, descolorir, desprender, ser pegajoso ou aderir na embalagem, prejudicar a qualidade sensorial e reagir com o alimento de maneira negativa, durante o manuseio e armazenamento (Baldwin et al., 2011).

Nas coberturas comestíveis podem ser acrescentados compostos ativos como conservantes, antioxidantes, inibidores de reações bioquímicas intrínsecas aos alimentos entre outros.

A utilização de antimicrobianos em coberturas comestíveis tem-se destacado pela crescente preocupação dos consumidores com a qualidade microbiológica dos alimentos. Assim, podem ser capazes de eliminar ou inibir microrganismos deterioradores e/ou patogênicos. Seu princípio básico de atuação é a adição de uma barreira extra (microbiológica) às barreiras físicas (oxigênio e umidade) (Han, 2003).

Nos alimentos uma das principais causas da deterioração é a oxidação lipídica e o crescimento microbiano dentro da embalagem tradicional, a utilização direta de componentes antioxidantes e/ou agentes antimicrobianos na formulação do alimento, pode modificar o sabor e/ou o aspecto do alimento fazendo com que a decisão sensorial do consumidor seja afetada no momento de escolha do produto (Baldino et al., 2017).

A escolha de um conservante, como antimicrobiano, para uma aplicação específica é baseada em fatores como, mecanismo de inibição, natureza química (solubilidade, pH, reatividade, toxicidade), cinética de migração e difusão do agente no alimento, características físico-químicas do alimento, tipo e população de microrganismos, fisiologia do microrganismo alvo, processo de fabricação do material de embalagem e aspectos relacionados à legislação (Han, 2000).

Antioxidantes naturais como óleos essenciais e extratos antioxidantes têm sido aplicados para a formulação de coberturas comestíveis como embalagens ativas, por exemplo, curcumina, não influenciando o sabor ou aspecto dos mesmos (Musso et al, 2017).

A curcumina (1,7-bis-(4-hidroxi-3-metoxifenil) -hepta-1,6-dieno-3,5-diona) (Figura 1) é obtida como principal componente da *Curcuma longa* (popularmente conhecida como açafrão-da-terra) e amplamente utilizado como aditivo alimentar. Esse composto fenólico hidrofóbico possui várias funcionalidades, como atividade antioxidante, antimicrobiana e demais atividades biológicas (Sun et al., 2002).

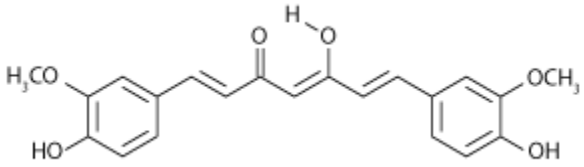

Figura 1. Estrutura molecular da Curcumina
Fonte: http://dx.doi.org/10.5935/0100-4042.20150035

A composição química do açafrão-da-terra pode ser influenciada por vários fatores como: cultivo, tipo de plantio, solo, disponibilidade hídrica, época de colheita, clima entre outros. Sendo apresentada uma composição química de 30 a 50% de amido, 6 a 10% de proteína, 6,5 a 8,5% de cinzas, 2 a 6% de fibras, 3 a 6% de óleo volátil e 2 a 8% de curcuminoides (Govindarajan e Stahl, 1980; Braga et al., 2003). A coloração amarela dos rizomas é atribuída pelos compostos fenólicos classificados como curcuminoides, compostos polifenólicos divididos em três principais substâncias, a curcumina (80%), a desmetoxicurcumina (DMC) (18%) e a bisdesmetoxicurcumina (BDMC) (2%), que são diferenciadas apenas pela quantidade de grupos metoxila (OCH3) presentes na estrutura química. A curcumina apresenta dois grupos metoxila, a desmetoxicurcumina contém apenas um grupo metoxila e a bis-desmetoxicurcumina não apresenta grupo metoxila (Anand et al., 2008; Goel e Aggarwal, 2010).

Devido as suas propriedades antimicrobianas a curcumina (Figura 1) vem sendo testada como alternativa para controlar e/ou reduzir a incidência de contaminação microbiana em alimentos. Estudos foram realizados para determinar a concentração mínima inibitória de curcumina, um dos principais componentes, para patógenos alimentares como as bactérias *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes* (Gram-positivas), *Escherichia coli*, *Pseudomonas aeruginosa* e *Samonella typhimurium* (Gram-negativas) e *Penicillium notatum* e *Aspergillus niger* (fungos) e apresentaram resultados que variam de 100-400 μg/mL para sua utilização e eficácia como agente antimicrobiano efetivo (Basniwal et al., 2011; Altunatmaz et al., 2016).

Estudos realizados por Niamsa e Sittiwet (2009) sobre a atividade antimicrobiana do extrato aquoso de *C. longa*, constataram que o extrato inibiu o crescimento de *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Kebsiella pneumoniae* ATCC 10031 e *Staphylococcus epidermidis* ATCC 12228. A concentração mínima inibitória apresentou-se de 4000 a 16000 μg/mL e a concentração mínima bactericida de 16000 a 32000 μg/mL.

As coberturas comestíveis com inclusão de microrganismos podem ser descritas também como forma de embalagem ativa, e estas apresentam alternativas para a melhora de algumas características nos alimentos, principalmente na sua conservação (Vargas et al., 2008). A utilização de bactérias ácido lácteas (BAL) além de fornecer benefícios diretamente a saúde dos consumidores também apresenta ação direta no produto, podendo inibir o crescimento de bactérias patogénicas e deteriorantes (Rokka e Rantamaki, 2010) pela capacidade antimicrobiana, produção de bacteriocinas, ou por competição *in situ* (Messaoudi et al., 2013). Esses eventuais benefícios trazidos para a saúde dos consumidores tornam o produto possível de alegação como produtos probióticos, que se definem por "microrganismos vivos que, quando administrados em número adequado conferem um benefício de saúde ao hospedeiro (FAO / OMS)", portanto, devem conter no mínimo 10^6-10^9 UFC / g ou UFC / mL de células viáveis no momento do consumo (Castro et al., 2015).

São considerados como potenciais probióticos grande número e espécies de microrganismos, sendo os mais comum e comercialmente disponíveis as do gênero *Lactobacillus* e *Bifidobacterium* (Holzapfel et al., 1998; Shah e Ravla, 2004).

As bactérias do gênero *Lactobacillus* compreendem um grupo taxonômico heterogêneo e grande de microrganismos que pertencem às bactérias ácido lácticas, possuem cerca de 201 espécies atualmente conhecidas (Bull et al., 2014), são consideradas seguras pois são colonizadores naturais do trato gastrointestinal humano e um gênero subdominante do cólon (Ren et al., 2013).

Apresentam-se na forma de bastonetes, retos ou curvos, ocorrendo isolados ou em cadeia, são catalase negativos, anaeróbios ou aerotolerantes, não esporulados, fastidiosos, mesofílicos (condições ótimas para sua multiplicação são de 35- 40°C), Gram-positivas e produzem ácido láctico como principal produto da fermentação de carboidratos (Goldstein; Tyrrell; e Citron, 2015), sobrevivem em ambientes mais ácidos. Algumas estirpes pertencentes ao gênero *Lactobacillus* são empregadas como probióticos, *L. acidophilus, L. casei, L. plantarum, L. reuteri, L. rhamnosus, L. paracasei, L. delbrueckii, L. johnsonni* (Tripathi e Giri, 2014).

L. acidophilus é um microrganismo homofermentador obrigatório e é capaz de utilizar uma variedade de fontes de carbono para o seu crescimento, garantindo sua competitividade no trato gastrointestinal humano (Bull et al., 2013). Manifesta-se na forma de bacilos curtos com pontas arredondadas, isolados ou em cadeia, gram-positivo, mesofílico (crescimento ideal entre 37 e 42°C), tolerante a meios ácidos (maior taxa de

crescimento em pH 5,5-6,0) (Gomes e Malcata, 1999; Bull et al., 2013), intolerante ao sal (Gomes e Malcata, 1999) e, microaerofílico sendo um dos lactobacilos menos resistentes ao oxigênio (Bull et al., 2013).

L, helveticus é um microrganismo homofermentativa, pertencente ao grupo das bactérias ácido lácticas termófilas (42 a 45 ºC), pH de 5,5 a 5,8, fastidioso (necessidades nutricionais complexas de aminoácidos, peptídeos, bases nucleicas, vitaminas, minerais, ácidos graxos e carboidratos) (Hebert, Raya, e Giori, 2000; Slattery et al., 2010). Por alguns autores pode ser considerado como uma bactéria probiótica pois tem grande importância para saúde humana (Slattery et al., 2010; Taverniti e Guglielmetti, 2012), os peptídeos formados durante a maturação dos queijos a partir da hidrólise das proteínas pelo *Lactobacillus helveticus* podem apresentar propriedades bioativas (Griffiths e Tellez, 2013).

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I- OBJETIVOS GERAIS

O presente trabalho teve por objetivo determinar os efeitos da utilização de *Streptococcus thermophilus* e *Lactobacillus helveticus*, no perfil de ácidos graxos de queijos maturados por 30 dias. Com o intuito de aumentar a vida de prateleira e propiciar melhoria na qualidade microbiológica dos queijos foram estudadas diferentes coberturas comestíveis aplicadas em queijos maturados (alginato de sódio com compostos naturais e com bactérias ácido lácticas).

Starter bacteria promoted fatty acids profile changes in cheese

ABSTRACT: The starter microorganisms use in ripened cheese production intended to improve the unsaturated fats profile in products. The objective of this work was to evaluate, through ripened cheeses production made from milk enriched with a polyunsaturated fat source, as well as the physicochemical, microbiological and fatty acid profile characteristics by fermentation promoted by *Lactobacillus helveticus* (Lh) and *Streptococcus thermophilus* (St) addition. The experimental design was completely randomized in a factorial scheme, with data analyzed using ProcMixed from SAS 9.3. The cheeses presented microbiological and physicochemical quality within the standards established by the relevant Brazilian legislation. There was a reduction in coliform values for both treatments. With respect to lactic acid bacterial counts, these remained viable until the 30th day of maturation. For antioxidant capacity, there were no differences between treatments in times and interaction between treatment x time. There was no significant difference between treatments in relation to the samples instrumental color. The cheese texture did not present significant difference between treatments, times and interaction in the evaluated parameters. The *S. thermophilus* and *L. helveticus* inclusion in the ripened cheese production was effective because it promoted an improvement in the unsaturated fatty acids profile and a decrease in the saturated (palmitic, stearic, oleic and linolenic acid).

Keywords: polyunsaturated fatty acid, lactic acid bacteria, lacteous derivative.

INTRODUCTION

Currently, there is a tendency to link health problems with poor diet caused by inferior ingredients. The association between dairy products consumption, especially cheese and health, is a factor to be considered in these food production and marketing (Terpou et al., 2017).

Cheeses are associated with high levels of long chain saturated fatty acids, considered harmful if consumed in large quantities in human diets, which are responsible for various metabolic disorders and diseases. However, using different production technologies, varying in milk composition, breed, lactation stages and animal diet, this

obstacle has been reduced. In the consumer market, the appearance of several dairy products, enriched or not with unsaturated fatty acids, healthy and potentially beneficial to consumers has become frequent (González-Martín et al, 2017).

The composition of fatty acids composition in foods is of great importance, especially in relation to polyunsaturated fatty acids, such as those from the omega-3 and omega-6 families, which are attributed numerous benefits to the human organism (Perini et al., 2010).

The fatty acids increase in milk can be accomplished through strategies such as the food sources addition in animal feed that may influence the sensory and chemical characteristics of the final product (Jones et al., 2005). Another way to improve the fatty acid profile in animal products is through the bacteria action, by synthesizing, from linoleic acid, in some bacteria strains such as bifid and lactic acid in fermented products (Gorissen et al., 2010).). Since cheese processing also involves bacterial fermentation, a study was conducted to observe the improvement made by bacteria in this type of product, as well as the process steps effect in maintaining unsaturated fatty acid content (Lucatto et al., 2014).

In addition to the improvement of dairy products in relation to the fatty acid profile, products fermented using lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium* also help in maintaining the intestinal microbiota, ensuring health and better welfare through their consumption.

The objective of this work was to evaluate, through the ripped cheese production with milk enriched with polyunsaturated fat source, the physicochemical and microbiological characteristics as well as the fatty acid profile by fermentation promoted by two commercial cultures addition containing *Lactobacillus helveticus* and *Streptococcus thermophilus*.

MATERIAL AND METHODS

The experiment was carried out at the Iguatemi Experimental Farm FEI, belonging to the Maringá State University (UEM), Maringá / Paraná / BR. The experimental protocol was approved by the Experimental Animal Ethics Committee of the Maringá State University, PR (nº 6450240117).

Milk from four Holstein cows with ± 120 days of lactation that were fed with a 60:40 roughage: concentrated diet, including annatto seeds (1.5% DM) and flaxseed oil (3% DM) were used. The milk was collected during five days, in four feeding periods (16 feeding days and 5 milk collection days, totaling 21 days) for cheese production, to analyze the chemical composition and fatty acid profile.

Four batches of cheese were produced consecutively over 4 periods, and 3 cheese triplicates were produced for each treatment (Lh and St) and for each evaluated time (0.10,20 and 30 days) totaling 96 units.

The samples for milk chemical analysis (Table 1) were placed in a plastic bottle containing Bronopol® preservative (2-bromo-2-nitropropane-1,3-diol) and analyzed in an automated Ekomilk Total equipment (Cap-Lab, São Paulo, Brazil).

To quantify milk fatty acids, the methodology proposed by Murphy et al. (1995) and method 5509 of ISO (1978) using the Agilent Model 7890a gas chromatograph (Table 2) were used.

"SH" dairy cultures (Lyofast SH 092 F, SACCO® containing *Streptococcus thermophilus* and *Lactobacillus helveticus*) and (LyofastLH 091, SACCO® containing *Lactobacillus helveticus*) were used to make cheese (05UC / 100 liters milk).

For cheese preparation, 48 liters of pasteurized milk (Pasteurizer Sulinox ®) (65 ° C / 30min), calcium chloride (50 ml per 100 liters of milk), LH or SH milk cultures and liquid coagulant (HA-LA®-CHR, Denmark) were used. After cutting, the mass was

heated to 45 °C, the whey was drained and then the mass was molded (JandaPlast, model RH-1000). After 12 hours, salting was performed at 2% per surface (m / m) and the cheeses were kept in a BOD greenhouse for 30 days / 12 ° C and relative humidity of ± 60.5%.

Water activity (Aqualab® 4TE, Decagon, Sao Paulo, Brazil), pH (digital pH meter, Tecnal Tec-5), titratable acidity (Lutz, 2008), color (Konica Minolta), dry matter, mineral matter, crude protein (AOAC, 1992) and fat content (Bligh and Dyer, 1959) were determined in cheese samples at 0, 10, 20 and 30 days of ripened.

The instrumental color was determined using a Konica Minolta chromometer (Konica Minolta, Model CR 400/410, Japan) using the CIELAB system (CIE, 1986). In the CIELAB color space defined by L *, a *, b *; where: L * (brightness), a * (+ a: red; −a: green) and b * (+ b: yellow; −b: blue). Measurements were made in triplicate with the previously calibrated apparatus on cheese rinds and inner parts.

The samples were diluted in peptone water for microbiological analysis (AOAC, 1992) and there were evaluated the lactic acid bacteria (MRS Lactobacillus, Himedia-De Man, Rogosa and Sharpe agar); proteolytic mesophilic bacteria (PCA-Himedia Agar added with 1% of reconstituted skim milk, Plate Count Agar) and total coliforms, (the VRB Agar Himedia - Violet Reb Bile Agar). At 30 days, the *Listeria monocytogens* and *Staphylococcus aureus* presence were also evaluated (AOAC, 1992).

The cheese lipid profile determination was performed according to Bligh and Dyer (1959) for samples lipids extraction. Subsequently, the lipids were esterified according to ISO (1978) 5509 for analysis using the Agilent autosampler, equipped with 250 ° C flame ionization detector and fused silica capillary column (100 m in length, 0, 25 mm internal diameter and 0,20 μm, Restek 2560). The gas flow was 1.5 mL / min H_2

(carrier gas), 30 mL / min for N_2 (auxiliary gas) and 35 and 350 mL / min respectively for H_2 and synthetic air (flame gases).

The initial column temperature was adjusted to 50 ° C, maintained for 4 minutes, then increased every 10 minutes to 200 ° C and remained for 15 minutes, these increased from 20 ° to 240 ° C and remained for 8 minutes, each race last 44 minutes. Spitless mode was injected with injection or temperature of 250 ° C, detector temperature of 250 ° C. The sample fatty acid quantification was performed by comparison with the fatty acid methylester concentration time of standard samples (Sigma Aldrich).

The antioxidant compounds were evaluated in the cheese samples with 0, 10, 20 and 30 storage days, using the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical sequestration method (ABTS) described by Re et al. (1999) expressed in ET μM (Zhu et al., 2002).

For texture profile analysis (TPA) Brookfield-TC III texture analyzer equipment (Engineering Laboratories, INC., Middleboro, MA, USA) was used in the following configurations: TPA; test speed: 1 mm / s; compression distance 5 mm; 38 mm acrylic cylindrical TA4 probe. The variables measured for TPA were hardness, chewability, cohesiveness and elasticity.

Data were analyzed at 5% significance in Tukey test by ProcGLM of SAS 9.3 (2013) testing the interaction between treatment and time and a linear and quadratic contrast study was performed for storage times.

RESULTS

For the evaluated times, dry matter (DM) contents increased in function of maturation days, with consequent decrease of moisture values ($P < 0.001$). Dry matter values were not significant for treatments and for interaction between treatment and time ($p > 0.05$) (Table 3). Regarding humidity, there was a reduction in the percentages during

maturation. For ash content, there was no significant effect on treatment, time and interaction ($p > 0.05$).

The fat values presented increasing behavior with the maturation time ($p < 0,05$), because with the humidity decrease and dry matter increase there was cheese components concentration, linearly increasing the present EE amount.

There was no significant difference between time and treatment x time interaction ($p > 0.05$) for pH and Aw values, however, pH values showed significant difference for treatments ($p < 0.05$), being higher mean observed in the treatment containing *L. helveticus* (5.801). The titratable acidity and pH values were not congruent, the acidity values were not significant for treatment, time and treatment x time interaction (Table 3).

For total coliform counts (Table 3) there was a significant difference for treatments ($p < 0.05$) and ripened times ($p < 0.05$), with a reduction in counts during maturation. For the lactic acid bacteria and proteolytic bacteria counts, no significant differences were observed for treatments, ripened times and for treatment-time interaction ($p > 0.05$).

Although for the total coliforms, there was a reduction in both treatments during the ripened periods ($p < 0.05$), the lowest total coliforms value was observed in the treatment containing *Streptococcus thermophilus* and *Lactobacillus helveticus* (5.61 log). that the pH value was lower (5.31). *Listeria* and *Staphylococcus aureus* were not present at 30 days of maturation.

For color (Table 3), there was no difference for parameters L *, a * and b *, therefore no color change was observed in function of cheese fermentation by *Streptococcus* or *Lactobacillus helveticus*.

The ABTS radical antioxidant capacity values in the Trolox equivalent (ETμM) or ABTS radical degradation percentage (%) showed no significant difference for treatment, time and treatment x time interaction (p> 0, 05), being found a smaller value in 20 days of maturation (428.703 EtμM).

The texture parameter values were not significant for treatment, time and treatment x time interaction for any of the observed parameters (Table 4).

Fatty acids such as C20: 3n6; C20: 3n3; C22: 1 n9; C20: 4 n6; C23: 0; C20: 5n3; C24: 1 and C22: 6 n3 were sporadically found but were used to calculate monounsaturated, saturated and polyunsaturated fatty acid values.

There was a significant difference in fatty acid profile between treatments (p <0.05), with *Streptococcus thermophilus* and *Lactobacillus helveticus* treatment with the highest levels of C18: 0 (Stearic Acid) observed with 11.877mg / g, C18: 1. n c (Oleic Acid) with 24,971 mg / g. The n3: n6 ratio was significant for treatment (p <0.05), with the highest values observed in Lh + St treated cheese.

The highest C18: 3 n3 (alpha-linolenic acid) content 0.7835 mg / g was in the Lh treatment compared to 0.1975 mg / g in the St + Lh treatment. The milk had an average of 0.2387 mg / g of this fatty acid, and the cheese produced with Lh with an average of 0.7835 mg / g, being considered a four times higher concentration, evidencing this fatty acid production by the metabolic pathways during the fermentation.

The PUFA: SUFA ratio was higher for Lh treatment (0.0926 mg / g), and there were no significant differences for treatment (p> 0.005) for polyunsaturated fatty acids.

Saturated fatty acids presented lower content in cheese produced with *L. helveticus* (27.9854 mg / g) compared to the treatment containing *S. thermophilus* and *L. helveticus*, (63.1508 mg / g). The monounsaturated fatty acids concentration was higher

in the Lh treatment 68.980 mg / g, and may be the influencing cause in the higher polyunsaturated fatty acids contents in the PUFA: SUFA ratio.

DISCUSSION

The evaluated cheeses were classified as containing medium and low humidity (BRASIL, 1996). According to Salazar-Montoya et al. (2018), evaluating the use of *Lactococcus lactis* during the Manchego cheese ripened, observed that, after 15 days of maturation, there was a gradual decrease of moisture, as a result of syneresis, provided by the protein network rearrangement and resulting in the whey expulsion, a behavior similar to that observed in the experiment.

The cheese produced showed a gradual reduction in moisture content up to 30 days of maturation. Cheese moisture is directly responsible for its consistency and, during the ripened, the dehydration intensity depends on the cheese size and shape, as well as the environment conditions under which ripened occurs (Beresford et al. 2001).

The ash content is in accordance with the recommended literature for fresh cheese, which ranges from 1.0% to 6.0% (Gomes, 1997). Ferreira and de Freitas Filho (2008) and Uliana and Rosa (2009) obtained ash content for colonial cheese and rennet from 3.85% to 4.31% and 2.77% to 2.87% respectively.

The pH values variation depends on the cheese's buffering capacity and also on the amount of protein and minerals (Narimatsu et al., 2003), and may be justified because there are differences between treatments for these parameters and variations over ripening time (Table 3). The acidification process continued during maturation, which, except for the high lactic acid production, mainly by *S.thermophilus*, is related to the low buffering capacity of the cheese mass (Havranek et al., 2014).

Regarding the increasing acidity levels these can be justified by the possible action of endogenous bacteria that degrade lactose, having as end product CO_2 and lactic acid, which increases cheese acidity (Faion et al., 2015).

Manufactured cheeses are generally pressed by hand, thus presenting whey retention, interfering with the lactose amount eliminated through the whey (Alinovi et al., 2018), thus there is less lactose elimination, with a higher substrate amount for fermentation with increased lactic acid production, and may justify the standardization lack for acidity.

Another major factor in the cheeses physicochemical composition is in relation to the AW of them that presented a fast decrease, which may be justified due to evaporative water loss, protein hydrolysis by peptides to amino acids and triglycerides to fatty acids (Beresford et al., 2001).

Lactic bacteria are important in cheese making, since the lactic acid production, which accelerates milk coagulation, aiding in syneresis, also contributes to taste, shape and texture (Awad et al., 2007). During cheese manufacture and ripening, the lactic microflora composition undergoes to various changes, depending on environmental conditions, such as increased lactic acid and decreased pH, which tend to decrease its counts (Di Cagno, et al., 2006).

According to Delamare et al. (2012), evaluating Serrano cheese in relation to mesophilic bacteria, group to which lactic acid bacteria belong, obtained values from 4.0 to 9.0 log CFU / g in cheeses produced with unpasteurized milk. According to Paiva et al. (2015), who evaluated the natural yeasts addition in the lactic acid bacteria count of Minas de Serro manufactured cheese during 60 days of ripened, they observed a decrease in LAB during the storage period from 8.20 log CFU / g to 7 .90 $\log_{10}$ on 30 day of

storage. The ripened cheeses presented both treatments (6.479 log_{10} and 6.683 log_{10}, respectively) viable over the storage time.

Although proteolytic bacteria did not show significant differences between the evaluated treatments ($p > 0.05$), they are important factors to be considered during cheese ripening. Proteolysis is a prerequisite for lactic acid bacteria growth and subsequent degradation of milk proteins (casein), leading to the release of peptides and free amino acids (Forsythe, 2002; Moulay et al., 2006). importance in cheese ripening and in the development of the flavor, aroma and texture characteristic of the finished product (Forsythe, 2002).

In milk fermentation, the *S. thermophilus* role is related to its rapid conversion of lactose to lactic acid, causing rapid pH decrease and production of other metabolites with important technological properties, such as exopolysaccharides and bacteriocins, which may be contributing factors to decreased coliform amount on products (Delorme, 2008).

The cheese color (Table 4) may be related to the different internal and external factors, the cheese opacity degree (L *) may be influenced by the internal aggregation degree of the cheese protein matrix, that the more hydrated and the lower the centers number that allow light scattering, making them darker, which can be attributed to the chemical changes that occur during ripening, since it is a biologically active product.

According to Ginzinger et al. (1999) the color parameter b * is strongly correlated with the yellow color that appears in cheese which may be related to the ripening time. Buffa et al. (2001) analyzed the color change of goat cheese with and without pasteurization during the 60-day maturation and found that the a * value, tending to red, remained constant until 30 days (0.55), the values of L decreased (91.53) and b *,

tending to yellow, increased (8.51). In the present experiment L * values decreased, a * values increased and b * values decreased.

Elasticity may be expressed as a measure of recovery from the original condition, undeformed, after the first compressive force is removed and cohesion is considered to be a measure of the extent to which cheese may be deformed before breaking (Ong et al., 2012). These parameters are affected by milk composition, cheese production and microorganism action, maturation conditions and mainly moisture, pH and soluble calcium (Lucey et al., 2003; McMahon et al., 2005). The elasticity values remained constant with the storage time, showing that the biochemical reactions that occurred inside the cheese were not sufficient to modify the final structure, giving higher or less flexibility and differentiating both treatments.

Pinho et al. (2004), evaluating the texture profile during 60 days of ripening of Terrincho cheese, observed that during the first 20 days of ripening there was an increase in hardness, fracturability, gomosity, chewability and, in contrast, decreased adhesiveness, elasticity, cohesiveness and after 20 days of ripening changes in these values. According to the authors, the change in texture after this ripening period was attributed to a decrease in pH below 5.5.

The ABTS radical sequestration method measures the antioxidant activity of compounds with hydrophilic and lipophilic nature (Gülçin et al., 2010; Karadag et al., 2009). The annatto seed supplied to animals in diet has a carotenoid, bixin (Bixa orellana L), which has antioxidant potential (Nozière et al., 2006), with extensive double bond chain, provides various electronic distributions that allow the free radicals addition to the adjacent carbons in the unsaturation, giving greater reactivity of these molecules to oxidizing agents, especially oxygenated derivatives, providing higher stability (Kiokias and Gordon, 2013).

Carotenoids from annatto can be degraded when exposed to light or subjected to high temperatures (Satyanarayana et al., 2003). Colonial cheeses during the manufacturing process are subjected to high temperatures and stored at low temperatures during ripening, which may have influenced the antioxidant capacity of annatto (Rocha Garcia et al., 2012). In the evaluated cheeses there was no significant difference for treatment ($p > 0.05$), time ($p > 0.05$) and for treatment x time interaction ($p < 0.05$).

Moreira (2013) verified the ABTS free radical inhibition percentage of the ethanolic extracts of three different annatto seeds, using as control the synthetic antioxidant BHT was 84.99 ± 1.01% inhibition, 82.46 ± 1.43% and 72.51 ± 1.15%.

In general, cheeses are related to the high saturated fatty acids concentration; However, they are also found to have the unsaturated fatty acids presence that are important to health, such as oleic acid and conjugated linoleic acid (CLA). The fatty acids composition in cheese varies according to animal breed, time of year, animal diet and species, as well as cheese manufacturing processes (yeast used and ripening time) (González-Martín et al., 2017).

However, factors such as ripening and starter bacteria use may be factors of changes in the cheese's lipid profile, Morrone et al. (2012) studied cheeses with longer ripening time (354 days) as "Pecorino" cheese, and found in the fatty acids profile, high monounsaturated and polyunsaturated fatty acids, mainly conjugated linoleic acid with 0.5%.

Other authors as Tonial et al. 2009; Aguilar et al. 2014 and Arslan et al. 2014 observed that the prevalence in the lipid profile of ruminant products, milk and various cheese varieties was of saturated fatty acids (SFA) followed by monounsaturated and polyunsaturated fatty acids.

Perotti et al., 2008 evaluated the free fatty acids profile (C6: 0 to C18: 2) at different ripening times (90 and 180 days) in "Reggianito Argentino" cheeses made with natural whey and with different strains of "*Lactobacillus helveticus*" (Lh 133, Lh 138 and Lh 209), and these did not present significant differences for the different yeasts types used but presented differences for the evaluated times, which may suggest that the bacteria types used in cheese production are not influencing the fatty acid profile in cheese.

The cheeses produced in this experiment with *S. thermophilus* and *L.helveticus* showed similar behavior for the high value observed for saturated fatty acids (SUFA: 63.1508 mg / g fat) as observed by Carafa et al., 2019 who evaluated 4 cheeses types produced with the action of 4 starter cultures on Mountain cheese, *Lactococcus lactis subsp. lactis 68, Streptococcus thermophilus 93* and *Lactobacillus rhamnosus BT68*, cheeses without culture addition, cheeses with culture addition in the vat and cheeses with low and high amount of culture were produced. The palmitic fatty acids (14: 0), myristic (16: 0) and stearic (18: 0) showed high concentrations, thus ensuring high amounts of saturated fatty acids (SUFA) in all treatments after 7 months of maturation (59.0, 61.2, 59.9 and 62.1g / 100g).

The recommended n6: n3 ratio for humans in diets should be in the ratio of 2: 1 to 3: 1, as it presents the possibility of greater conversion of alpha-linolenic acid to docosahexaenoic acid (ADH), which reaches its maximum value around 2.3: 1 (Masters, 1996). Diets based on n-6: n-3 ratios of less than 1: 1 are not recommended because they inhibit the transformation of linoleic acid into very long chain polyunsaturated fatty acids (Martin et al., 2006).

CONCLUSION

Ripened cheeses produced with *L. helveticus* and *S. thermophilus* showed desirable physicochemical and microbiological characteristics, maintaining product quality for 30 days. Regarding the fatty acid profile, the ripened cheese produced with the *Lactobacillus helveticus* addition obtained lower saturated fatty acids content and higher monounsaturated fatty acids with higher PUFA: SUFA ratio.

APPENDICES

Table 1. Physicochemical composition of milk used for ripened cheese production.

	TREATMENT			
Variables	P1	P2	P3	P4
Milk Production (kg / day)	12.43	10.96	15.40	17.80
Total Solids (% m / m)	10.74	11.22	11.86	10.20
Fat (% m / m)	3.80	3.75	4.41	3.11
Lactose (% m / m)	3.76	3.94	4.06	3.86
Protein (% m / m)	3.18	3.53	3.39	3.23
pH	6.62	6.51	6.59	6.78

* averages are presented in columns by periods; P1: period1; P2: period 2; P3: period 3; P4: period 4.

Table 2. Fatty acid profile in milk used to produce ripened cheeses.

Variables	mg/g	Variables	mg/g
C6:0	0.1637	C18:2 n6c	1.4497
C8:0	0.3016	C20:0	0.1651
C10:0	1.5347	C18:3 n6	0.0409
C11:0	0.0502	C20:1	0.0503
C12:0	2.4334	C18:3 n3	0.2387
C13:0	0.1005	C21:0	0.7526
C14:0	10.5757	C20:2	0.0289
C14:1	0.7296	C20:3 n6	0.0298
C15:0	0.8831	C20:3 n3	0.0046
C15:1	0.0101	C20:4 n6	0.0944
C16:0	28.2870	C23:0	0.0042
C16:1	1.7830	C24:0	0.0129
C17:0	0.7405	C20:5 n3	0.0101
C17:1	0.2226	C24:1	0.0092
C18:0	14.0820	SUFA	60.0872
C18:1 n9t	5.5840	MUFA	37.8462
C18:1 n9c	29.4287	PUFA	2.0665
C18:2 n6t	0.1981		

*SUFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids; C6: 0: Caproic acid; C8: 0 Caprylic acid; C10: 0 Capric acid; C11: 0 Hendecanoic acid C12: 0 Lauric acid; C14: 0: Myristic acid; C14: 1 Myristoleic acid; C15: 0: Pentadecylic acid; C16: 0 Palmitic acid; C16: 1: Palmitoleic acid; C17: 0 Marginic acid C17: 1:; C18: 0: Stearic acid; C18: 1 n9t: Elaidic acid; C18: 1 No 9t: Oleic acid; C18: 2 n6t: Linoleic acid; C21: 0; C20: 2: 11.14-Eicosadienoic acid; C20: 3 n6: Dihomo-y-linolenic acid; C20: 3 n3: Dihomo- (α-) linolenic acid; C20: 4 n6:

Arachidonic acid; C23: 0; C24: 0: Lignoceric acid; C20: 5 n3: 5.8, II, 14.17-eicosapentaenoic acid (EPA); C24: 1: Nervic acid.

Table 3. Physicochemical composition of cheese.

Parameters	TREATMENTS		TIME					P-value		
	St+Lh	Lh	0	10	20	30	SEM	Treat	Time	Treat *Time
DM	61.827±12.420	61.105±10.147	45.414±3.286	66.234±5.165	68.111±3.351	66.182±10.481	2.913	0.2273	<.0001	0.0834
MOISTURE	37.590±12.237	39.595±8.625	53.903±3.987	35.015±6.231	33.317±4.493	35.150±10.621	2.671	0.0611	<.0001	0.0386
ASH	5.830±4.547	4.237±1.128	3.709±0.779	4.986±1.017	5.746±1.306	6.358±6.755	0.771	0.0979	0.2324	0.4065
CP	23.445±3.913	25.434±12.30	26.404±7.240	26.694±14.723	21.654±4.769	22.847±5.102	2.2704	0.3919	0.3540	0.2342
FAT	25.414±8.765	24.621±6.922	19.449±4.646	28.085±6.923	23.630±8.541	27.814±7.644	2.0521	0.6444	0.0121	0.4625
pH	5.318±1.008 [b]	5.801±0.401 [a]	5.872±0.742	5.561±0.480	5.478±0.265	5.338±1.331	0.1804	0.0108	0.2319	0.1478
Aw	0.8837± 0.0326	0.934±0.038	0.9758±0.015	0.938±0.040	0.929±0.014	0.888±0.027	0.0678	0.5217	<.0001	0.2731
ACIDITY	23.692±3.050	22.322±1.152	10.437±0.621	21.928±0.720	25.357±1.130	7.960±1.574	0.5615	0.2076	0.0801	0.1125
COLIFORMS (Log $_{10}$)	5.613±0.924 [b]	6.372±1.060 [a]	6.278±0.743	6.518±1.043	5.943±1.014	5.227±0.992	0.2499	0.0011	0.0007	0.2764
Proteolitc Bac (Log$_{10}$)	5.978±1.727	5.491±1.664	6.266±1.500	4.909±1.564	6.138±1.857	5.263±1.606	0.4491	0.3952	0.1663	0.9790
LAB (Log $_{10}$)	6.479±1.104	6.683±0.653	6.286±0.325	7.166±0.599	6.564±0.818	6.433±1.385	0.2347	0.4667	0.0697	0.2716
Color L*	76.517±17.924	78.982±10.29	83.039±8.154	79.768±8.694	76.351±12.110	68.790±15.457	1.928	0.3369	0.3369	0.338
Color a*	1.274±3.589	0.287±1.224	0.706±1.182	0.715±0.634	0.280±0.955	1.666±6.032	3.7376	0.0782	0.5076	0.1963
Color b*	13.735±5.390	14.615±3.223	13.645±4.321	14.976±5.701	14.788±2.590	12.675±5.405	1.17208	0.3998	0.6943	0.5565
ABTS (%)	17.915±10.659	20.453±11.940	17.671±9.805	21.369±7.133	16.934±12.978	20.761±14.328	2.824	0.3854	0.6316	0.7520
ABTS (Eq)	448.544±215.742	499.924±241.682	443.605±198.453	518.466±144.377	428.703±262.690	506.162±290.004	57.178	0.3854	0.6316	0.7520

*the averages are present in the lines; Tukey test P <0.05; Aw: 0.97426x-0.00279 R^2: 0.3467; DM: 44.94076x^2+ 2.65068x-0.06984 R^2: 0.6416; Humidity: 55.0524 x^2-2.65068 x + 0.06984 R^2: 0.6416; FAT: 19.9777 x^2 + 0.43245 x-0.01082 R^2: 0.0344; COLIFORMS: 6.2633 x^2 + 0.04827x - 0.00303 R^2: 0.2798.

Table 4. Instrumental texture parameters of ripened cheese.

Parameters	TREATMENTS		TIME					P-value		
	St+Lh	Lh	0	10	20	30	SEM	TREAT	TIME	TREAT *TIME
HARDNESS (N)	13456± 1162	10028± 1151	1498.33±552.45	13826±12463	15405±11745.0	16958±11109.2	2362	0.4816	0.1239	0.8854
COHESIVINESS	0.7669± 0.175	1.095±0.836	1385± 1.1505	0.7925±0.067	0.737±0.227	0.8250±0.157	0,1848	0.1883	0.2176	0.5717
ELASTICITY	4.061± 0.492	4.193±0.284	4.120± 0.198	4.243± 0.304	3.994±0.656	4.122±0.283	0,8249	0.7335	0.8025	0.2637
GOMOSITY	8914.8±7542	7643.83±9156	1154.33±602.62	9440.75±531.5	9987.00±7043.9	13814.50±587.3	1660	0.6647	0.1156	0.9440
CHEWABILITY (N)	366.000±323.95	321.36±392.43	473.83±25.406	412.987± 436.4	403.014± 301.1	551.275±327.9	68,91	0.6751	0.1505	0.9188

* Treatment St+Lh: *Lactobacillus helveticus* and *Streptococcus thermophilus*; Treatment Lh: *Lactobacillus helveticus*.

Table 5. Fatty acid profile in ripened cheese samples (mg / g fat).

Parameters	TREATMENTS		TIME					P-value		
	St+Lh	Lh	0	10	20	30	SEM	TREAT	TIME	TREAT *TIME
C6:0	0.6277±0.237 [b]	0.7385±0.246 [a]	0.4891±0.329	0.7839±0.117	0.7479±0.191	0.7113±0.225	0.049	<.0001	0.4624	0.7874
C8:0	0.6912±0.196 [b]	2.2427±0.548 [a]	1.2338±0.039	1.5683±0.966	1.6097±0.979	1.4560±0.748	0.181	<.0001	0.4624	0.7874
C10:0	2.2205±0.534 [a]	0.2010±0.093 [b]	0.9614±0.984	1.1737±0.037	1.3208±0.230	1.3871±0.360	0.224	<.0001	0.2212	0.3151
C11:0	0.1919±0.059 [b]	3.1563±0.578 [a]	1.5099±1.623	1.7653±1.761	1.7948±1.758	1.6263±1.547	0.319	<.0001	0.6748	0.8049
C12:0	3.1154±0.612 [a]	0.14380±0.026 [b]	1.4436±1.511	1.4825±1.463	1.7532±1.810	1.8391±1.892	0.321	<.0001	0.3003	0.3218
C13:0	0.1413±0.024 [b]	13.1013±2.001 [a]	6.0316±6.572	6.811±7.605	7.0874±7.613	6.5548±7.114	1.380	<.0001	0.6717	0.6854
C14:0	12.462±1.880 [a]	1.0595±0.223 [b]	7.1147±6.268	6.1613±5.542	7.12158±6.694	7.2830±6.933	1.218	<.0001	0.4603	0.4977
C14:1	0.9875±0.1011 [b]	1.1488±0.1585 [a]	0.9904±0.1170	1.091±0.148	1.1053±0.191	1.0856±0.162	0.031	0.0122	0.4896	0.7224
C15:0	1.0582±0.053	0.0628±0.0962	0.5865±0.5239	0.5818±0.525	0.53044±0.567	0.5433±0.583	0.104	<.0001	0.5463	0.4305
C15:1	0.0698±0.1555 [b]	33.202±4.6182 [a]	15.1071±6.547	16.6094±8.616	17.985±9.688	16.841±8.741	3.515	<.0001	0.5554	0.6005
C16:0	2.0827±0.529 [a]	0.80517±0.136 [b]	1.4291±0.837	1.5731±0.906	1.4024±0.714	1.3709±0.7433	0.153	<.0001	0.8460	0.6440
C16:1	2.0827± 0.529 [a]	0.8051± 0.136 [b]	1.429± 0.837	1.573± 0.906	1.402±0.714	1.370± 0.743	0.135	<.0001	0.8460	0.6440

C17:0	0.7155±0.086 [a]	0.3883±0.088 [b]	0.54048±0.240	0.5700±0.207	0.5617±0.174	0.5354±0.171	0.038	<.0001	0.8915	0.2933
C17:1	0.3323±0.0886 [b]	12.4666±2.093 [a]	5.9254±0.105	6.1623±0.527	6.9124±0.415	6.5978±0.126	1.299	<.0001	0.7138	0.6485
C18:0	11.787± 1.252 [a]	6.077± 0.748 [b]	9.2845± 3.386	8.853± 2.674	9.805± 3.596	9.4285± 3.513	0.628	<.0001	0.5854	0.7335
C18:1 n9t	5.776±0.816 [b]	20.806±8.192 [a]	16.0257±11.012	13.2657±11.203	10.686±6.957	13.1875±10.387	1.951	<.0001	0.5303	0.6486
C18:1 n9c	24.971±5.435 [a]	0.3241±0.099 [b]	11.9172±14.281	14.2151±15.239	12.4843±13.770	11.974±12.806	2.681	<.0001	0.7602	0.7549
C18:2 n6t	0.3183±0.088 [b]	1.5241±0.328 [a]	0.7943±0.5197	0.9206±0.741	1.0139±0.798	0.9561±0.714	0.134	<.0001	0.4763	0.4473
C18:2 n6c	1.3368±0.258 [b]	0.1445±0.035 [b]	0.8326±0.804	0.7311±0.651	0.7064±0.630	0.6926±0.609	0.129	<.0001	0.5766	0.4008
C20:0	0.1303±0.026 [a]	0.0507±0.015 [b]	0.1019±0.066	0.0787±0.040	0.0940±0.0408	0.0876±0.039	0.009	<.0001	0.2857	0.3267
C18:3 n6	0.0422±0.013	0.0539±0.019	0.0492±0.0152	0.0463±0.0102	0.0500±0.023	0.0467±0.022	0.003	0.0840	0.9684	0.0638
C18:3 n3	0.1975±0.051 [b]	0.7385±0.447 [a]	0.4169±0.3195	0.4308±0.3136	0.5489±0.5452	0.4754±0.536	0.085	0.0021	0.9202	0.8901
C20:1	0.2199±0.067 [a]	0.0445±0.016 [b]	0.1119±0.4645	0.1369±0.121	0.1671±0.114	0.15250.117	0.020	<.0001	0.2857	0.3267
C21:0	0.5884±0.2386 [a]	0.0445±0.0164 [b]	0.4113±0.464	0.3302±0.358	0.2641±0.254	0.2603±0.230	0.065	<.0001	0.3788	0.3404
C20:2	0.0328±0.007	0.0412±0.015	0.0379±0.005	0.0336±0.010	0.0362±0.014	0.0348±0.013	0.002	0.2526	0.9547	0.9307
C22:0	0.0361±0.010	0.1069±0.292	0.1989±0.409	0.0243±0.008	0.0276±0.009	0.0246±0.006	0.048	0.6166	0.6830	<.0001
C22:2	0.0046±0.002 [b]	0.0106±0.003 [a]	0.0069±0.004	0.0080±0.003	0.0085±0.005	0.0110±0.003	0.009	0.0623	0.9448	<.0001
C24:0	0.0128±0.007	0.0110±0.005	0.0139±0.008	0.0101±0.003	0.0135±0.006	0.0096±0.006	0.001	0.9071	0.8172	0.7259
SFA	63.1508±5.124[a]	27.9854±2.355[b]	45.7236±20.556	44.0263±17.414	46.5781±19.479	45.9444±21.079	3.751	<.0001	0.7646	0.6247
MUFA	34.2610±5.209 [b]	68.980±2.266 [a]	51.5107±20.938	53.0547±17.066	50.7322±19.071	51.1861±20.506	3.707	<.0001	0.8096	0.6284
PUFA	2.011±0.366	2.561±0.789	2.2107±0.484	2.2292±0.533	2.4316±0.880	2.2742±0.835	0.135	0.0553	0.9329	0.3910
PUFA: SUFA	0.0319±0.005 [b]	0.0926±0.031 [a]	0.0573±0.030	0.0604±0.032	0.0669±0.049	0.0644±0.046	0.007	<.0001	0.9135	0.6763
n6: n3	0.2235±0.084 [a]	0.0851±0.027 [b]	0.1787±0.136	0.1647±0.102	0.1277±0.051	0.1462±0.080	0.019	<.0001	0.5281	0.2849

* SUFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids; C6: 0: Caproic acid; C8: 0 Caprylic acid; C10: 0 Capric acid; C11: 0 Hendecanoic acid C12: 0 Lauric acid; C13: 0: -; C14: 0: Myristic acid; C14: 1 Myristoleic acid; C15: 0: Pentadecylic acid; C15: 1: -; C16: 0 Palmitic acid; C16: 1: Palmitoleic acid; C17: 0 Marginic acid C17: 1 -:; C18: 0: Stearic acid; C18: 1 nt: Elaidic acid C18: 1 nt: Oleic acid; C18: 2 n6t: Linolelaidic acid; C18: 2 n6c Linoleic acid; C18: 3 n6: Y-linolenic acid; C18: 3 n3: (α) linolenic acid; C20: 0: - C21: 0-; C20: 2: 11.14-Eicosadienoic acid; C20: 3 n6: Dihomo-y-linolenic acid; C20: 3 n3: Dihomo- (α-) linolenic acid; C20: 4 n6: Arachidonic acid; C22: 0: Behenic acid; C22: 2: 13.16-docosadienoic acid C23: 0: -; C24: 0: Lignoceric acid; C20: 5 n3: 5.8, II, 14.17-eicosapentaenoic acid (EPA); C24: 1: Nervic acid

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Sodium Alginate with Turmeric Bioactive coating for Ripened Cheeses

ABSTRACT: The bioactive edible coating use appears as technological innovation in the dairy derivatives market to improve quality and increasing the products shelf life. The objective of this work was to evaluate the physicochemical and microbiological characteristics of ripened cheese with sodium alginate and turmeric coating addition produced with commercial culture of *Lactobacillus helveticus*. The coatings were evaluated for mechanical properties, water steam permeability and sorption isotherm. The experimental design was completely randomized and the treatments consisted of sodium alginate and turmeric 1% (AGAT) edible cover and the other one without edible cover (SEMC), data were analyzed by the SAS 9.3 program. The total coliform count result was significant for storage period. Lactic acid bacteria remained viable in both treatments and were reduced according to the storage time of 30 days. For instrumental color, there was no significant difference between treatments. Coverage significantly altered hardness, gomosity, chewability and cohesiveness over time, while elasticity was not affected. The coating presence was not significant for water steam permeability and mechanical properties. The sodium alginate and 1% turmeric solution coating application on ripened cheeses did not effectively improve microbiological quality, however, coated cheese samples showed increased lactic acid bacteria, increased water activity and improved cheese texture, making them softer, with less elasticity, cohesion and chewing.

Keyword: active packaging, *Lactobacillus helveticus*, microbiological quality.

1. INTRODUCTION

In the food sector there is great interest in the development and characterization of edible coatings, by their potential to include natural / synthetic molecules that provide product improvements, increasing shelf life and improving their sensory characteristics

(Elizondo, Sobral & Menegalli, 2009). The use of edible coatings to cover surfaces is a treatment that involves product quality and safety protection during its processing and commercialization, until the final consumer (Youssef, 2013).

Due to the growing demand for sustainability and environmental safety, studies are developed to improve food packaging materials and replace materials that may damage the environment (Majeed et al., 2013).

Edible coatings can be made with proteins, polysaccharides and lipids, in individual or combined mixtures, each one presenting their particularities, advantages and limitations (Follain et al., 2013).

Among the most used polymers stands out sodium alginate. This forms a strong edible coating, despite the negative charge on the molecule (Nieto, 2009) and has been shown to be promising ecologically correct because of its biodegradability without harming the environment (Tang, Kumar, Alavi, & Sandeep, 2012).

The active packaging may include synthetic or natural molecules that are responsible for providing improvements to the final product, in the microbiological conditions and consequent increase the shelf life. Curcumin is a natural colorant found in *Curcuma longa L.* rhizomes and is one of the components found in turmeric that has important biological activities in food preservation (Cecilio Filho, Souza, Braz, & Tavares, 2000), antimicrobial, antifungal, insecticides, anti-inflammatory and antioxidants properties (Ferreira et al., 2013).

For cheese, packaging must provide common product protection against mechanical damage and poor environmental conditions through handling and distribution. Ideal packaging solutions can prevent or minimize quality changes, resulting in longer shelf life and quality preservation (Youssef, El-Sayed, Salama, El-Sayed, & Dufresne, 2015).

The objective of this work was to evaluate the ripened cheese production containing commercial culture of *Lactobacillus helveticus* and cover application, its physicochemical and microbiological characteristics during the storage period for 30 days. The edible coatings properties obtained from alginate cover with or without turmeric 1% were also evaluated.

2. MATERIAL AND METHODS

The experiment was carried out at the milk quality laboratory, which belongs to the Mesoregional Center for Milk Excellence and Technology (CMETL-UEM).

2.1 CHEESES PREPARATION

During two days, the milk was collected at the Iguatemi Experimental Farm FEI, for cheese production and for chemical composition analysis. The culture "Lyofast LH 091", SACCO® (*Lactobacillus helveticus*), 05UC / 100 liters of milk was used.

To prepare cheese, 25 liters pasteurized milk (65 ° C / 30min), calcium chloride (50 ml per 100 liters milk), LH culture (05UC / 100 liters) and liquid coagulant (9 ml coagulant for 10 liters of milk, HA-LA®-CHR brand, Denmark) were used. After cutting, the mass was baked at 45 ° C and then formed (JandaPlast, model RH-1000). The treatments were cheese without edible coating (SEMC) and with sodium alginate plus 1% turmeric solution (AGAT) coating. The cheeses were kept in BOD for 30 days / 12 ° C and relative humidity of ± 60.5%.

2.2 PREPARING THE EDIBLE COVERAGE SOLUTION

For coating preparation it was used 2% (w / w) sodium alginate and 2% (w / w) calcium chloride according to methodology described by Meneghel, Benassi, & Yamashita, (2008). It was included in the edible cover an turmeric alcoholic solution prepared with 10 grams of turmeric (produced in Maringá-Paraná region, non-commercial) and the powder solubilized in 100 ml of ethyl alcohol remained in constant

stirring for 24 hours without light, and then filtered through a qualitative filter and added in the amount of 1% in the sodium alginate solution.

2.3 CHEESE ANALYSIS

At ripened time of 0, 15 and 30 days, the water activity (Aqualab® 4TE, Decagon, São Paulo, Brazil), pH (digital pH meter, Tecnal Tec-5), titratable acidity (Lutz, 2008), color (Konica Minolta), dry matter, mineral matter (AOAC, 1992) and instrumental texture analysis were determined in cheese samples.

The instrumental color was determined through a Konica Minolta chromometer (Konica Minolta®, Model CR 400/410, Japan) using the CIELAB system (CIE, 1986). The measurements were made in triplicate with the equipment previously calibrated, using internal and external cheese part.

The Brookfield-TC III Texture Analyzer (Engineering Laboratories, INC., Middleboro, MA, USA) was used to analyze the cheese texture profile (TPA) in the following configurations: TPA; test speed: 1 mm / s; compression distance 5 mm; 38 mm acrylic cylindrical TA4 probe. The variables measured for TPA were hardness, chewability, cohesiveness and elasticity.

For microbiological analysis, samples were diluted in 0.1% peptone water and lactic acid bacteria (MRS Lactobacillus, Himedia-De Man, Rogosa and Sharpe agar) and coliforms seeded on VRB agar (VRB agar, Himedia - Violet Reb) were evaluated. Bile Agar), both incubated at 35 ° C for 48 hours (AOAC, 1992).

2.4 COATING ANALYSIS

The treatments evaluated were sodium alginate coating (PAG) and sodium alginate coating + 1% turmeric solution (PAGAT).

The following analyzes were performed: coating thickness evaluation, mechanical properties (Pavlath, Voisin, & Robertson, 1999), water stem permeability (SP), sorption isotherm and electron microscopy (SEM).

In order to characterize the coating hygroscopic behavior and mechanical properties, the prepared solution (2% sodium alginate) was deposited in suitable containers in a quantity of 150 ml (acrylic plates, rectangular format 20 x 20 cm) and oven dried. at 45ºC for 24 hours for coating formation.

The coatings thickness was evaluated manually by a digital micrometer (Mitutoyo, resolution 0.01 mm - São Paulo - SP). Ten random points of the coating sample area were evaluated, and the final result was the measurements arithmetic mean.

The samples were conditioned at 53% relative humidity at 25 ° C for three days in B.O.D, to determine mechanical properties. Tensile properties were determined using a Stable Micro System (TAXT2i - England) instrumental texturometer using a methodology based on ASTM (1996). The tensile properties determined were: maximum tensile strength at break (MPa), elongation at break (%) and elasticity modulus (MPa).

Water steam permeability was determined gravimetrically according to the ASTM (1995) method with some modifications. The relative humidity gradient used was 2-53% at 25 °C, where samples fixed in aluminum capsules were weighed until the mass gain rate was constant. The water vapor permeability rate was determined according to equation 1.

$$\mathrm{TPVA} = \frac{\mathrm{m}}{\mathrm{t}} . \frac{1}{\mathrm{A}} \quad (1)$$

Where m / t is the angular coefficient of the mass gain line (g) versus time (h) and A (m2) is the coating permeability area. Thus, the water steam permeability value can be calculated according to equation 2:

$$PVA = \left[\frac{TPVA \,.e}{p_s \,.(RH_{out} - RH_{ins})}\right] x100^{(2)}$$

Being PVA the water steam permeability (gm/m2.Pa.h.), TPVA (water steam permeability rate) (g/m2.h), e is the average coating thickness (average of 6 measurements) (m), ps is the vapor saturation pressure at the test temperature (Pa), RH_{out} the relative humidity outside the capsule (%) and RH_{ins} the relative humidity inside the capsule.

For sorption isotherm analysis, samples of about 0.5 g were conditioned for 20 days in anhydrous calcium chloride, after the drying period the samples were evaluated in different desiccators containing 11.3% (sodium chloride), 33% (magnesium chloride), 43% (potassium carbonate), 53% (magnesium nitrate), 64% (sodium nitrate), 75% KCL and BaCl (GAB) and LiCl. The Guggenheim-Anderson-de-Boer model was used. (GAB) to calculate sample concentration and to adjust data (Takahashi et al., 2017) (EQUATION 3).

$$Xw = m0.C.K.Aw/[(1 - K.Aw).(1 - K.Aw + C.K.Aw)] \quad (3)$$

Where Xw (g water / g dry matter) is the moisture contend equilibrium; m0 is the value of the monolayer; aquatic activity; C and K, Guggenheim constants representing the sorption heat in the first layer and the sorption heat of the multilayers, respectively. All tests were performed in triplicate.

For microstructure analysis by electron microscopy, the Quanta 250 Scanning Electron Microscope (Fisher Scientific-FEI, Oregon, USA) was used on the 1000-fold lens. The samples were previously dried in calcium chloride and then surface and fracture areas were evaluated, where the fracture was obtained by sample cryogenic freezing (liquid N_2) followed by breaking.

2.5 STATISTICAL ANALYSIS

The data were analyzed using Proc GLM SAS 9.3 (2013) testing the treatments, times and the interaction between treatment and time.

3.RESULTS

For coatings characterization, samples of sodium alginate (PAG) and sodium alginate and 1% turmeric (PAGAT) (Tables 1 and 2) were analyzed and the cheese evaluations were performed with uncoated samples (SEMC) and covered with 1% sodium alginate and turmeric (AGAT).

The values of rupture stress, elongation, Young's modulus, thickness and water steam permeability (SP). showed no significant differences for the PAG control (without addition of 1% turmeric alcohol solution) and PAGAT treatment (with addition of 1% alcohol solution).

The adsorption isotherms values of sodium alginate (PAG) and sodium alginate and turmeric 1% (PAGAT) were considered good because they had relative average deviations below 5% and therefore, the model tested was perfectly adapted to experimental data, GAB model. The adsorption isotherm (Figure 2 A and B) for evaluated coating was sigmoidal with a slight increase in moisture content due to the water activity increase.

In the evaluated cheeses, the dry matter (DM) content increased in function of storage days with consequent decrease of moisture values ($p < 0.0001$). The water activity (Aw), total coliforms (TC) and lactic acid bacteria (LAB) values were significant for treatments. For ripened times, values were significant for DM, humidity, pH, Aw and coliforms (Table 3).

For lactic acid bacterial counts, significant differences were observed for treatment ($p = 0.0469$) and for the interaction between treatment x time ($p < 0.0001$)

remaining constant and viable throughout the storage time. In total coliform counts, there were significant differences for treatments ($p < 0.0001$) and ripened times ($p < 0.0001$), with reduction in counts during the ripened time (Table 3).

For color, there was no difference between treatments for parameters L *, a * and b *, only in relation to storage times. For the treatment x time interaction, the parameter a * color was significant ($p = 0.0014$) (Table 4).

The hardness, gomosity and chewability parameters values were significant for treatment, for storage time, the hardness, cohesiveness, gomosity and chewability were significant (Table 4).

1A Surface photos and 1B of 1% sodium alginate and turmeric coating fraction were obtained by scanning electron microscope (SEM) at 500-fold magnification.

4. DISCUSSION

Sodium alginate is a natural linear polysaccharide that has good moisture retention, gel formation and biocompatibility (Skurtys et al., 2014).

The observed values for rupture tensile strength (MPa) for PAG treatment (39.66MPa) and PAGT treatment (42.28 MPa), when compared with values reported in the literature for edible coating of crosslinked alginate by immersion in aqueous $CaCl_2$ solution were smaller. For this system, the authors determined tensile strength values in the range of 68 to 80 MPa, when the salt concentration in the solution ranged from 1 to 3 g/100 mL, in the present study it was used 2 g/100 mL (Rhim, 2004).

This difference obtained in this study can be explained by the high viscosity found for sodium alginate and 1% turmeric solution coating, presenting higher cohesiveness to the coating, and can be explained as a function of sodium alginate being D-manururonic acid, and L-guluronic acid, which has substantial hydrophilic groups. With added water, water molecules immersed in crystalline sodium alginate networks

were then distributed between two hydrophilic layers and formed three-dimensional networks (Davidovich-Pinhas & Bianco-Peled, 2010).

Higher tensile stresses and elongation rates result in Young's higher modulus coatings (Al-Hassan & Norziah, 2012). The edible coating containing turmeric alcohol solution (26.59 ± 3.76MPa) presented higher value for Young's modulus than the treatment containing only sodium alginate (15.41 ± 2.03MPa), being that the higher is Young's modulus, the higher is the material hardness. (Galdeano, 2007).

Water steam permeability (SP) is an important component to evaluate when choosing products for edible coatings development, as it corresponds to the water steam transport vapor from the atmosphere to the product or mixture and from food to the atmosphere. This effect is responsible for ensuring quality and shelf life (Youssef et al., 2019).

For treatment without 1% turmeric addition, the permeability value was 1.19 (x10-15g./m.pa.s), being higher than the value for treatment with sodium alginate, which was 0.38 (x 10 -15g./m.pa.s); the ethanol turmeric solution presence may have influenced the polymer segmental mobility, reducing water steam permeability (Rhim, 2004), and the value for the edible coating prepared with sodium alginate was 1 .42 (10-9 g / m2 s Pa). Water steam transfer occurs through the coating hydrophilic portion, SP decreases with increasing hydrophobic compound fraction; thus, SP depends on the hydrophilic-hydrophobic ratio of edible coating constituents (Mei, Yuan, Wu, & Li, 2013).

The water adsorption isotherms were adjusted using the GAB model, in the GAB equation there are three theoretical parameters based on physical phenomena occurring during water steam adsorption and is considered as the most suitable model to describe the experimental data in 0,10 to 0.90 considered the interval of greatest interest in food (Aguirre-Loredo et al., 2018). In the GAB model, the moisture content in the

monolayer (mo) is the water amount that is strongly adsorbed at specific sites in the material and can be used to measure the active sites availability for water adsorption. The mo values for sodium alginate and turmeric 1% coverage were 0.0952 and 0.02334 (g / 100g dry matter). R2 values were higher than 0.99 indicating adequate adjustment of experimental data.

Regarding the isothermal curve data, for alginate and turmeric 1%, exponential growth was observed in the region corresponding to Aw <0.2, where the water adsorption in the monolayer (mo) is described. For treatment with sodium alginate different behavior was observed, since the increase occurred from the area above the value previously mentioned (Figures 2A and B).

In the study by Aguirre-Loredo, Rodríguez-Hernández, Morales-Sánchez, Gómez-Aldapa, & Velazquez (2016), the adsorption isotherm for the edible chitosan coating showed a slight increase in moisture content at Aw⩽ 0.6, and subsequently increased which is similar to the present study. According to Perdomo et al. (2009) and Srinivasa, Ramesh, & Tharanathan (2007) this behavior is related to edible coatings with hydrophilic characteristics.

The difference between treatments for coating thickness can be explained by the occurrence of concurrent reactions, where alginate dissolution in solution and non-solubilization in edible coatings, formed crosslinking between Ca^{2+} and carboxyl groups on the coating surface. This occurred when edible coatings were immersed in $CaCl_2$ solutions, because when Ca^{2+} concentration is low, alginate dissolution would be dominant to reduce coating thickness (Pavlath, Voisin, & Robertson, 1999).

The edible coating thickness values can also influence the permeability, mechanical properties and transparency of coatings (Kurt & Kahyaoglu, 2014). Control

of the coating thickness is difficult, especially when the production process occurs through the fusion type (Sobral, 2000).

According to Chambi & Grosso (2006), the edible coatings mechanical properties are largely associated with the distribution and density of intermolecular and intramolecular interactions, which depend from the polymer chains arrangement and orientation.

In semi-hard cheeses, an important factor affecting stability is water activity (Aw), which is mainly dependent on moisture and salt content. During cheese ripening, Aw decreases until the surface is in equilibrium with the surrounding atmosphere, thus influencing the cheese chemical and microbiological reactions (Saurel, Pajonk, & Andrieu, 2004).

In the present work water activity decreased during storage time and dry matter values increased. Cheese releases CO_2 and simultaneously consumes O_2, requiring gas exchange control to maintain quality and increase shelf life (Cerqueira et al., 2010), this fact may be influenced by the coating permeability produced with alginate, thus reducing changes with the environment.

According to Rolim (2008), the interaction of alcohol or turmeric components with cheese proteins makes them more hydrated, making it difficult to remove water, a fact that can be confirmed by the results obtained from water activity in both treatments.

The pH values were significant for time ($p < 0.0001$), decreasing over 30 days of storage, similar to the work of Lucera et al. (2014) in which different edible coatings (potassium sorbate (PS), sodium benzoate (SB), calcium lactate (CL) and calcium ascorbate (CA)) were tested to maintain the Mozzarella quality. The pH was monitored for 8 days and remained between 6.50 and 6.30. Fox, Law, McSweeny, & Wallace (1999)

reported that pH reduction was expected in the early ripened stages by the metabolism of residual lactose to lactic acid, followed by pH increase depending on cheese type.

According to Mushtaq, Gani, Gani, Punoo & Masoodi (2018) who developed zein coatings with different concentrations of pomegranate extract (0, 25, 50 and 75 mg / ml coating) in developing edible packaging for Himalayan cheese, showing beneficial evolution of microflora (LAB). In samples during 5 days of storage there was no significant difference observed in LAB counts for all samples.

The activity of some compounds is related to the wide variety presence of secondary metabolites, these active compounds may act by breaking microbial membranes; in turmeric, phenolic compounds are present in the extract and oil (Aly & Gumgumjee, 2011). The authors found that *Curcuma longa L*. methanolic extract "in vitro" showed action on several bacteria, among them *Escherichia coli*. However, the values obtained in the present study showed a not so effective action of turmeric solution, because the treatment containing 1% turmeric was the one with the highest growth of these microorganisms.

In the color parameters, opacity means lower transparency, being important to control the light incidence in cheese (Cuq, Gontard, Cuq, & Guilbert, 1996). The values obtained in the present study in sodium alginate and turmeric edible coatings, the cheeses became darker, the parameter b * showed no significant difference with turmeric use, although the opposite was expected by the curcuminoids presence in the turmeric alcoholic solution.

For texture parameters, it was observed that the coated cheese hydration may have contributed to greater softness compared to uncoated cheese (Guerra-Martínez, Montejano, & Martín-del-Campo, 2012). Cheese hardness increased significantly over time with increasing dry matter ($p < 0.0001$) and decreasing moisture ($p < 0.0001$).

Zhong, Cavender and Zhao (2014) studying edible coatings for Mozzarella cheese also found that coatings generally slow down the cheese hardening process and produce softer textured cheeses.

Chewability is the energy required to chew a solid food to the point of being swallowed, and gomosity is defined as the energy required to disintegrate a semi-solid food to the point of being swallowed (Augusto, 2003). In this study, coated cheeses presented lower chewability and consequently lower hardness.

Figures 1A and B show the surface and cross-sectional area of edible coatings. According to Jiménez, Fabra, Talens & Chiralt (2010), solvent evaporation causes changes in component concentrations and viscosity of the emulsion's liquid phase, leading to lipid aggregation, affecting the internal structure and surface of the edible coating and, consequently the barrier, mechanical and optical properties, turning the coating microstructure analysis interesting.

Figure 1A shows the dense and regular surface, without cracks and pores, and contributed to the satisfactory properties of this barrier. In the cross section (Figure 1 B) the structure is dense and cohesive. According to Blácido (2006), edible coatings with sodium alginate and turmeric showed characteristics that resulted in coatings with higher tensile strength, as observed.

5. CONCLUSION

Cheeses ripened with *L.helveticus* containing edible cover of sodium alginate and turmeric at 1% for 30 days, showed an increase in lactic bacteria and water activity, being softer, with gomosity, cohesiveness and chewability reduction. However, the use of 1% turmeric alcoholic solution as antimicrobial agent was not effective to reduce total coliforms.

6. APPENDICES

Table 1. Mean values of tensile strength at break, Elongation, Young's modulus, Water stem permeability and thickness of sodium alginate (PAG) and sodium alginate with turmeric 1% (PAGAT) coatings.

Parameters	PAG	PAGAT
Rupture Tensile (MPa)	39.66±5.31	42.18±5.97
Elongation (%)	130.29±1.86	126.38±0.28
Young's modulus (MPa)	15.41±2.03	26.59±3.76
URE gradient(%)	2 – 53	2 – 53
SP ($x10^{-15}$) (g/m.Pa.s)	1.19±1.17	0.38±0.118
Thickness ($x10^{-3}$) (m)	0.044±0.03	0.022±0.007

* PAG: sodium alginate; PAGAT: Sodium alginate and turmeric 1%; the means followed by the same letters in the line did not differ significantly by the ANOVA and Tukey tests, with a significance level of 5%; URE: relative humidity gradient; SP: water steam permeability.

Table 2. Constant values of the GAB equation at 25 °C, calculated by nonlinear regression for sodium alginate (PAG) and sodium alginate and turmeric 1% (PAGAT).

Parameters	PAG	PAGAT
m_0	0.0952	0.02334
C	0.1352	15.7908
k	0.8636	0.9129
R^2	0.99	0.99

* Control: sodium alginate; T1: Sodium alginate and turmeric 1%; Mo: monolayer water content; C: Guggenheim constant; K: Measurement of multi-layer water sorption heat.

Table 3. Physicochemical and microbiology composition of cheeses without covering (SEMC) and with sodium alginate and turmeric coating application (AGAT).

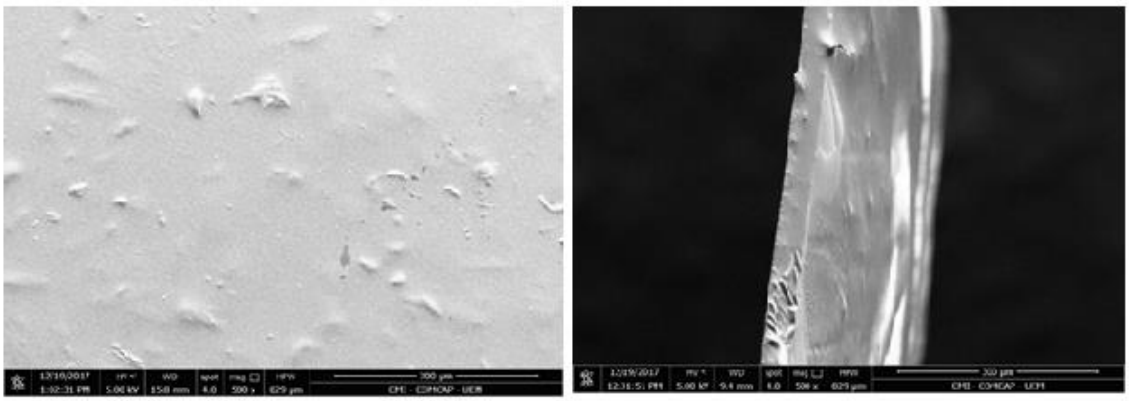
Parameters	TREATMENTS		TIME				P-value		
	SEMC	AGAT	0	15	30	SEM	TREAT	TIME	TREAT *TIME
DM	65.835±12.40	64.916±13.07	50.904±4.18	66.231±2.40	80.938±2.03	0.41914	0.342	<.0001	0.865
MOISTURE	34.164±12.40	35.083±13.07	49.095±4.18	33.768±2.40	19.061±2.03	0.48721	0.342	<.0001	0.865
pH	6.277±0.352	6.331±0.274	6.591±0.319	6.089±0.157	6.224±0.190	0.03270	0.477	<.0001	0.617
Aw	0.908±0.05[b]	0.936±0.04[a]	0.983±0.009	0.908±0.013	0.872±0.042	0.00028	0.0001	<.0001	0.057
COLIFORMS (Log $_{10}$)	6.538±0.638[b]	7.042± 0.506[a]	7.233±0.220	6.913±0.638	6.170± 0.377	0.09676	<.0001	<.0001	0.006
LAB (Log $_{10}$)	6.170±0.891[b]	6.585±0.735[a]	6.405±1.086	6.584±0.670	6.114±0.655	0.21168	0.046	0.158	<.0001

* SEMC: uncoated; AGAT: coated with sodium alginate + turmeric 1%; DM: dry matter; Aw: water activity; COLIFORMS: total coliforms LAB: lactic acid bacteria; TREATMENTS: significance level for treatment; TIME: significance level for time; INTERACTION: significance level for the interaction between treatment and time; Regression Equations: DM: 50.904 + 1.04 x R^2: 0.9452; MOISTURE = 49.095-1.04 x R^2: 0.9452; pH = 6.591-0.0543x R^2: 0.2488; Aw = 0.9832-0.00629 x R^2: 0.7334; MRS = 6.

Table 4. Color parameters and instrumental texture observed for cheeses with and without the 1% turmeric cover application.

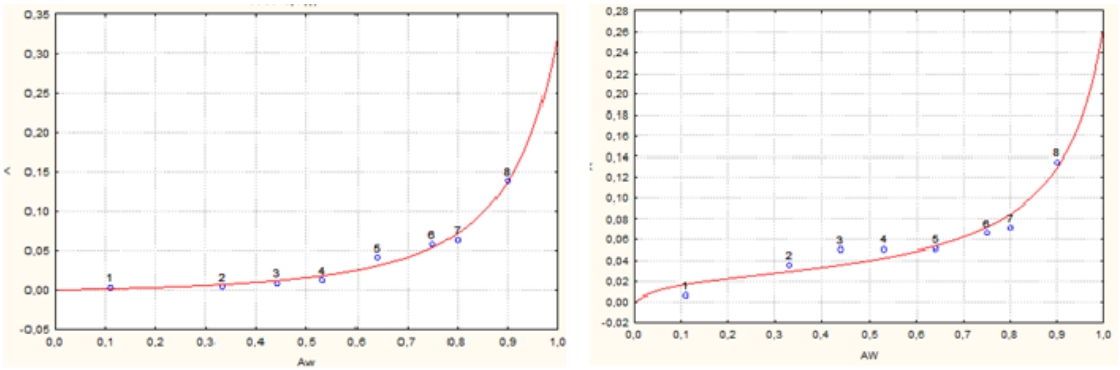
Parameters	TREATMENTS		TIME				P-value		
	SEMC	AGAT	0	15	30	SEM	TREAT	TIME	TREAT *TIME
color L*	78.465±10.41	77.588±10.97	86.512±3.259	82.358± 2.809	64.385± 6.739	0.55042	0.274	<.0001	0.645
color a*	0.714±1.22	0.982±0.839	1.633±0.243	1.014±0.645	-0.174±1.126	0.26343	0.204	<.0001	0.001
color b*	15.452± 3.450	16.297±2.918	13.356±2.039	15.958±2.019	18.442±3.224	0.46072	0.191	<.0001	0.217
HARD	9049.09±7142.9[a]	5047.91±3660.0[b]	2243.12±767.9	8026.87±4718.1	11136.42±6835.0	710.559	0.015	0.001	0.163
COE	0.890±0.129	0.893±0.110	0.957±0.087	0.915±0.070	0.791±0.131	0.0209	0.829	0.023	0.894
ELAS	6.622±9.490	4.209±0.287	8.137±10.88	4.161±0.265	3.567±1.226	0.63842	0.451	0.341	0.376
GOM	8376.181[±]5710.8[a]	4755.916±4106.7 [b]	2168.375±1289.3	8251.375±5231.9	9407.285±4996.9	633.148	0.015	0.001	0.080
CHEW	375.581[a]±217.0	193.883[b]±158.0	145.350±148.0	336.312±216.9	372.100±192.6	30.737	0.012	0.024	0.329

* SEMC: uncoated; AGAT: coated with sodium alginate + turmeric 1%; TREATMENTS: significance level for treatment; TIME: significance level for time; Interaction: significance level for interaction between treatment and time; Regression equations for color: L * = 86.512 + 0.188 x -0.031 x^2 R^2: 0.8301; a * = 1.633-0.024x R^2: 0.4771; b * = 13.356 + 0.176x R^2: 0.4372. HARD: hardness; COE: cohesiveness; ELAS: elasticity; GOM: gomosity; CHEW: chewability; Regression equations for texture: HARD = 2243.12 + 474.72 x; COE = 0.957-0.00013x-0.000180 x^2; ELAS = 8.137-0,377x; GOM = 2168 + 569.76 x; CHEW = 145.35 + 17.9.



(A) (B)

Figure 1 A and B. Surface and fraction photos of the sodium alginate and 1% turmeric coating obtained by scanning electron microscope (SEM) at 500-fold magnification.



(A) (B)

Figure 2 A and B. Moisture sorption isotherm obtained for sodium alginate, sodium alginate and 1% turmeric coatings.

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Probiotic coating for ripened cheeses with *Lactobacillus acidophilus* and *Lactobacillus helveticus* inclusion

ABSTRACT: The cheese enrichment with technologies in production has had a major impact on consumers' health. The use of beneficial "probiotic" microorganisms to humans, appears promising for dairy products improvement. The objective of this work was to evaluate the edible coverage as a vehicle for lactic acid bacteria by sodium alginate addition in ripened cheese. Chesse were evaluated in relation to physical-chemical characteristics, microbiological stability, viability and resistance to gastrointestinal tract passage. The intrinsic properties of the coating were evaluated by mechanical properties, thermal steam permeability and sorption isotherm. The experimental design was completely randomized, four treatments (uncoated cheeses (SEMc), sodium alginate coated cheeses (AG), sodium alginate and *L. acidophilus* coated cheeses (AGLA) and sodium alginate and *L. helveticus* coated cheeses (AGLH) at four storage times (0.5, 10 and 15), analyzed by the SAS 9.3 program There was a reduction in coliform values and the lactic acid bacteria remained viable for both treatments by day 15. In the identification using Random Amplified Polymorphic DNA (RAPD) technique, *Lactobacillus helveticus* strains were isolated, suggesting the microorganism migration to inside the cheese. The parameters of water steam permeability, thickness and Young's modulus were significant ($p < 0.05$). In the gastrointestinal conditions, there was a reduction in LAB according to the storage time, not being resistant to passage through the gastrointestinal tract. However, it suggested the microorganism (*L. helveticus*) permeability added in the cover to the cheese interior, ensuring that the cover can be a vehicle for dairy bacteria.

Keywords: lactic acid bacteria; dairy products, mechanical properties, microbiological quality, gastrointestinal simulation

1. Introduction

Cheese is a traditional food that can be made from different types of milk, is diverse in textures, aromas, flavors and shapes, and is part of the regular diet of most people by its composition. Its consumption has increased significantly over the years and, as a result, the cheese industry has evolved, seeking to improve some key product features such as increased shelf life and quality and safety promotion. Some modifications have been highlighted to improve sensory quality such as the inclusion of different packaging systems (Costa, Maciel, Teixeira, Vicente & Cerqueira 2018).

Coatings appear as an environmentally viable alternative for cheese preservation and packaging, acting as individual packaging, but also as an added

protection if used in combination with other compounds. The use of edible packaging and coating materials has been explored as a possibility because, like conventional coating materials, they allow the deterioration prevention, the shelf life extension and the water loss reduction. Acting as antimicrobial agents and bring several advantages over conventional coatings, such as better spreading, diffusivity and solubility (Ramos et al., 2012).

Probiotic microorganisms can be used as a solution to improve cheese composition quality and shelf life. They are defined as living microorganisms that, when administered in adequate amounts, confer a benefit on the host's health (Food and Agriculture Organization, 2001; Sanders, 2003). The recommendation in foods to be beneficial to humans is to be present in the amount of 106 live microorganisms per g or mL at the time of consumption (Chapel, Hay, & Shah, 2006, Mokarram, Mortazavi, Najafi & Shahidi 2009, Picot & Lacroix, 2004, Manojlović, Nedović, Kailasapathy & Zuidam, 2010).

Lactic bacteria (LAB) are the most commonly studied probiotics in recent decades. Belonging to desirable gastrointestinal tract microflora (TGI) they are therefore "considered safe" (Sanders, 2003) and are involved in the fermentation of most dairy products such as cheese and yogurt. They play an essential role in food preservation and inhibit spoilage microorganisms or foodborne pathogens by producing lactic acid, acetic acid, H_2O_2, bacteriocin, diacetyl and CO_2 (Yuksekdag & Aslim, 2010).

The objective of this work was to evaluate the probiotic coating as a vehicle for lactic acid bacteria (*Lactobacillus acidophilus* and *Lactobacillus helveticus*) in mature cheese. Microbiological stability, viability, resistance to gastrointestinal tract passage, cell morphology by electron scanning microscope (SEM) and intrinsic coating properties were evaluated.

2. Material and Methods

The experiment was carried out at the Milk Quality laboratory, which belongs to the Mesoregional Center for Excellence and Milk Technology (CMETL-UEM).

The cheeses were produced with raw milk, without pasteurization process and ripened for 20 days, circular shape weighing 500g. The cheeses were then subjected to the four treatments: uncoated cheeses (SEMc), sodium alginate (AG) coated cheeses, sodium alginate and *L. acidophilus* (AGLA) coated cheeses (0.001%) and cheeses coated sodium alginate and *L. helveticus* (AGLH) (0.001%). During the analyzes the cheeses were kept in a BOD greenhouse for 15 days / 12°C and relative humidity of ± 60.5%.

For the edible coating (filmogenic solution) the procedure was performed by solubilization of 2% sodium alginate macromolecules and 2% calcium chloride in sterile water. Lyophilized cultures of *L. acidophilus* (SACCO®) and *L. helveticus* (SACCO®) were activated in sterile MRS agar, respectively, both at 37 ° C for 24 hours. After this period they were centrifuged (6000 rpm for 15 minutes) and the precipitates were washed with sterile distilled water and centrifuged again (2 times), resuspended in sterile distilled water and added to the coating (Liserre, Re, & Franco, 2007), 1 ml of the concentrate was used for each 1000 ml of sodium alginate solution. After this step, the coating was applied to the cheese samples by dipping. It was determined by deep sowing on MRS agar, incubated at 35ºC for 48 hours, the lactic acid bacteria which count was 10^7.

In cheese samples, at 0, 5, 10 and 15 days of storage, water activity (Aqualab® 4TE, Decagon, São Paulo, Brazil), pH (digital pH meter, Tecnal Tec-5) and titratable acidity were determined (Lutz, 2008).

For microbiological analyzes, samples were diluted in peptone water and lactic acid bacteria (MRS Lactobacillus, Himedia-De Man, Rogosa and Sharpe agar) were

evaluated and for coliforms seeded on VRB agar (VRB agar, Himedia - Violet Reb Bile Agar), incubated at 35 ° C for 48 hours (AOAC, 1992).

To characterize the coating hygroscopic behavior and mechanical properties, the filmogenic solution was deposited in suitable containers (rectangular shaped acrylic plates containing 150 ml gel) and oven dried at 60ºC for 24 hours.

For the coverage evaluations, the following analyzes were performed: coating thickness evaluation, mechanical property (tensile test), water steam permeability (SP), adsorption isotherm and scanning electron microscopy (SEM).

The coating thickness was evaluated manually using a digital micrometer (Mitutoyo, resolution 0.01 mm - São Paulo - SP). Ten random points of each coating sample were evaluated.

For tensile properties determination, the samples were conditioned at 53% relative humidity at 25 ° C for three days in B.O.D. Tensile properties were determined using a Stable MicroSystem texturometer (TAXT2i model - England) using a methodology based on the American Society for Testing and Material ASTM (1996). The tensile properties determined were: maximum tensile strength at break (MPa), elongation at break (%) and elastic modulus (MPa).

Steam vapor permeability was determined gravimetrically according to the American Society for Testing and Material (1995) method with some modifications. The relative humidity gradient used was 2-53% at 25 ° C, where samples fixed in aluminum capsules were weighed until the mass gain rate was constant. The water steam permeability rate was determined according to equation 1.

$$\mathrm{TPVA} = \frac{\mathrm{m}}{\mathrm{t}} \cdot \frac{1}{\mathrm{A}} \qquad (1)$$

* m/t: angular coefficient of the mass gain line (g) versus time (h), and A (m^2) coating permeation area.

Thus, the water steam permeability value was calculated according to equation 2:

$$SP = \left[\frac{\text{TPVA} .e}{p_s .(UR_{ext} - UR_{int})}\right] x100 \quad (2)$$

* SP: water steam permeability (gm/m^2.Pa.h.), TPVA (water steam permeation rate) (g/m^2.h), and average coating thickness (average of 6 measurements) (m), p is the steam saturation pressure at the test temperature (Pa), URext the relative humidity outside the capsule (%) and URint the relative humidity inside the capsule (%).

For sorption isotherm analysis, samples of about 0.5 g were conditioned for 20 days in anhydrous calcium chloride, after the drying period the samples were evaluated in different desiccators containing 11.3% (sodium chloride), 33% (magnesium chloride), 43% (potassium carbonate), 53% (magnesium nitrate), 64% (sodium nitrate), 75% KCL and BaCl (GAB) and LiCl. The Guggenheim-Anderson-de-Boer model was used. (GAB) to calculate sample concentration and to adjust data (Takahashi et al., 2017) (EQUATION 3). All tests were performed in triplicate.

For coatings isothermal adsorption analysis, 0.5 g samples were kept for 20 days in anhydrous calcium chloride for pre-drying, after the samples were placed in different desiccators containing 11.3% (saturated saline sodium), 33% (magnesium chloride), 43% (potassium carbonate), 53% (magnesium nitrate), 64% (sodium nitrate), 75% KCL and BaCl (GAB) and LiCl .The Guggenheim model Anderson-de-Boer (GAB) (Equation 3) was used to calculate sample concentration and to adjust data (Takahashi et al., 2017). All tests were performed in triplicate.

$$Xw = m0.C.K.Aw/[(1 - K.Aw).(1 - K.Aw + C.K.Aw)] \quad (3)$$

* Xw (g of water / g of dry matter) is the equilibrium moisture contend; m0 the monolayer value; aw water activity; C and K, the constants of Guggenheim which represent the sorption heat in the first layer and the sorption heat of the multilayers, respectively.

Microorganisms were also tested for resistance to passage through the gastrointestinal tract on days 0 and 15 of storage, following the methodology described

by Rao et al. (1989) in which 1 g of cheese from treatments containing microorganism (AGLA and AGLH) were weighed and placed in a tube containing 10mL of simulated gastric juice (0.08 M HCl containing 0.2% NaCl, pH 1.55) and incubated. at 37 ° C for 120 min. After incubation, the samples were removed and placed in 9 mL of simulated intestinal juice (0.05 M KH_2PO_4, pH 7.43). The tubes were incubated at 37 ° for 150 min. At the incubation end, 1 ml of each vial was used for microbiological analysis, and 1 ml of each solution was added to test tubes containing 9 ml of 0.1% homogenized peptone water, followed by serial dilutions of inoculated in MRS agar.

To verify the LAB migration of the edible cover inside the cheese, colonies isolated from both treatments were subjected to purification and confirmed by Gram stain. Then the bacterial isolates were genotypically analyzed using the Random Amplified Polymorphic DNA (RAPD) technique and the OPM1 primer (5'GTTGGTGGCT3 '). Reactions were performed in a 25 μL Techne-Tc3000 thermal cycler containing 1 μL DNA, 1.0U Taq DNA polymerase (Invitrogen), Taq Buffer, 3mM $MgCl_2$, 0.25mM dNTPs and 2.48 pmol of the oligonucleotide. initiator. Amplification conditions were 2 minutes for initial denaturation at 94 ° C, 30 cycles of 94 ° C at 1 minute, pairing at 42 ° C for 20 seconds, 72 ° C for 2 minutes, and final extension at 72 ° C for 10 minutes. The RAPD product was analyzed on 1.5% agarose gel containing ethidium bromide (0.5 g.ml-1) and analyzed by the L-PIX ST (LOCCUS) photo documentation apparatus.

The RAPD profiles obtained by using the two oligonucleotides were combined in a matrix and compared using the Jaccard coefficient; The coefficients correlation was calculated by the Unweighted pair-group method with arithmetical averages (UPGMA) through the Numerical taxonomy system of multivariate programs (RoTS, 2000).

For coatings microstructure analysis by electron microscopy, the Quanta 250 Electron Scanning Microscope (Fisher Scientific-FEI, Oregon, USA) was used. The

previously prepared coating was frozen in liquid nitrogen (-196ºC) after, the samples were lyophilized for 24 hours, prepared in the stub with the thin layer of gold deposition and evaluated under magnifying glass 500 and 1000 times.

Data were analyzed using the SAS REG Proc 9.3 (2013).

3. Results

There was a significant difference ($p < 0.05$) for Young's modulus (Mpa) of the coatings being the highest value for the treatment containing only sodium alginate, the LAB cells presence decreased on average the Young's modulus.

There was a significant difference for water steam thickness and permeability, being the highest thickness values in the treatment containing sodium alginate and the highest permeability value presented in the treatment of sodium alginate and *L. helveticus*.

The average values for coat adsorption isotherm showed no significant difference ($p > 0.05$) for the evaluated parameters (Table 2).

Figures 1 A, B and C show the isotherms, they are sigmoid, and it is observed that the moisture content of the coating slowly increased with increasing humidity up to $\alpha w \sim 0.64$.

The pH, acidity (° D), water activity (Aw), total coliform count (VRB) and lactic acid bacteria (MRS) values were significant for interaction treatments and times and for time ($p < 0.05$). For treatments these were significant ($p < 0.05$) for pH, acidity, AW and MRS (Table 3).

For total coliform counts, there were significant differences for treatment and time interaction ($p < 0.0001$), and for ripened times ($p < 0.0001$), with a reduction in counts during the evaluation period, as for LAB viability. Although for total coliforms there was a reduction in both treatments during ripened periods ($p < 0.0001$), a significant difference was observed for pH values in storage times (Table 3).

Table 4 presents the interaction of treatments and times for acidity, water activity, pH, total coliforms (VRB) and lactic acid bacteria (MRS) parameters.

For gastrointestinal simulation, treatments containing *L. acidophilus* (AGLA) and *L. helveticus* (AGLH) were evaluated at 0 and 15 days (Table 5).

Figure 5 shows the dendogram obtained by the results of cheese samples with and without coating with the microorganisms' presence. The amplified bars profile with OPM1 primer (5'GTTGGTGGCT3 ') refers to the treatments used in the research (SEMc, AG, AGLA and AGLH) and the controls are lyophilized pure cultures, LA: *L.acidophilus* and LH: *L. helveticus* (SACCO ®).

The surfaces micrographs of sodium alginate, sodium alginate and *L. acidophilus* and sodium alginate and *L. helveticus* coverings, non-crosslinked, are shown in figures 2,3 and 4.

4. Discussion

The edible cover thickness values were significantly different ($p < 0.005$). However, Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk (2014) found no significant effect on thickness by the *L. rhamnosus GG* cells addition to prebiotic fibers. Thickness can be considered as an important parameter that determines factors such as coating transparency, water steam permeability and mechanical properties, improving the coating capacity in relation to the food mechanical integrity (Ghanbarzadeh & Almasi, 2011).

From the rupture stress values, it was observed that the microorganism inclusion had little influence on sodium alginate coatings although the cover made with sodium alginate without microorganism inclusion was more fragile (16.192 Mpa). In the cover ability to elongate, no significant difference was observed for values obtained between the treatments. Coverage treatment containing sodium alginate tended to increase

Young's modulus (22.704 Mpa) and decrease tensile strength, a behavior already observed in the literature on polysaccharide and protein coatings (Jo, Kang, Lee, Kwon, & Byun, 2005). in the other coverings with microorganism inclusion, there was increased tensile strength and decreased Young's modulus, thus reducing the coatings flexibility.

In the study by Pereira et al. (2016), the probiotic microorganism incorporation in coatings led to a slight decrease in the rupture stress parameter value, similar results were also obtained by Kanmani and Lim (2013), through the probiotic microorganism addition in the pullulan and starch coatings that resulted in breaking stress reduction.

In the Aziz, Salama, & Sabaa (2018) study in which they used sodium alginate and different castor oil (0, 1, 2 and 3%) percentage to produce antimicrobial coverage and to evaluate mechanical properties, values, the values for treatment containing only sodium alginate were for breaking stress (Mpa) 17.35 ± 4.36, elongation (%) 10.04 ± 5.10 and Young's modulus (Mpa) 33.73 ± 0.79 higher than those found in this study.

Water steam permeability (SP) values refer to the transfer of water from food to the environment or from the environment to food; coverings should have a lower SP to reduce food dehydration as much as possible and thus keep them fresh (Gontard, Guilbert, & Cuq, 1993). The lowest SP value was observed for treatment without microorganism inclusion and with higher coverage thickness. Ebrahimi et al. (2018) observed SP increase of up to 50% by the probiotic addition (*Lactobacillus acidophilus*, *L. casei*, *L. rhamnosus* and *Bifidobacterium bifidum*), compared to the coverage of carboxymethyl cellulose - CMC, without microorganisms.

Sánchez-González, Saavedra, &, Chiralt (2013) evaluated different polysaccharides and proteins for edible coverings with the *L. plantarum* microorganism inclusion, found that the lactic acid bacteria incorporation induced a significant increase in SP, regardless of the hydrocolloid nature. It could be justified by the bacteria presence

that introduces discontinuities in the polymeric matrix, and could promote the mass transfer of water molecules, corroborating the data found in this experiment.

In the Gialamas, Zinoviadou, Biliaderis, &, Koutsoumanis (2010) study, the *Lactobacillus sakei* cells inclusion in sodium alginate and calcium chloride coatings was evaluated to determine mechanical and chemical properties. the coating water sorption isotherm behavior was not affected by culture presence in any of the methods used for bacterial cell incorporation (casting and by aspersion), which can be attributed to the fact that the added content is smaller. compared to the coating dry matter and, in this sense, it is not expected to influence the coating water sorption isotherm behavior.

The lactic acid bacterial (LAB) count values at the zero time of evaluation showed that there was an initiating microbiota in both coated and uncoated cheeses having the highest value in treatments for cheese with sodium alginate and *Lactobacillus acidophillus*, so the coating presence may favor the growth or survival of these bacteria, reducing oxygen permeability and increasing Aw, factors that would limit their growth in uncoated cheese (Ramos et al., 2012).

Higher counts were also found at 10 days of storage for the lactic acid bacteria presence in the cheeses of referred treatment. According to Perreira et al. (2016) evaluating two different microorganisms inclusion and their viability in different storage times at different temperatures, *Bifidobacterium animalis* Bb-12® and *Lactobacillus casei-01*, whey protein isolate coating by 0, 3, 5, 10, 40 and 60 days at 23 and 4 °C, observed that from the initial concentration of 10^9 CFU / g coating, a viability loss of 3 log cycles (reaching 106 CFU / g) was observed within 10 days of storage at 23 °C for both probiotics and was kept stable thereafter, with slight reduction after 40 days of storage.

In Ningtyas, Bhandari, Bansal & Prakash (2019) study, the survival of *Lactobacillus rhamnosus* encapsulated with sodium alginate and free in cream cheese mass was studied during 35 days of storage at 4 °C. Both treatments and storage days ranged from 8.28 to 6.63 log CFU / g respectively, effectively showing that the encapsulation may be a more effective way for microorganism survival.

Ramos et al. (2012) developed different edible coverings based on whey protein isolate, glycerol, guar gum, sunflower oil and Tween 20 along with various antimicrobial compounds combinations. Edible covering made with antimicrobials, lactic acid and COS (chitosan oligosaccharide) use in "Saloio" semi-hard ripened cheeses showed a protective effect against spoilage and pathogenic microorganisms (*Staphylococcus spp*., *Enterobacteriaceae*, *Pseudomonas spp*.) increasing the cheese shelf life and conserving it for up to 60 days.

The highest average values were observed for total coliforms presence (VRB) for uncoated cheeses at 10 days. At 15 days, values in both coated and uncoated treatments decreased by about 2 logs during storage. The highest coliforms value on day zero of storage and their decrease during storage may summarize the idea that this contamination could come from milk used for cheese production due to the pasteurization absence (Zottola & Smith, 1993).

At 10 days of storage the highest *L. acidophilus* counts (8.58 log) led to a decrease in the average pH values and consequently increased acidity with a consequent reduction in coliform counts (7.59 log), due to the increase in lactic acid production (Fathollahi, Hesari, Azadmard, & Oustan, 2010).

At 10 days the highest pH values between treatments was observed in cheese without the use of edible coating (5.64) and was statistically different for the other

treatments with coating inclusion. However, Kanmani & Lim (2013) studied starch coatings with the probiotic strains inclusion and did not observe differences in pH.

The water activity (Aw) was significant for the treatments ($p < 0.05$) and also for the storage time, Ramos et al. (2012) evaluated the effectiveness of antimicrobial edible coatings in cheese coverage over 60 storage days and realized that Aw was not constant over storage time for coated and uncoated cheeses, and that only after 20 days of storage could the surface reach equilibrium with the surrounding atmosphere; In this experiment the values stabilized at the 10th day of storage, and the lowest value was observed for uncoated cheese (0.8017), so it can be said that there was higher water loss in uncoated cheese.

Cheeses are presented as an alternative vehicle to maintain sufficient viable probiotic bacteria after passage through the gastrointestinal tract in order to promote human health (Burns et al., 2008). Cheeses have a higher pH than yogurt and milk which can help maintain probiotic viability during storage and improve their buffering capacity. Capacity in combination with high protein lipid dense matrix may offer additional protection to microbial cells during passage through the stomach to the intestine (Cruz, Buriti, de Souza, Faria, & Saad, 2009; Hayes, Coakley, O'sullivan, & Stanton, 2006; Phillips, Kailasapathy, & Tran, 2006).

There was a decrease in LAB count at different evaluation times in relation to gastric simulation. With a reduction of 5.78 log, not being considered resistant to the microorganism passage through the gastrointestinal tract.

The values observed by Oliveira et al. (2014) for viable cell count of *L. acidophilus* (LA-5), *L. casei subsp. paracasei* (L. casei-01) and *B. lactis* (BB 12) incorporated in goat cheese after exposure to simulated gastrointestinal conditions showed significant differences between the treatments ($P < 0.05$). At the beginning of in

vitro digestion, all evaluated probiotic strains incorporated into cheese had viable cell counts between 7 and 8 log CFU / g; however, samples obtained at the end of in vitro digestion showed a viable cell count of 6.0 log CFU / g (± 0.25) for *L. acidophilus*, 5.7 log CFU / g (± 0.19) for *L. casei subsp. paracasei* and 5.5 log CFU / g (± 0.21) for *B. lactis*, decreasing its survival as it passes through the gastrointestinal tract.

According to Dos Santos et al. (2015) evaluating eight *L. rhamnosus* and *L. plantarum* strains isolated from manufactured rennet cheese observed a reduction close to or greater than 50% at the end of the in vitro assay, a reduction of 2.46 log CFU / mL at the end of the assay, starting from an initial inoculum of 9.10 log CFU / mL.

In the Eckert et al. (2018) study some probiotic lactic acid bacteria, *Lactobacillus plantarum* ATCC8014, *L. paracasei* ML33 and *L. pentosus* ML82, were encapsulated with serum-alginate pectin (WAP) or permeated serum-alginate-pectin (PAP) to verify resistance to the gastrointestinal tract and storage conditions. Some *Lactobacillus* strains are unable to survive at low pH values because these conditions inhibit the metabolic activity of them, thereby reducing its viability (Sultana et al., 2000). The results showed that non - encapsulated microorganisms are more sensitive to simulated gastric conditions since none of the isolates survived these conditions, reducing 3 log cycles and maintaining the viability of 6 log UFCmL $^{-1}$).

Cheeses containing microorganisms added to the coating were analyzed by the RAPD-PCR technique to verify the bacterial migration capacity of the surface into the cheese. The RAPD-PCR reveals the diversity and dynamics of some lactic acid bacteria strains, which have been useful to identify the presence of some microorganisms in complex environments such as cheese (Lazzi et al., 2009; Mancini, Lazzi, Bernini, Neviani, & Gatti, 2012; Randazzo, Caggia, & Neviani, 2009).

According to Figure 5 the isolates obtained from the cheese interior (Lh, T3 and T4) belong to the same species, since they are 100% similar. The similarity percentage between *Lactobacillus acidophilus* (La) and T3 strains was 20%, being considered low, not suggesting this microorganism migration into the cheese interior.

In the Andrighetto, Marcazzan, & Lombardi (2004) study the microorganisms present in the whey for the production of Grana Padano cheese were evaluated by RAPD-PCR, this analysis was useful to evaluate the *Lactobacillus* and its biodiversity, mainly the *L.helveticus*.

Through the use of the scanning electron microscope it was possible to verify the complete distribution of both microorganisms by the sodium alginate matrix, as shown in figures 2, 3 and 4. It was also verified that the bacterial cells were incorporated in the cover matrix. without alteration of its morphology (rods), being well distributed and able to remain embedded in the coating, finding that the edible sodium alginate coating is a promising matrix for incorporation and delivery of both lactic acid bacteria.

In the study of Pereira et al. (2016) was observed by scanning electron microscopy analysis of serum protein isolate coatings with bacterial cells, visualization of *B. animalis Bb-12*® and *L. casei-01*. The probiotic microorganism incorporation into the edible coatings did not give any noticeable modification to the structural conformation and the microorganism presence confirmed by rod-like shapes found in the protein matrix; Thus, the coating is considered as protection to maintain the microorganism viability in a similar way to that observed in this work.

The images obtained by the scanning electron microscope (SEM) in the work of Ebrahimi et al. (2018) showed that the polymeric structure of carboxymethyl cellulose (CMC) was homogeneous, uniform and compact, without micropores and the covers with included microorganisms showed a larger number of holes than control coatings, bacterial

cells were embedded in the coating matrix (small rod-like shapes) and could result in increased cell protection effects of the coatings.

5. Conclusion

The *L. acidophilus* and *L. helveticus* inclusion in edible cheese coverings reduced the total coliforms presence at 10 days, but did not effectively improve the microbiological quality of the product in relation to its presence. However, it suggested the possibility of microorganism permeability, *L. helveticus*, added to the cheese interior, ensuring that the cover can be a good vehicle for lactic acid bacteria.

6. References

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7. APPENDICES

Table 1. Characterization of sodium alginate coatings and microorganisms.

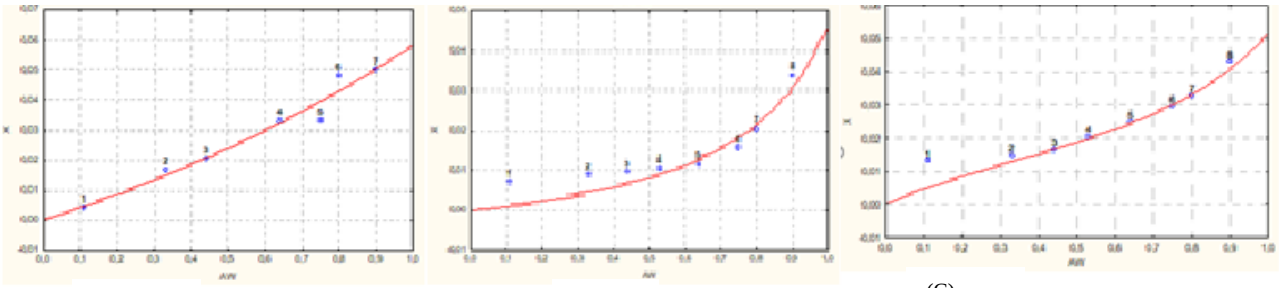
Parameters	AG	AGLA	AGLH
Rupture stress (MPa)	16.192 ± 7.4391	17.025 ± 3.7982	18.100 ± 2.950
Elongation (%)	5.1950 ± 1.9197	5.2883 ± 2.129	5.2960 ± 1.551
Young' modulus (MPa)	22.794^{a} ±12.324	6.534^{b}± 4.068	5.976^{b} ± 1.264
RHG gradient(%)	2-53	2-53	2-53
SP ($x10^{-15}$) (g/m.Pa.s)	1.017^{c} ± 1.851	4.716^{b} ± 1.362	6.046^{a} ± 5.258
thickness ($x10^{-3}$) (m)	0.0798^{a} ± 0.0177	0.0398^{b}± 0.0069	0.0046^{c}± 0.0004

* AG: sodium alginate coating; AGLA: sodium alginate + *L. acidophilus* coating and AGLH: sodium alginate + *L. helveticus* coating; the means followed by the same letters in the line did not differ significantly by the ANOVA and Tukey tests, with a significance level of 5%; RHG: relative humidity gradient; SP: water steam permeability.

Table 2. Values of GAB equation constants at 25 ° C, calculated by nonlinear regression for sodium alginate and microorganisms.

Parameters	AG	AGLA	AGLH
m_0	0.18646	0.01358	0.01741
C	0.81829	0.91831	4.48303
k	0.26688	0.77635	0.69275
R^2	0.9786	0.9132	0.9481

* AG: sodium alginate coating; AGLA: sodium alginate + *L. acidophilus* coating and AGLH: sodium alginate + *L. helveticus* coating; Mo: water content of the monolayer; C: Guggenheim constant; K: measure of the water sorption. heat in the multilayers.



(A) (B) (C)

Figures 1A, 1B and 1C. Moisture sorption isotherm obtained for sodium alginate, sodium alginate and *L. acidophilus* and sodium alginate and *L. helveticus* coatings.

Table 3. Physicochemical and microbiology composition of cheeses with and without film application.

Variables	Treatments				Time					P-value		
	SEMc	AG	AGLA	AGLH	0	5	10	15	SEM	Treat	Time	treat*time
pH	5.6483±0.1253[a]	5.5458±0.1366[b]	5.4933±0.1489[b]	5.5541±0.1876[b]	5.7500±0.0969	5.5708±0.0697	5.5141±0.0952	5.4066±0.1210	0.0226	0.0001	<.0001	0.0572
acidity (°D)	27.583±0.635[ab]	24.833±0.5890[b]	27.416±0.5664[ab]	28.666±1.025 [a]	18.833±0.3639	29.916±0.4737	31.583±0.8039	28.166±0.3785	0.1038	0.0170	<.0001	<.0001
Water activity	0.8017±0.0141[c]	0.8245±0.0230[b]	0.8260±0.0207[b]	0.8470±0.0477[a]	0.8407±0.0446	0.8437±0.0240	0.8153±0.0201	0.7995±0.0107	0.0004	<.0001	<.0001	<.0001
Coliforms (Log 10)	7.0390±0.9445	6.9995±0.9907	7.1353±0.6107	7.0210±0.7274	7.6107±0.2544	7.0585±0.2281	7.7008±0.3589	5.8250±0.3163	0.0116	0.0710	<.0001	<.0001
LAB (Log 10)	7.4943±0.7004[ab]	7.2975±0.7645[ab]	7.7121±1.1636[a]	7.2169±0.7946[b]	8.3334±0.5117	6.7945±0.6623	7.9646±0.6822	6.9126±0.4802	0.1277	0.0135	<.0001	<.0001

* LAB: lactic acid bacteria; SEMc: uncoated; AG: sodium alginate coating; AGLA: sodium alginate + *L. acidophilus* coating and AGLH: sodium alginate + *L. helveticus* coating; SEM: standard error of mean, Treat: Treatment, P-value <0.05. Regression equations: Acidity: SEM: y = -0.02178 x2 + 0.38222x + 2.133333 R2: 0.7262; AG: y = -0.02200 x2 +0.40333 x + 1.63333 R2 = 0.9633; AGLA: y = 0.05667 x + 2.31667 R^2: 0.3412; AGLH: y = -0.02933 x2 + 0.53867 x + 1.3933 R^2: 0.8735; Water activity: AG: y = -0,0008933x2 +0,01220 x + 0.81933 R^2: 0.8735; AGLA: y = -0.00056000 x2 + 0.00661x + 0.82540 R2: 0.7494; AGLH: y = -0.00793 x + 0.90650 R^2: 0.9408; pH: SEMc: y = 0.00342x2-0.07044x + 5.83667 R2: 0.9011; AG: -0.02114x + 5.65433 R2: 0.7792; AGLA: y = -0.02147 x + 5.65433 R2: 0.7792; AGLH: y = -0.02953 x + 5.77567 R2: 0.8411; VRB: SEMc: y = -0.01049 x 2 + 0.04242 x + 7.29702 R2: 0.9996; AG: y = -0.13898x + 7.60338 R2: 0.9600; AGLH: y = -0.0085x2 + 0.01653x + 7.6943 R2: 0.8963; MRS: SEM: y = 0.01935 x2-0.37020 x + 0.35015 R2: 8503; AG: y = -0.10171 x + 7.97561 R2: 0.7744; AGLH: y = 0.01853x2-0.32457 + 8.02947 R2: 0.48.

Table 4. Treatment x time interactions unfold for evaluated parameters.

	COLIFORMS						
	Time					P-value	
	0	5	10	15	SEM	linear	Quadratic
SEMc	7.2970±0.0220	7.2467±0.0235[a]	8.0406±0.0897 [a]	5.5719± 0.0135 [b]	0.2726	<.0001	<.0001
AG	7.7105±0.1169	6.7476±0.1039 [c]	7.9678±0.0536 [a]	5.5722±0.2365 [b]	0.2859	<.0001	0.0780
AGLA	7.7085±0.2239	6.9729±0.0167 [b]	7.5924±0.1537 [b]	6.2676±0.0498 [a]	0.1762	0.0100	0.2816
AGLH	7.7266± 0.3076	7.2667± 0.0223 [a]	7.2023± 0.0592 [c]	5.8882±0.0117[b]	0.2099	<.0001	0.0189
SEM	0.0734	0.0658	0.1036	0.0913			
P-value	0.0783	<0.0001	<0.0001	0.0003			
	LAB						
	Time					P-value	
	0	5	10	15	SEM	linear	Quadratic
SEMc	8.3501±0.1267	6.9828±0.5145[ab]	7.4943±0.2516[b]	7.1500± 0.1138	0.2021	0.0719	0.0051
AG	7.8636±0.3395	7.6349±0.0399[a]	7.3481± 0.0332 [b]	6.3939±0.5871	0.2206	0.0017	0.3909
AGLA	8.8533±0.7413	6.4013±0.3475[b]	8.5812±0.1484 [a]	7.0125±0.5979	0.3359	0.2865	0.5347
AGLH	8.2664± 0.1262	6.1590± 0.2754 [b]	7.3481± 0.0332 [b]	7.0940±0.0261	0.2293	0.2764	0.0310
SEM	0.1477	0.1912	0.1969	0.1386			
P-value	0.1054	0.0031	0.0001	0.1844			
	ACIDITY						
	Time					P-value	
	0	5	10	15	SEM	linear	Quadratic
SEMc	21.333±0.1527[a]	35.000±0.2645 [a]	24.333±0.2309 [b]	29.666±0.6658	0.1835	0.3159	0.0117
AG	16.333±0.1154[b]	31.000±0.1732 [a]	24.666±0.3055 [b]	27.333±0.1527	0.1700	0.1123	<.0001
AGLA	22.666±0.1527[a]	23.333±0.2309 [b]	35.666±0.0577 [a]	28.000±0.2645	0.1635	0.0461	0.1414
AGLH	1.5000±0.2000[b]	30.333±0.1527 [a]	41.666±0.3511 [a]	27.666±0.4509	0.2960	0.0573	0.0001
SEM	0.1050	0.1367	0.2320	0.1092			
P-value	0.0008	0.0010	<0.0001	0.9109			
	WATER ACTIVITY						
	Tempo					P-value	
	0	5	10	15	SEM	linear	Quadratic
SEMc	0.8080±0.0111[b]	0.8066±0.0162 [b]	0.7883±0.0177 [b]	0.8040±0.0045	0.0004	0.6586	1.000
AG	0.8193±0.0015[b]	0.8580±0.0110 [a]	0.8196±0.0040[ab]	0.8013±0.0147	0.0006	0.2185	0.0011
AGLA	0.8220±0.0052[b]	0.8546±0.0065 [a]	0.8253±0.0020 [a]	0.8020± 0.0131	0.0005	0.0957	0.0022
AGLH	0.9136±0.0051[a]	0.8556±0.0015 [a]	0.8280± 0.0193 [a]	0.7906±0.0077	0.0137	<.0001	0.1500
SEM	0.0128	0.0006	0.0005	0.0003			
P-value	<0.0001	0.0008	0.0221	0.4751			
	pH						
	Time					P-value	
	0	5	10	15	SEM	linear	Quadratic
SEMc	5.8366±0.0503	5.5700±0.0264	5.6366±0.0305 [a]	5.5500±0.0721	0.0361	0.0187	0.0042
AG	5.7366±0.0838	5.5166± 0.0404	5.5333±0.0450 [b]	5.3966±0.0503	0.0394	0.0016	0.0516
AGLA	5.6600± 0.0435	5.5600±0.1053	5.3966±0.0321 [c]	5.3566±0.1327	0.0429	0.0006	0.5663
AGLH	5.7666± 0.1270	5.6366±0.0472	5.49000±0.0360[cb]	5.3233±0.1001	0.05417	<.0001	0.7050
SEM	0.0279	0.0201	0.0275	0.0349			
P-value	0.1509	0.2104	0.0003	0.0739			

* SEMc: uncoated; AG: sodium alginate coating; AGLA: sodium alginate + L. acidophilus coating and AGLH: sodium alginate + L. helveticus coating; SEM: standard error of mean, Treat: Treatment, P-value <0.05.

Table 5. Lactobacillus counts (log10) in relation to gastrointestinal resistance of microorganisms at 0 and 15 days.

Variabless						P-value		
	AGLA	AGLH	0	15	SEM	Treat	Time	treat*time
SG	2.190± 0.432	2.247± 0.416	2.554± 0.0667	1.877± 0.333	0.4329	0.6089	<.0001	0.4508

* AGLA: sodium alginate + L. acidophilus coating and AGLH: sodium alginate + L. helveticus coating. SEM: standard error of mean, P-value = 0.05. AGLA regression equations: y = 0.04103x + 1.926 R2: 0.5329; AGLH: y = 0.05107 + 1.8100 R2: 0.8969; SG: gastric simulation.

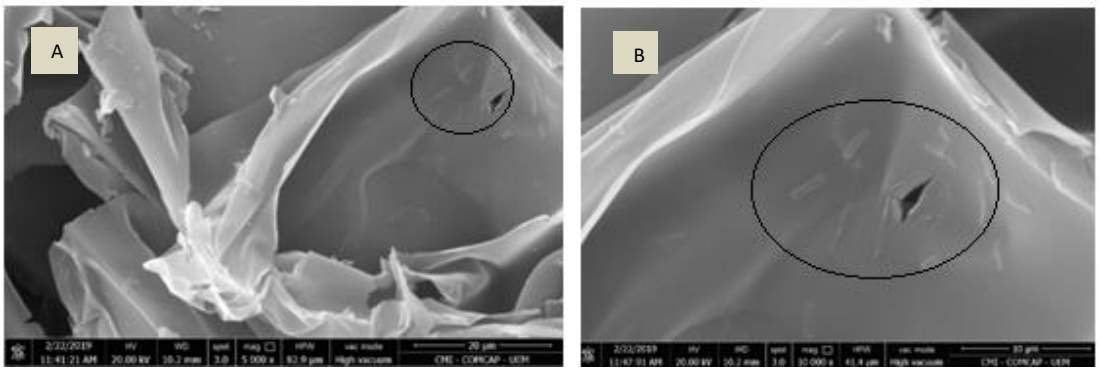


Figure 2 A and B. Sodium alginate and L. acidophilus coating 5,000 and 10,000 times.

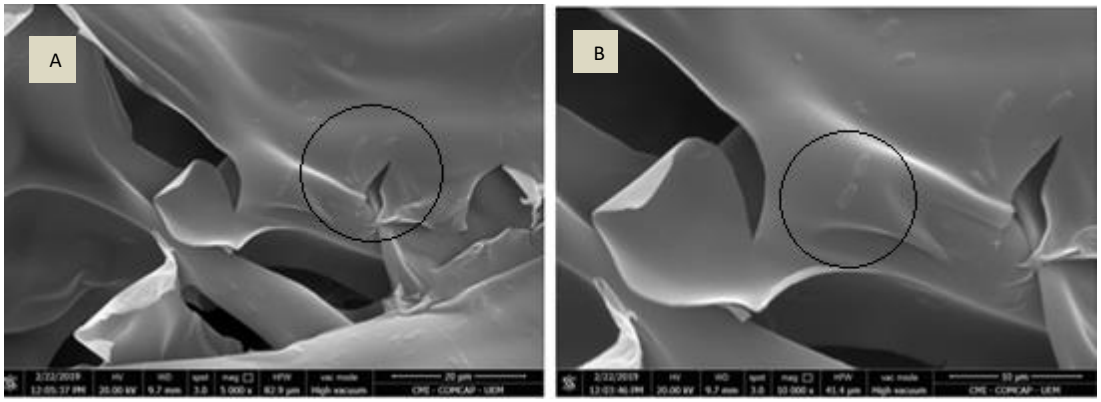


Figure 3 A and B. Sodium alginate and *L. helveticus* coating 5,000 and 10,000 times.

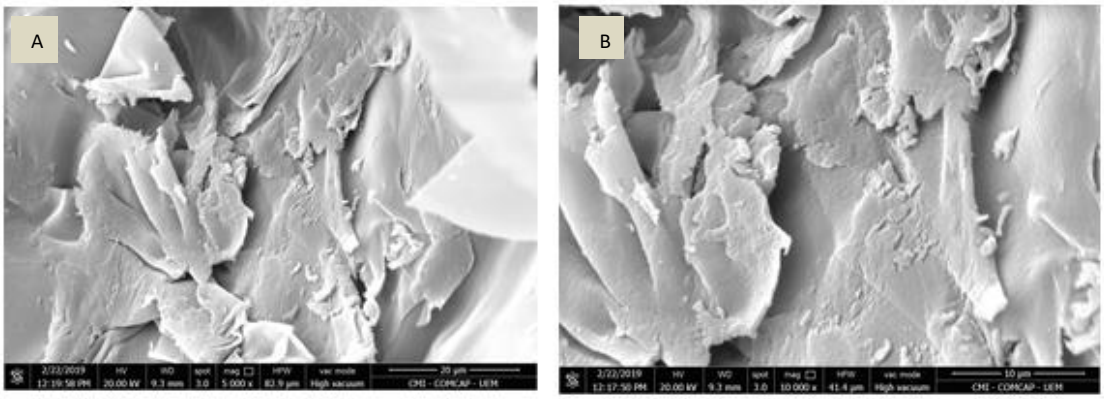


Figure 4 A and B. Sodium alginate coating 5,000 and 10,000 times.

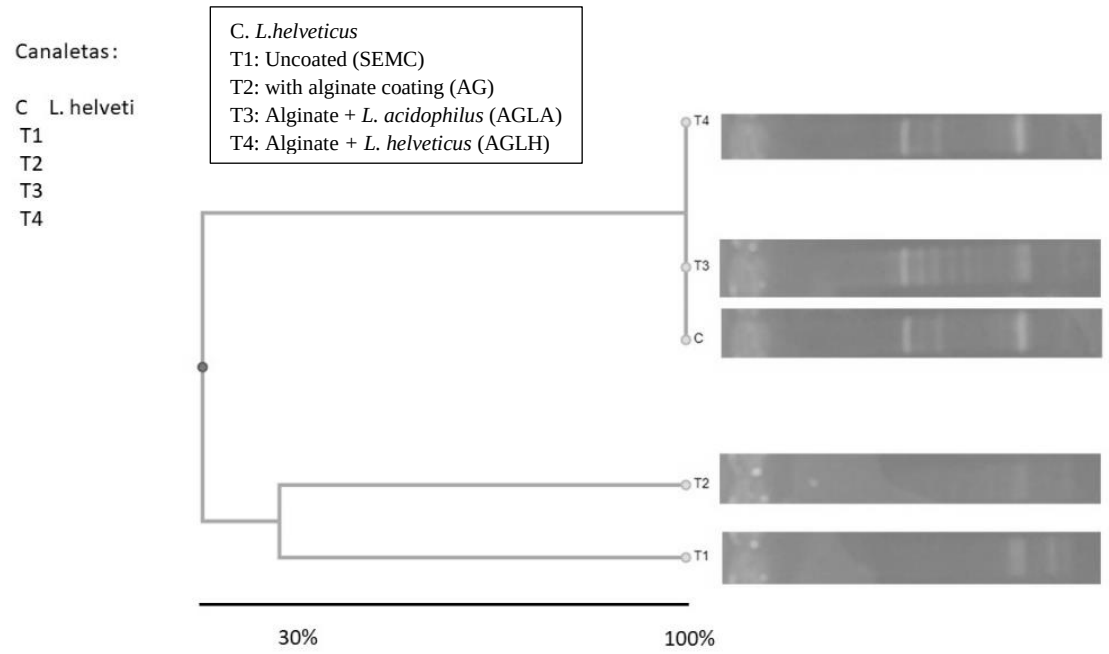


Figure 5. Dendogram of cheeses with and without coating application.

ARTIGO VULGARIZADO

QUEIJOS ARTESANAIS UM MERCADO PROMISSOR!!

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Atualmente, os queijos vêm se destacando no setor do Agronegócio, os artesanais principalmente, sendo estes elaborados com leite cru e produzidos de forma artesanal e muitas vezes familiarmente, a partir do leite recém-ordenhado, sem o processo de pasteurização.

Originalmente diversos queijos em todo o mundo são fabricados com leite cru, entretanto no Brasil, com exceção de alguns estados como Minas Gerais, Santa Catarina e Paraná, as normas sanitárias impedem que estes queijos sejam produzidos com o leite sem sofrer o processo de pasteurização.

Queijos artesanais e sua fabricação

No histórico do queijo acredita-se que este surgiu entre os rios Tigre e Eufrates, no antigo Iraque, cerca de 8.000 anos atrás, quando o homem começou a domesticar animais e utilizar certas plantas como alimento. O queijo era produzido pelos sumérios, babilônios, egípcios, gregos, romanos e celtas há milênios, no entanto, o grande crescimento de produção e comercialização aconteceu no final do século XIX na Suíça, França, Alemanha, Itália, países baixos, Escandinávia, Grã-Bretanha, Estados Unidos, Canadá, Austrália entre outros.

No Brasil, os queijos artesanais são caracterizados por regiões de produção, no estado de Minas Gerais (Serro, Serra da Canastra, Cerrado (antigo Alto Paranaíba), Araxá e Campo das Vertentes), na Serra Catarinense, região Campos de Cima da Serra no Rio Grande do Sul, no Nordeste e no Mato Grosso do Sul.

Os queijos artesanais são preparados com leite cru e o processo de produção tradicionalmente tem sido passado de geração em geração em muitas regiões brasileiras. Diferenciam-se da produção industrial pelo fato de não usarem processos mecanizados de produção nem de pasteurização do leite, entre outros. O modo de fazer destes queijos está associado ao modo de vida dos produtores e à bagagem cultural das regiões produtoras.

Alguns estados do Brasil, podem produzir e comercializar legalmente o queijo artesanal, como Minas Gerais, Santa Catarina e mais recentemente o estado do Paraná.

A lei Nº 20549 DE 18/12/2012 *dispõe sobre a produção e a comercialização dos queijos artesanais de Minas Gerais e* considera queijo artesanal o queijo produzido com leite integral, fresco e cru, em propriedade que mantenha atividade de pecuária leiteira, e, para Santa Catarina, Lei Nº 17.486, DE 16/01/2018, este é definido como aquele elaborado com leite cru da própria fazenda, com métodos tradicionais, com vinculação ao território de origem, conforme Regulamento Técnico de Identidade e Qualidade (RTIQ)

estabelecido para cada tipo e variedade, sendo permitida a aquisição de leite de propriedades rurais próximas desde que atendam todas as normas sanitárias pertinentes.

No Paraná foi sancionada a lei 19.599/2015 que permite a produção e comercialização do queijo artesanal no estado. O projeto autoriza a comercialização de queijo artesanal em todo o território nacional mediante critérios higiênico-sanitários, como a exigência de certificação de propriedade livre de tuberculose e o controle da potabilidade da água usada nos processos de elaboração do queijo e nas atividades de ordenha.

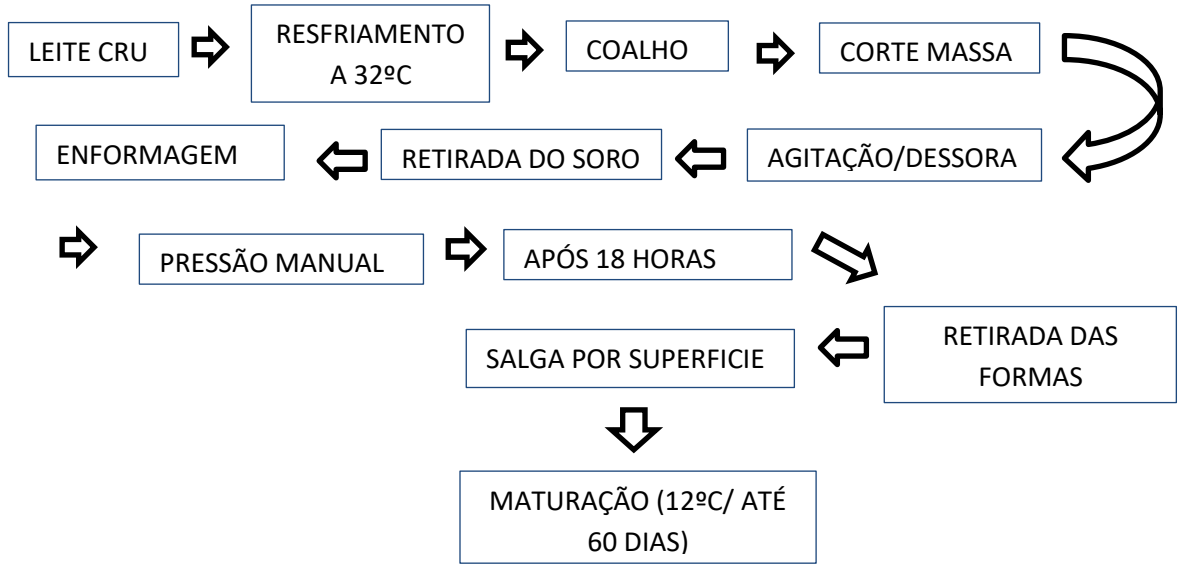
Queijo COLONIAL?

Embora a concepção de "colonial" ainda esteja em construção, este remete aos imigrantes Europeus e seus descendentes. Colonial faz referência a cultura e a tradição do "saber-fazer dos imigrantes" (Dorigon & Renk, 2010).

Entende-se por queijo colonial o produto obtido pela coagulação do leite bovino por meio do coalho / outras enzimas coagulantes, podendo ser consumido fresco ou em diversos graus de maturação. As características físicas mais comuns são o formato arredondado, com peso ao redor de 1 kg, pode não apresentar a casca quando imaturo, casca fina e amarela quando maduro e casca mais grossa e dura quando submetido a maturação mais longa, quando for recoberto com banha e colorau (Mariot, 2002).

"COLONIAL" remete principalmente às relações familiares, TRADIÇÃO, COSTUMES, sucessão de gerações, Relações de confiança e fidelidade.

Cronograma de fabricação do queijo colonial:



Utilização de fermentos lácteos

A microbiota dos queijos pode ser dividida em dois grupos: bactérias láticas iniciadoras (BLI) e microrganismos secundários.

As primeiras são responsáveis pela transformação de lactose em ácido lático durante a produção do queijo, suas enzimas também contribuem no processo de maturação, estando envolvidas na proteólise e na conversão de aminoácidos em substâncias voláteis responsáveis pelas propriedades sensoriais do produto. Por serem de crescimento rápido, estes microrganismos podem alterar o leite por acidificação se sua ação não for controlada. Por outro lado, são indispensáveis para a fabricação dos queijos.

As bactérias lácticas iniciadoras (BLI) podem ser adicionadas no início da produção em queijos produzidos com leite pasteurizado, ou pode-se utilizar somente aquelas que já ocorrem naturalmente no leite, a partir de leite não pasteurizado como no caso do queijo colonial artesanal. São chamadas de culturas starters e são definidas por conterem grande número de microrganismos, os quais podem ser adicionados para acelerar o processo de fermentação.

As culturas secundárias compreendem as bactérias lácticas não iniciadoras (BLNI), que crescem no interior dos queijos e outras bactérias, leveduras e/ou fungos que crescem, tanto no interior, quanto na parte externa dos queijos. Dentre estes microrganismos estão os proteolíticos, os lipolíticos e os produtores de gás, responsáveis pelas características de aromas e sabor diferenciado nos queijos.

Queijos e seu perfil de ácidos graxos

Produtos de origem animal como leite e derivados lácteos sempre foram considerados como vilões na dieta humana, devido a sua composição apresentar percentual de gordura saturada. No entanto estudos têm mostrado uma nova visão sobre esses produtos, pois alguns compostos biológicos benéficos a saúde humana tem sido descobertos, agregando assim uma capacidade nutricional desejável a estes alimentos.

Entre estes compostos benéficos, pode-se citar o ácido linoleico conjugado (CLA) que é encontrado principalmente nos produtos lácteos e carnes bovina, sendo estimado entre 56 diferentes isômeros geométricos e de posição do CLA. O cis- 9, trans -11 e trans -10, cis-12 são os únicos isômeros biologicamente ativos e vêm despertando cada vez mais o interesse dos profissionais da saúde humana.

Estes podem ser produzido no organismo de animais ruminantes ou por meio de síntese microbiana diretamente nos queijos, ocorrendo a produção de ácido linoleico por algumas linhagens de bifidobactérias e bactérias láticas; opção bastante interessante na produção de derivados fermentados.

Uma vez que o processamento de queijos também envolve fermentação bacteriana, alguns estudos têm sido conduzidos visando observar a síntese do CLA pelas bactérias neste tipo de produto, bem como o efeito das etapas de processo

na manutenção do teor de CLA. Entretanto, estudos se mostram necessários para comprovação da sua eficácia em produção de CLA além disso, a origem do leite, a variação sazonal de produção, além das condições de processamento e maturação podem ser influenciadores do teor de CLA nos queijos.

Em estudos conduzidos no Universidade Estadual de Maringá no ano de 2016/2017, pelo grupo de microbiologia e tecnologia dos derivados lácteos, pela doutoranda Paula Martins Olivo sob orientação da Professora Doutora Magali Soares dos Santos Pozza- Programa de Pós-graduação em Zootecnia, foi testada a utilização do microrganismo *Lactobacillus helveticcus* na produção de queijos de massa semidura com período de maturação por 30 dias com o objetivo de melhora no perfil de ácidos graxos benéficos a saúde humana e um possível incremento no ácido linoleico conjugado.

Os resultados indicaram que os níveis de poli-insaturados foram aumentados nos queijos com adição do microrganismo *L. helveticus*, comprovando que a inclusão de tal bactéria ácido láctica pode ser efetiva promovendo o desenvolvimento de produtos com características desejáveis aos consumidores.

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