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LEITE FERMENTADO CAPRINO PROBIÓTICO: AMINAS BIOGÊNICAS E ANÁLISE SENSORIAL

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PROBIOTIC FERMENTED GOAT'S MILKS: BIOGENIC AMINES AND SENSORY ANALYSIS

Dissertação apresentada ao Programa de Pós-Graduação em Medicina Veterinária da Universidade Federal Fluminense, Área de concentração: Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal, como requisito parcial para obtenção do título de Mestre.

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"Nossa maior fraqueza está em DESISTIR.
O caminho mais certo de vencer é tentar
mais uma vez."

Thomas Edison

RESUMO

O leite caprino é um excelente substituto do leite bovino na alimentação humana, podendo ser utilizado na elaboração de diferentes produtos lácteos, como por exemplo, os leites fermentados. Contudo, este leite, quando comparado às demais matrizes lácteas, possui um sabor e aroma característicos devido à elevada concentração dos ácidos graxos caprílico, cáprico e capróico, os quais influenciam de forma negativa na aceitação dos derivados caprinos frente a consumidores não habituais. Algumas estratégias sensoriais podem ser utilizadas para aumentar a aceitação dos derivados caprinos, dentre elas esta a exposição repetida. O iogurte atualmente é o principal veículo de culturas probióticas. Entretanto estas culturas podem possuir a capacidade de produzir a enzima descarboxilase, a qual propicia a formação de aminas biogênicas. Os processos fermentativos favorecem a formação destas aminas, em virtude de propiciarem, entre outros fatores, à maior atividade proteolítica e a presença de microrganismos. Por estes motivos, o controle das aminas biogênicas em leites fermentados é importante, podendo ser usadas como indicador de qualidade da matéria prima, como uma forma de controle de qualidade do produto final, além de poderem ser utilizadas como critério de seleção de cultura starter e de cepas probióticas. Os resultados do primeiro experimento (**Artigo 1**) indicam que o processo fermentativo favorece a formação de aminas biogênicas, em ambos os leites fermentados (bovino e caprino). Sendo das aminas avaliadas, a tiramina foi a de maior importância, podendo esta ser utilizada como índice de qualidade para estes leites fermentados. Além disso, foi comprovada a maior aceitação do leite fermentado bovino em comparação ao caprino. Com o intuito de melhorar a aceitação do iogurte de cabra, no segundo experimento (**Artigo 2**) foi realizada uma rápida exposição repetida, a qual demonstrou ser suficiente para a familiarização dos consumidores ao produto. Contudo, não foi suficiente para aumentar a aceitação. No entanto, o aumento das sessões de exposição pode ser uma estratégia para aumentar a aceitação, uma vez que houve correlação entre o aumento da aceitação e os dias de exposição. Além disso, algumas alternativas podem ser usadas para melhorar o desempenho sensorial dos iogurtes da cabra, como o desnate do leite e da adição de polpa de fruta.

Palavras chave: Leite de cabra. *Lactobacillus acidophilus* LA-5®. *Bifidobacterium lactis* BB-12®. Exposição repetida. Produtos lácteos.

ABSTRACT

The goat milk is an excellent substitute for cow milk in human nutrition, and it can be used in development of different dairy products, such as fermented milk. However, when compared to other milks, the goat milk has a tipic aroma and flavor, due to the high concentration of caprylic, capric and caproic acids, which influence negatively the acceptance of goat's products for unusual consumers. Some strategies could be used to increase the acceptance of goat's derivatives, among them the repeated exposure. The yogurt is currently the main vehicle for probiotic cultures. Nevertheless these cultures may have the ability to produce biogenic amines. The presence and accumulation of these compounds in foods are influenced by several factors, such as the fermentation process that it favors the formation of these amines for provide, among other factors, the increased proteolytic enzyme activity and the presence of the microorganism. For these reasons, the control of biogenic amines in yoghurt and others fermented milk is important. Biogenic amines can be used as an indicator of quality of raw milk, as a criterion for selection of probiotic strains, in addition to being a form of quality control of the final product. The results of the first experiment (**Article 1**) indicate that the fermentation process favors the formation of biogenic amines in both fermented milks (bovine and caprine). Of the amines evaluated, tyramine was the most important, which can be used as a quality index for these fermented milks. Moreover, it was proven the greater acceptance of cow's fermented milk compared to goat's fermented milk. In order to improve the acceptance of goat's yogurt, in the second experiment (**Article 2**) was performed a rapid repeated exposure, which proved to be enough to consumers' familiarity with the product. However, it was not sufficient to increase the acceptance. Nevertheless the increase of exposure sessions can be a strategy to enhance the acceptance, once there was a correlation between the growth of acceptance and the days of exposure. Besides that some alternatives may be used to improve the sensory performance of the goat's yogurt, such as the use of skim milk and the addition of fruit pulp.

Keywords: Goat milk. *Lactobacillus acidophilus* LA-5®. *Bifidobacterium lactis* BB-12®. Repeated exposure. Dairy products. HPLC.

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1 INTRODUÇÃO

O leite é considerado um dos alimentos mais completos, uma vez que é composto por elementos importantes para a nutrição humana (FAO, 2012). Neste contexto, o leite de cabra vem conquistando mercado, devido ao fato de apresentar alta digestibilidade, alcalinidade e ser hipoalergênico quando comparado ao leite de vaca (PANDYA; GHODKE, 2007 PARK, 1994; PARK et al., 2007), sendo muito utilizado na nutrição de crianças e idosos, e como alternativa de consumo para indivíduos alérgicos ao leite bovino. Entretanto, o leite caprino quando comparado aos demais, apresenta elevada concentração de ácidos graxos de cadeia média e curta (caprílico, cáprico e capróico). Estes compostos conferem aos derivados lácteos desta matriz um sabor característico, o qual muitas vezes influencia de forma negativa na aceitação destes produtos frente a consumidores não habituais (MARTIN-DIANA et al., 2003; MOWLEM, 2005; SLACANAC et al., 2010). Por este motivo, apesar de ser um excelente substituto do leite de vaca na alimentação humana, faz-se necessário o uso de estratégias tecnológicas, ou até mesmo sensoriais, na utilização do leite de cabra para elaboração de diferentes produtos lácteos, como os leites fermentados.

Os leites fermentados são obtidos por meio da ação de microrganismos específicos que acidificam o meio (CODEX ALIMENTARIUS, 2010). Estes são os produtos de escolha pela indústria alimentícia como veículo de culturas probióticas, sendo comercialmente os principais alimentos que contém estas características funcionais (SÁNCHEZ et al., 2009). Segundo a FAO (2001) os probióticos são microrganismos vivos, que quando ingeridos em concentrações adequadas conferem algum benefício á saúde de quem os consomem. Dentre os benefícios à saúde atribuídos aos probióticos estão: o melhoramento da saúde intestinal por meio da regulação da microbiota; a estimulação e o desenvolvimento do sistema imunológico; a redução de sintomas de intolerância à lactose; e a redução do risco de outras doenças, como o câncer (OELSCHLAEGER, 2010).

No entanto, alguns dos gêneros destes microrganismos possuem a capacidade de formar aminas biogênicas (GLORIA et al., 2011). Estas aminas são formadas durante a transformação dos alimentos pela ação de enzimas descarboxilases endógenas e pelas enzimas descarboxilases produzidas por microrganismos, ambas sobre aminoácidos específicos. A presença e o acúmulo de aminas biogênicas nos alimentos são influenciadas por inúmeros fatores, tais como o perfil e a disponibilidade de aminoácidos livres, a atividade de água, a temperatura, o pH do meio e principalmente a presença de microrganismos descarboxilase positiva (SCHIRONE et al., 2011; SCHIRONE et al., 2012).

Deste modo os processos de fermentação podem favorecer a formação de aminas biogênicas, em virtude de propiciarem, diversos fatores, entre eles, à maior atividade enzimática proteolítica que favorece a formação destas aminas. Os tipos e os teores destes compostos em produtos lácteos fermentados variam de acordo com a matéria prima (perfil de aminoácidos), o tipo de produto, o tempo de fermentação, a atividade proteolítica, as condições do processo de fabricação e os grupos de microrganismos presentes (GLORIA et al., 2011; SCHIRONE et al., 2011). Além disso, estes compostos podem ser utilizados como indicadores de qualidade de diversos produtos fermentados, tais quais os leites fermentados. As aminas biogênicas também podem ser utilizadas como critério de seleção de cepas com potencial probiótico (BOVER-CID; HOLZAPFEL, 1999; BOVER-CID; IZQUIERDO-PULIDO; VIDAL-CAROU, 2000).

Objetivou-se nesta pesquisa avaliar o comportamento de algumas aminas biogênicas, incluindo tiramina, putrescina, cadaverina, espermidina e histamina, em leites fermentados probióticos elaborados a partir de leite de vaca e de cabra durante os primeiros dez dias de armazenamento a 4 ± 2°C, além de realizar um teste sensorial afetivo a fim de avaliar a aceitação global destes mesmos produtos. Por fim, objetivou-se ainda, investigar se a metodologia da exposição repetida aumenta a aceitação de iogurte (probiótico e convencional) elaborado com leite de cabra.

2 REVISÃO DE LITERATURA

2.1 CAPRINOCULTURA LEITEIRA

A origem da cabra não é verdadeiramente conhecida. Estima-se que os caprinos foram os primeiros animais domésticos com a finalidade de produzir alimentos (RIBEIRO, 1997). Quanto à origem, acredita-se que seja europeia, partindo da Ásia e Pérsia, porém sem relatos precisos. A *Capra hircus*, provavelmente a primeira espécie a ser domesticada, esteve com o homem, fornecendo leite, carne e couro. Na Bíblia, a cabra aparece como animal domesticado que servia ao homem. Seus produtos eram vastamente usados como alimento, vestuário, e também como forma de negociação. Até o tabernáculo era revestido com peles e panos feitos com o couro de cabras (MONTINGELLI, 2005).

Segundo a "Food and Agricultural Organization" o rebanho caprino leiteiro mundial, em 2010, foi de aproximadamente 198 milhões de animais, com uma produção leiteira de 18 toneladas (FAO, 2012). Quanto ao Brasil, no último censo agropecuário, o rebanho caprino brasileiro possui cerca de sete milhões de cabeças (BRASIL, 2009). Destes, aproximadamente 90% estão concentrados no Nordeste. Entretanto, a criação de caprinos tem despertado o interesse de outras regiões do país, como Sul e Sudeste, que são voltadas principalmente para o mercado de leite e derivados.

A média de produção de leite de cabra no Brasil é de 30 kg/cabra/ano, estando muito atrás da média mundial, que é de 80 kg/cabra/ano (EMBRAPA, 2007). Essa produção tem origem basicamente em duas regiões: no Nordeste com mais de 75%, principalmente nos estados do Rio Grande do Norte e Paraíba, e na Região Sudeste com 17%, concentrada nos estados de Minas Gerais e Rio de Janeiro. Contudo, no Brasil a caprinocultura leiteira ainda é um importante elemento de subsistência para famílias de baixa renda que habitam as zonas rurais (FURTADO, 1984). Porém esse quadro tem mudado devido à criação do centro de estudos de caprinos no Nordeste e no Centro-Sul do país, o que tem proporcionado reconhecimento e valorização da cabra como um animal leiteiro.

Desta forma, a caprinocultura leiteira vem despontando como uma atividade dotada de potencial, podendo proporcionar significativas contribuições ao desenvolvimento socioeconômico da agropecuária no Brasil. Além do fato desta produção pode ser desenvolvida em pequenas propriedades, utilizando mão de obra familiar, completando a renda junto com outras atividades agrícolas de renda (VIEIRA et al., 2009). Existem hoje novos núcleos de pequenos produtores, estruturados em associações, no Rio de Janeiro, Minas Gerais e Rio Grande do Sul, fornecendo o leite de cabra para indústrias de Laticínios, que o beneficia em leite longa vida, em leite em pó e em queijos (CAPRILAT, 2012).

2.1.1 Leite de cabra

O leite de cabra, pela legislação brasileira, é o produto oriundo da ordenha completa, ininterrupta, em condições de higiene, de cabras sadias, bem alimentadas e descansadas, o qual apresenta alto valor nutritivo e qualidade dietética. É um alimento que apresenta diversos compostos necessários à nutrição humana, como: carboidrato, proteínas, lipídios, vitaminas e minerais (BRASIL, 2000), representando grande importância nutricional ao homem.

A proteína do leite caprino apresenta alto valor biológico, sendo fonte de aminoácidos essenciais, possuindo maiores concentrações de treonina, isoleucina, lisina, cisteina, tirosina e valina quando comparado ao leite de vaca (CEBALLOS et al., 2009; HAENLEIN, 2004; PARK et al., 2007). A micela de caseína do leite de cabra e de ovelha difere do leite de vaca em diâmetro, hidratação e mineralização. As micelas de caseína do leite caprino são menores, e contém maior concentração de cálcio, fósforo inorgânico, sendo menos solvatada e menos estável ao calor quando comparadas a bovina. A composição das frações da caseína no leite é influenciada por polimorfismos genéticos sobre os genes α1-, α2-, β- e κ-caseína (PARK et al., 2007). E segundo ALBENZIO et al., (2012) o leite de cabra possui composição proteica diferente do leite de vaca, principalmente com relação a fração α-caseína.

Este leite é fonte de ácidos graxos essenciais, excedendo o leite de vaca em ácidos graxos monoinsaturados, poliinsaturados e triglicerídeos de cadeia média, que são todos conhecidos por serem benéficos à saúde humana. Além de seu conteúdo mineral e vitamínico, (CEBALLOS et al., 2009; HAENLEIN, 2004; PARK et al., 2007).

O sabor e o aroma do leite caprino estão associados aos lipídios, nos quais os ácidos graxos de cadeia média e curta, representados principalmente pelos ácidos capróico (C6:O), caprílico (C8:O) e cáprico (C10:O). Desta forma, estes compostos são de grande importância para o sabor e aroma típicos dos derivados lácteos desta matriz (FURTADO, 1984). Chilliard et al., (2003) em sua revisão concluiu que a composição lipídica é um dos mais importantes componentes da qualidade nutricional do leite caprino, o que implica em um bom rendimento e firmeza para produção de queijos e leites fermentados, bem como na coloração, sabor e odor dos produtos caprinos. Contudo, esta elevada concentração de ácidos graxos de cadeia curta influenciam, muitas vezes, de forma negativa na aceitação do leite de cabra e seus derivados frente a consumidores não habituais (MARTÍN-DIANA et al., 2003; MOWLEM 2005; SLACANAC et al., 2010).

A cor branca do leite de cabra é decorrente da ausência de β-caroteno, devido a um processo fisiológico das cabras, no qual há a conversão desta substância em vitamina A, (FISBERG et al., 1999; PARK et al., 2007).

O leite caprino comparado ao bovino apresenta maior digestibilidade, alcalinidade distinta, maior capacidade tamponante, além de ser hipoalergênico (PARK, 1994; PARK et al., 2007). A maior digestibilidade do leite caprino esta relacionada ao elevado percentual de ácidos graxos de cadeia curta e média, facilitando a digestibilidade e favorecendo o esvaziamento gástrico. A alcalinidade e a maior capacidade tamponante estão correlacionadas à concentração das caseínas e ao arranjo diferente dos fosfatos na micela de caseína. E a hipoalergenicidade ocorre devido a menor concentração da fração proteica α-S1-caseína (ALBENZIO; SANTILLO, 2011; ALBENZIO et al., 2012; PARK, 1994), o qual esta relacionado ao polimorfismo genético desta fração proteica nesta espécie.

Alguns estudos têm associado o leite de cabra, quando consumido de forma regular, a diferentes efeitos funcionais como participar da manutenção da saúde, reduzir doenças crônicas e ter efeitos benéficos nas funções fisiológicas, sendo ótimo alimento na nutrição humana (CORREIA; CRUZ, 2006; OSMARI, 2006; ROCHA, 2007).

2.1.2 Produtos derivados de leite de cabra

A indústria láctea se destaca, entre as indústrias de produtos de origem animal, como a que apresenta maior diversidade entre o seus produtos derivados, no entanto observa-se uma carência de novos produtos neste setor econômico quando a matriz utilizada é o leite caprino. O leite de cabra pode ser consumido fluido ou transformado em queijos finos, leite em pó, manteiga, ricota, doce de leite, leites fermentados, além de outros produtos. O processo tecnológico transforma o leite de cabra, aumentando os derivados lácteos produzidos a partir deste produto, atingindo as necessidades do consumidor o qual está cada vez mais informado, exigente e voltado ao consumo de produtos com alto valor nutricional (AMARAL; AMARAL; MOURA NETO, 2011). Produtos como queijos, iogurtes, doce de leite e bebidas lácteas, podem ser obtidos a partir do leite de cabra, utilizando se de processos simples e acessíveis aos pequenos produtores, sendo essa uma alternativa para o aumento no consumo de produtos de origem caprina e para a agregação de valores a tais produtos (SANTOS et al., 2011).

Os leites fermentados elaborados com leite de cabra agregam as características nutricionais e tecnológicas do leite caprino. A fragilidade do coágulo formado neste tipo de produto, como o iogurte, é comprovada por alguns autores (EISSA et al., 2010; MARTIN-DIANA et al., 2003; TAMIME et al., 2011; VARGAS et al., 2008), o qual esta relacionada com diversos fatores, tais como o menor tamanho da micela de caseína, a menor concentração da fração proteica α-s^1-caseína, a maior dispersão micelar e a presença de cálcio coloidal (PARK, 2007; PARK et al., 2007). O iogurte produzido a partir do leite de cabra é tradicionalmente produzido na península do Mediterrâneo, no Oriente Médio, no sul da Rússia e no subcontinente indiano (TAMIME; ROBINSON, 2007). Segundo SLACANAC et al., (2010) os leites fermentados caprino apresentam elevado potencial de comercialização. E estes produtos quando acrescidos de culturas probióticas representam um grupo de alimentos com grandes perspectivas atuais e futura, no que diz respeito às suas propriedades funcionais e terapêuticas.

2.2 LEITES FERMENTADOS

O leite fermentado foi produzido pela primeira vez acidentalmente por nômades que estocavam o leite proveniente da ordenha em recipientes ou sacolas feitas de estômago de pequenos ruminantes. Esta estocagem era favorecida pelo clima árido e seco da região da Eurásia, o que proporcionou a proliferação de bactérias, as quais modificaram a estrutura daquele alimento, tornando-o sensorialmente mais atrativo para aqueles indivíduos, além de ser uma forma de conservação do leite (HAENLEIN, 2007; YILDIZ, 2010). Por este motivo, estes países possuem uma longa tradição na elaboração destes derivados lácteos (SAXELIN et al., 2003a, 2003b).

No Brasil, de acordo com a legislação vigente, os leites fermentados são "produtos adicionados ou não de outras substâncias alimentícias, obtidas por coagulação e diminuição do pH do leite, ou leite reconstituído, adicionado ou não de outros produtos lácteos, por fermentação láctica mediante ação de cultivos de microrganismos específicos. Estes microrganismos específicos devem ser viáveis, ativos e abundantes no produto final durante seu prazo de validade" (BRASIL, 2007). Os leites fermentados compreendem uma série de produtos lácteos, como o iogurte, os leites fermentados ou cultivados, o leite acidófilo, o kefir, o kumys, a coalhada, e o "buttermilk" obtidos pela fermentação do leite por microrganismos específicos (SAXELIN, 2008). Estes produtos possuem como característica comum, a produção de ácido lático resultante da fermentação da lactose (CARNEIRO et al., 2012).

Atualmente, os leites fermentados são considerados um derivado lácteo com elevado potencial para o desenvolvimento de novos produtos, principalmente por estarem associados à saúde, o que vem sendo explorado pelas indústrias de laticínios. Este fator esta relacionado principalmente com três características: (1) as propriedades tecnológicas da matriz láctea, como permitir a viabilidade funcional das culturas probióticas e de ingredientes prebióticos ao produto; (2) a elevada praticidade de consumo destes derivados lácteos; (3) e a relação que os consumidores fazem dos produtos lácteos com o aspecto de saudabilidade (COSTA et al., 2013). Diversos benefícios são atribuídos a estes produtos lácteos fermentados e, principalmente àqueles contendo bactérias probióticas, destacando-se: redução da intolerância à lactose, efeitos contra diarreia, estimulação do sistema imune, atividade antitumoral, atividade antimutagênica, redução do colesterol sérico, efeitos na candidíase (VASILJEVIC; SHAH, 2008).

Entre os leites fermentados, o iogurte é o que possui maior aceitação no mercado brasileiro. Este produto tem como vantagens, o baixo custo de produção, pois não necessita de equipamentos sofisticados para ser elaborado, ser de fácil preparo, e ser uma forma de conservação do leite agregando valor ao produto (MARTINS et al., 2007). Desta maneira, o iogurte conquistou uma importância econômica considerável no mundo inteiro, em virtude da sua imagem de alto valor nutricional, benefícios à saúde e pelo seu sabor atrativo (PENG et al., 2009). Segundo a legislação brasileira, entende-se por iogurte o produto incluído na definição de Leites Fermentados, "cuja fermentação se realiza com cultivos proto-simbióticos de *Streptococcus salivarius* subsp. *thermophillus* e *Lactobacillus delbrueckii* subsp. *bulgaricus,* aos quais podem ser acompanhados, de forma complementar, por outras bactérias ácido-lácticas que, por sua atividade, contribuem para determinação das características do produto final" (BRASIL, 2007).

Na fermentação do iogurte, inicialmente o *Streptococcus thermophilus* metaboliza a lactose produzindo ácido lático, que diminui o pH favorecendo o crescimento dos lactobacilos. Por sua vez, o *Lactobacillus delbrueckii* subsp. *bulgaricus* libera, a partir da degradação de proteínas, diversos aminoácidos, os quais estimulam o crescimento dos cocos. Sendo assim, uma bactéria favorece o desenvolvimento da outra estando estas em simbiose (SIEUWERTS et al., 2010). Estas bactérias são responsáveis pela formação do sabor e aroma, além da textura característica deste produto. Ambas as bactérias possuem o sistema β-galactosidase, o qual propicia a hidrolise da lactose em glicose e galactose. A glicose é metabolizada em piruvato, e este é convertido a ácido lático. Enquanto a galactose é parcialmente metabolizada pelo *L. bulgaricus*, uma vez que o *Streptococcus thermophilus* não tem enzimas capaz de metaboliza-la. Os produtos da fermentação que contribuem para o sabor e aroma característicos do iogurte são o ácido lático, acetoaldeído, ácido acético e diacetil (CHENG, 2010; GÜRAKAN; ALTAY, 2010).

O iogurte elaborado a partir do leite de cabra difere em algumas propriedades importantes do iogurte elaborado a partir do leite de vaca, como a firmeza do coágulo, que tende a ser suave e menos viscoso no iogurte caprino. Fato este que está diretamente relacionado com as diferenças físico-químicas e reológicas destes leites. Pode ser explicado, principalmente, pela diferença no tamanho e estrutura da micela de caseína e no tamanho dos glóbulos de gordura (BO\VZANIĆ; TRATNIK; MARIĆ, 1998; KARADEMIR et al., 2002). Essa diferença na micela de caseína do leite caprino propicia a maior retenção de água, o que reflete na menor sinérese do iogurte caprino (HAENLEIN, 2004).

2.3 PROBIÓTICOS

Os probióticos são micro-organismos vivos, que administrados em quantidades adequadas conferem benefícios à saúde do hospedeiro (FAO, 2001). Uma distinção deve ser feita entre culturas probióticas e culturas iniciadoras. Para as culturas serem denominadas probióticas é essencial que seja comprovado os seus benefícios à saúde, enquanto para cultura iniciadora é necessário confirmar sua capacidade de fermentar alimentos. Sendo assim, nem toda cultura iniciadora é probiótica, e por este motivo, nem todo alimento fermentado deve ser considerado probiótico (SANDERS, 2009).

Vários gêneros bacterianos e algumas leveduras são utilizados como micro-organismos probióticos, incluindo os gêneros *Lactobacillus, Leuconostoc, Bifidobacterium, Propionibacterium, Enterococcus* e *Saccharomyces*, no entanto, estudos têm demostrado que as principais espécies com características probióticas são o *Bifidobacterium* spp., *L. acidophilus* e o *L. casei*. Atualmente, as principais culturas utilizadas pela indústria como probióticos incluem lactobacillos e bifidobactérias que possuem um longo histórico na produção de derivados lácteos e também são encontradas como parte da microbiota gastrointestinal do homem, além da levedura *Saccharomyces cerevisiae Boulardii* (SHAH, 2007).

Os efeitos dos probióticos podem ser classificados em três categorias: (1) capacidade de modular a defesa do hospedeiro; (2) efeito direto sobre outros micro-organismos, comensais ou patogênicos, o que os tornam importantes na prevenção e tratamento de infecções e restauração do equilíbrio da mucosa intestinal; e (3) efeito baseado na eliminação de produtos resultantes do metabolismo microbiano, como toxinas, o que resulta na desintoxicação do intestino de quem os consomem (OELSCHLAEGER, 2010).

Uma série de benefícios à saúde são atribuidos aos produtos que possuem probióticos, incluindo: atividade antimicrobiana; controle de micro-organismos patogênicos; hidrólise da lactose; modulação da constipação; atividade antimutagênica e anticarcinogênica (DENIPOTE; TRINDADE; BURINI, 2010; KUMAR et al., 2012); redução do colesterol sanguíneo, melhora do quadro de pacientes com diabetes tipo 2 (resistência a insulina) e obesidade (AN et al., 2011; ARONSSON et al., 2010; NAITO et al., 2011); modulação do sistema imune; melhoria na doença inflamatória do intestino; e supressão de *Helicobacter pyloriinfection* (MYLLYLUOMA et al., 2005; SALMINEN et al., 2010). Alguns destes benefícios já são bem estabelecidos, como a modulação da constipação e hidrólise da lactose, enquanto outros benefícios têm mostrado resultados promissores em modelos animais, necessitando ainda de mais estudos clínicos.

Deve-se salientar que estes benefícios à saúde são transmitidos por linhagens probióticas específicas, e não por espécie ou gênero específicos. E ainda, que cada linhagem esta relacionada com um determinado benefício. Desta forma, nenhuma cepa irá fornecer todos os benefícios propostos. Como por exemplo, o *Lactobacillus casei* linhagem Shirota, no qual há evidências suficientes que apoiam a visão de que sua administração por via oral é capaz de auxiliar na digestão e absorção dos nutrientes e restabelecer o equilíbrio normal da microbiota intestinal (CATS et al., 2003). Outro fator relevante é o número de células viáveis destes micro-organismos no produto comercializado.

2.4 ANÁLISE SENSORIAL

Análise Sensorial é a uma ciência usada para evocar, medir, analisar e interpretar reações às características dos alimentos e materiais, como são percebidas pelos sentidos da visão, olfato, gosto, tato e audição (ABNT, 1993).

Então, “análise sensorial é realizada em função das respostas transmitidas pelos indivíduos às várias sensações que se originam de reações fisiológicas e são resultantes de certos estímulos, gerando a interpretação das propriedades intrínsecas aos produtos. Para isto é preciso que haja entre as partes, indivíduos e produtos, contato e interação. O estímulo é medido por processos físicos e químicos e as sensações por efeitos psicológicos. As sensações produzidas podem dimensionar a intensidade, extensão, duração, qualidade, gosto ou desgosto em relação ao produto avaliado. Nesta avaliação, os indivíduos, por meio dos próprios órgãos sensórios, numa percepção somato-sensorial, utilizam os sentidos da visão, olfato, audição, tato e gosto” (BRASIL, 2008).

Esta ciência é muito importante, uma vez que fornece subsídios fundamentais para a produção e comercialização de produtos, entre eles os alimento, conseguindo caracterizar as preferências e exigências dos consumidores. (SILVA; DUARTE; CAVALCANTI-MATA, 2010).

2.4.1 Aceitação

O teste de aceitação é um teste afetivo. Este é uma importante ferramenta, visto que acessa diretamente a opinião do consumidor frente a um produto já estabelecido ou o potencial de um novo produto, e por isso também é chamado de teste de consumidor. Muito utilizado quando o objetivo é avaliar o grau com que os consumidores gostam ou desgostam de um produto (BARBOSA et al., 2010; SANTOS et al., 2008; SILVA et al., 2007)

2.4.2 Exposição Repetida

A exposição repetida, em inglês “repeated exposure”, é uma metodologia que tem sido reconhecida como uma excelente estratégia para aumentar a aceitação de uma determinada classe ou tipo de alimentos por um grupo específico ou para toda a população, sem restrição (WILLIAMS et al., 2008). Esta técnica foi e é muito utilizada como estratégia, com o intuito de aumentar a ingestão de alimentos por crianças. Diversos são os estudos em que crianças foram repetidamente expostas a um alimento específico (ANZMAN-FRASCA et al., 2012; LAKKAKULA et al., 2010; LIEM; GRAAF, 2004; WARDLE et al., 2003a; WARDLE et al.,2003b). Atualmente este método tem sido muito utilizado em alimentos no qual visasse a redução de sódio, como é o caso de sopas instantâneas (LIEM; TORAMAN AYDIN; ZANDSTRA, 2012; LIEM et al., 2012; METHVEN; LANGRENEY; PRESCOTT, 2012)

2.5 AMINAS BIOGÊNICAS

Quimicamente, o termo aminas é descrito como sendo bases orgânicas derivadas da amônia. Na biologia, o termo está relacionado a compostos formados ou degradados durante os processos metabólicos normais dos seres vivos, apresentando diferentes funções fisiológicas e, por isso, chamadas de “aminas bioativas” ou “aminas biologicamente ativas”. Sendo assim, as aminas bioativas são compostos nitrogenados, em que um, dois ou três átomos de hidrogênio da molécula de amônia foram substituídos por grupos alquila ou arila (HALÁSZ et al., 1994; KALAVC; VSVECOVÁ; PELIKÁNOVÁ, 2002). Estas podem ser classificadas, de acordo com a via biossintética em naturais, formadas durante a biossíntese "in situ", e em biogênicas, formadas por reações de descarboxilação no alimento (GLORIA, 2005).

Na tecnologia de alimentos, o termo “aminas biogênicas” está relacionado a uma categoria de compostos presentes em determinados tipos de alimentos e descritos como potencialmente perigosos, devido ao fato de apresentarem propriedades vasoativas, psicoativas e toxicológicas (BOVER-CID; HOLZAPFEL, 1999; LATORRE-MORATALLA et al., 2007). A denominação destes compostos é realizada a partir dos nomes dos aminoácidos precursores, como por exemplo, a histamina é originada da histidina, a tiramina da tirosina, e triptamina do triptofano, ou de acordo com a origem de onde foram descobertas, como é o caso da espermidina e espermina, que foram primariamente isoladas de fluido seminal (PONS SÁNCHEZ-CASCADO, 2005).

Desta forma, as aminas biogênicas são substâncias formadas durante a fase de transformação dos alimentos, pela ação de enzimas descarboxilases endógenas, ou por enzimas descarboxilases produzidas por microrganismos, que atuam sobre aminoácidos específicos (KALAVC, 2006). As aminas, em alimentos, podem ser intrínsecas do produto (via biossintética - naturais), ou serem formadas por microrganismos adicionados, como no caso das culturas starter, ou contaminantes, introduzidos devido às condições higiênico-sanitárias inadequadas. Por este motivo essas substâncias podem ser utilizadas como parâmetro ou critério de qualidade, refletindo as condições da matéria prima, ou também as condições higiênico-sanitárias durante a fabricação de certos produtos (GLORIA, 2005; HALÁSZ et al., 1994; KALAVC; VSVECOVÁ; PELIKÁNOVÁ, 2002). Uma importante vantagem da utilização destas aminas como critério de qualidade é pelo fato de serem termorresistentes, permanecendo no alimento, mesmo depois tratamento térmico (GLORIA, 2005). Além disso, estes compostos podem ser utilizados na seleção de culturas starter e cepas probióticas (AMMOR; MAYO, 2007; PRIYADARSHANI; MESTHRI; RAKSHIT, 2011; RUBIO et al., 2013).

Os derivados lácteos fermentados favorecem a formação de aminas biogênicas, uma vez que estes promovem as condições ideais para a formação destas aminas (LINARES et al., 2012). Os tipos e as concentrações de aminas biogênicas presentes em produtos lácteos fermentados, assim como em qualquer outro produto fermentado, variam no que diz respeito à matéria-prima, o tipo de produto, o tempo de maturação/ fermentação, os tipos de microrganismos adicionados e a atividade proteolítica, além das condições higiênico-sanitárias da produção (DEEPIKA PRIYADARSHANI; RAKSHIT, 2011; SANTOS et al., 2003). Nos produtos lácteos a tiramina é a aminas biogênicas maior importância, estando presente em maiores concentrações (ANDIC; GENCCELEP; KOSE, 2010; BUŇKOVÁ et al., 2010, 2013; FERNANDEZ et al., 2007; PACHLOVÁ et al., 2012; VALSAMAKI; MICHAELIDOU; POLYCHRONIADOU, 2000).

3 DESENVOLVIMENTO

3.1 ARTIGO 1: PROBIOTIC FERMENTED COW'S AND GOAT'S MILKS: DETERMINATION OF BIOGENIC AMINES AND SENSORY ACCEPTANCE. SUBMITTED TO INTERNATIONAL JOURNAL OF DAIRY TECHNOLOGY (PAPER I)

Probiotic fermented cow's and goat's milks: determination of biogenic amines and sensory acceptance

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ABSTRACT

The aim of this study was evaluate the behavior of biogenic amines in fermented cow's and goat's milks with probiotic bacteria and an overall acceptance test. Initial elevated tyramine levels were observed in both products, which increased throughout the storage period. The highest production of biogenic amines occurred between the first and fifth day of storage, decreasing in the content of such compounds occurred thereafter. A higher overall acceptance of the fermented cow's milk was observed. The results suggested that the content of biogenic amines may be a criterion for selecting lactic acid bacteria to develop fermented milks.

Keywords: caprine milk, bovine milk, HPLC, overall acceptance, tyramine.

INTRODUCTION

Milk is considered one of the most complete foods once it presents several important essential nutrients for humans (Drewmonwski and Fulgoni 2008), and the most produced type is cow's milk (FAO 2012). However, goat's milk has gained market due to its beneficial aspects of functional food as high digestibility and because it is hypoallergenic, being commonly consumed by individuals who are allergic to cow's milk, as children and elderly people, as well as by those individuals allergic to cow's milk (Albenzio *et al*. 2012).

Fermented milks are a traditional food created as a means of preserving fresh animal milk, and particularly, the use of goat's and cow's milk as raw materials for the processing of fermented milks is well established in the modern dairy industry (Tamime *et al*. 2011). The addition of probiotic bacteria into fermented milks adds value with respect to their potential functional benefits (Granato *et al*. 2010). The use of mixed cultures, as *Lactobacillus acidophilus* and *Bifidobacterium lactis* has been successfully used for fermentation of milk. According to Kongo *et al*. (2006), during the first 10 days of stored, occurs a high viability of probiotic strains of fermented goat's milk. In this stage, probiotics produce substances that may provide beneficial effects on human health. However, others sorts of metabolics, such as biogenic amines, may be also produced by probiotics strains at this period.

Bioactive amines are nitrogen-based compounds, that are naturally present in plants, animals and humans (Gloria 2005), and are classified, according to the origin, as natural and biogenic (Bover-cid *et al*. 2006). The biogenic amines can be formed in food during processing or during the period of storage, primarily due to processing of some specific amino acids, by the action of decarboxylases produced by microorganisms (Kalac 2006). Some genera of microorganisms with potential probiotic characteristics possess the ability to form biogenic amines (Bover-cid *et al*. 2006).

The ingestion of foods containing high levels of biogenic amines may be deleterious since they have vasoactive, psychoactive and toxicological properties (Latorre-Moratalla *et al*. 2007). The presence and accumulation of these substances are influenced by numerous factors, such as the composition and availability of free amino acids, water activity, temperature, pH of the medium and especially the presence of decarboxylase positive microorganisms (Pine *et al*. 2001; Schirone *et al*. 2012).

The fermentation and storage processes of fermented milks favor the formation of biogenic amines, once these factors promote a higher proteolytic activity of microorganisms that increase the amount of free amino acids in the products (Linares *et al*. 2011). Thus, the types and contents of biogenic amines present in fermented dairy products vary with respect to feedstock, type of product, time of ripening/fermentation, types of microorganisms used to prepare the food, proteolytic activity and also depend on the manufacturing conditions (Santos *et al*. 2003; Andic *et al*. 2010; Deepika *et al*. 2011).

Based on these facts, the purpose of this study was to evaluate the behavior of some biogenic amines, which included tyramine, putrescine, cadaverine, spermidine and histamine, in probiotic fermented cow's and goat's milks just during the first ten days of storage at 4 ± 2°C. Once a higher viability of probiotic strains occurs at the first ten days refrigerate storage. Additionally, a sensory test was carried out in order to assess the products' overall acceptability.

MATERIALS AND METHODS

Processing of fermented milks

For the preparation of fermented milks were used five liters of UHT cow's and five liters of UHT goat's milk (cow's milk - Macuco®, Rio de Janeiro, Brazil; goat's milk Caprilat®, Paraná, Brazil) and $4x10^8$ CFU/mL of lyophilized *Lactobacillus acidophilus* LA-5®, *Bifidobacterium lactis* BB-12® and *Streptococcus thermophilus* (Chr Hansen, Valinhos, Brazil), in the DVS form (direct vat set). For the fermentation process, the samples remained in the oven at 40 ± 2°C for 8 hours, and the fermentation process was interrupted when the pH reached 4.5. Finally, the product was packaged in 200 mL plastic pots sterilized and stored at 4 ± 2°C.

Physicochemical analyses

All analyses in this study were performed in triplicate. The samples of UHT milks were analyzed as follows: relative density, cryoscopy, fat (ISO 488 / IDF 105, 2008), titratable acidity (AOAC, 1999) and pH. And the fermented milks were analyzed for pH (AOAC 2005), titratable acidity and biogenic amines after the fermentation process (day 0) and each day during the first 10 days of chilled storage (4 ± 2°C). For the pH analyses were used a digital pHmeter (pH Model, Brazil).

The determination of biogenic amines was carried out by high-performance liquid chromatography (HPLC) using the method modified by Cunha *et al*. (2012), which is based on the acid extraction and derivatization was assayed as described by Rodríguez *et al*. (2001) and Mei (1994), respectively. The samples were homogenized with 5% perchloric acid (%v/v). The homogenates were kept under refrigeration (4±2°C) for 1 h and shaken continuously; then, the mixture was centrifuged at 503 x *g* for 10 min at 4±1°C (Hermle Z 360 K) and filtered. The filtered were alkalinized with 2 N NaOH until pH > 12 was reached, after that they were derivatized using benzoyl chloride (40 µL), homogenized (vortex, 15 seconds), and kept at room temperature for 20 min. The mixture was extracted with diethyl ether. The ether layer was aspirated and evaporated to dryness under a stream of nitrogen (Sample Concentrator Techne®, Cambridge, UK). Finally, the residue was dissolved in mobile phase and stored at 4±1°C.

The chromatographic system used was from Shimadzu® model LC/10 AS, coupled to UV detector SPD/10 AV, with integrator C-R6A Chromatopack, using a column Teknokroma, Extrasil Tracer ODS2 (15 x 0.46 cm, id. 5 mm) and Supelco precolumn, C18 Ascentis (2 x 0.40 cm, id. 5 µm). Exactly 20 µL of the prepared sample was injected into the HPLC. The mobile phase consisted of acetonitrile: water 42: 58 (%v/v), which was performed isocratically at a flow rate of 1.0 mL min^{-1}. The peaks were detected at 198 nm.

Five biogenic amines were quantified among them: tyramine ($C_8H_{11}NO$), putrescine ($C_4H_{12}N_2$), cadaverine ($C_5H_{14}N_2$), spermidine ($C_{14}H_{47}N_6O_{12}P_3$), and histamine ($C_5H_9N_3$). Standards of biogenic amines were purchased from Sigma-Aldrich® (St. Louis, MO, USA). Stock solutions for each amine were prepared in 0.1

N HCl and stored at 4±1°C. The quantitative analysis of biogenic amines was carried out using an external standard curve. For the quantification of the amines, standards solutions of individual biogenic amines were chromatographed separately and mixed to determine the retention times and the response of each biogenic amine (Figure 1). The standard curves with correlation coefficient of stock solutions were obtained by external standard method. All the results were expressed in $mg.kg^{-1}$.

Consumer test

The sensory evaluation of the two fermented milks (bovine and caprine) was performed one day after their manufacture and the overall acceptance of samples was assessed by a hedonic test. For this purpose, a nine-point hedonic scale (Drake 2007; Cruz *et al*. 2012) was used a total of 40 consumers (17-61 years). These panelists consisted of students randomly recruited from the Fluminense Federal University, Brazil. The criterion of inclusion to enroll the study was the regular consumption of dairy products, and people with allergy or intolerance to dairy products were not recruited.

The sensory analysis was performed on the second day of storage of the products, and the samples (20 mL) were coded with three-digit codes and presented monadically according to a randomized complete block design (MacFie *et al*. 1989). And the panelists performed the test in individual booths. They were asked to evaluate the overall acceptability of the fermented milks (bovine and caprine), based on a 9 point hedonic scale: like extremely = 9, like very much = 8, like moderately = 7, like slightly = 6, neither like nor dislike = 5, dislike slightly = 4, dislike moderately = 3, dislike very much = 2, dislike extremely = 1.

Statistical analysis

The results from the physicochemical and sensory tests were subjected to one-way analysis of variances (ANOVA) followed by Tukey test using the software GraphPad Prisma 5. A p-value below 5% ($p<0.05$) was regarded as significant.

RESULTS AND DISCUSSION

Physicochemical analyses

The results of cow's and goat's milk were: relative density 1.031 and 1.029; cryoscopy -0.549°H and -0.581°H; fat 3.0% and 3.1%; titratable acidity 16°D and 17°D; 6.71 and 6.7 pH, respectively. Thus, the physical properties and average composition of basic nutrients of cow's and goat's milk are in accordance to other studies (Park *et al*. 2007; Ceballos *et al*. 2009).

Initially (day 0), titratable acidity were 81.9°D and 89.9°D and, after storage time (day 10), were 106.9°D and 110.4°D, respectively for fermented cow's and goat's milk. As well as, the titratable acidity increased in both fermented milks at storage period. However, the acidity of fermented cow's milk was lower than fermented goat's milk, during the first to the last day of storage time. Yoon *et al*. (2013) had the same acidic behavior in yogurt beverage and stirred-type yogurt in Korea.

The initial pH of cow's and goat's milks (6.71 e 6.70, respectively, $p<0.05$) were reduced to, respectively, 4.51 and 4.48 after the end of fermentation process. These final pH values are in-line with the growth of both starter culture and probiotic bacteria (Navarro-Alarcón *et al*. 2011; Han *et al*. 2012). During storage, the mean pH value for fermented cow's milk was 4.50 and 4.51 for fermented goat's milk ($p>0.05$), suggesting the absence of post acidification, corroborating the results obtained by Martín-Diana *et al*. (2003). This finding was probably due to the absence of *Lactobacillus delbrueckii bulgaricus* in the fermented milks, once this bacterial strain is responsible for the post acidification (lactic acid and hydrogen peroxide) during refrigerated storage (Shihata and Shah 2000; Cruz *et al*. 2012; Cruz *et al*. 2013).

Fernandez *et al*. (2007) observed a relation between an acidic pH and an increasing of biogenic amines synthesis. This apparel could explain the fact that decarboxylase enzymes have demonstrated an optimum pH of around 5.0 (Moreno-Arribas and Polo 2008).As well as pH values, another factor related to the increase of biogenic amines amount is the bacteria growth. Therefore, this factor rises the production of decarboxylase enzyme, which increases the contents of biogenic amines.

In relation the determination of biogenic amines for high-performance liquid chromatography, the standard curves with correlation coefficient of 0.9981 (tyramine), 0.9977 (putrescine), 0.9997 (cadaverine) 0.9921 (spermedine) and 0.9343 (histamine) were obtained by external standard method. Thus, a quantitative analysis of biogenic amines was carried out using an external standard curve. All five biogenic amines were well separated in 15 min total run time with good peak resolution, sharpness and symmetry.

Regarding to content of biogenic amines during the first 10 days of storage at 4 ± 2°C, which are high survival of the probiotic culture (Kongo *et al*. 2006), tyramine was the most abundant compound present in both fermented milks throughout the storage period. And this result is in accordance with the contents found in other dairy products (Valsamaki *et al*. 2000; Fernández et al. 2007; Andic *et al*. 2010; Bunková *et al*. 2010; Özdestan and Üren 2010; Pachlová *et al*. 2012; Bunková *et al*. 2013). Up to the fourth day of storage, the tyramine content was higher in fermented goat's milk as compared to fermented cow's milk, demonstrating values 1073.11 $mg.kg^{-1}$ and 293.55 $mg.kg^{-1}$, respectively. This biogenic amine content in goat's fermented milk remained stable until the eighth day of storage. While the tyramine content in cow's fermented milk increased linearly throughout storage, it can be observed in Figure 2.

This different behavior between cow's and goat's fermented milk up to the eighth day of storage may correlate principally with: a different protein composition in particular as casein fractions (Albenzio *et al*. 2012); the initial different content of free amino acids; the relation of amino acids in each milk and the velocity of proteolysis in milk of these ruminants (Nuñez and Medina, 2011). In addition, the high level of tyramine in both products at the end of the 10 days in storage period may be attributed to the production of free tyrosine that is further decarboxylated by microbial enzymes to produce tyramine (Özdestan and Üren 2010).

The cow's fermented milk presented lower concentration of putrescine compared with goat's fermented milk. At day 0, putrescine was 83.29 $mg.kg^{-1}$ and 104.09 $mg.kg^{-1}$; and, in day 10, it was 20.26 $mg.kg^{-1}$ and 22.92 $mg.kg^{-1}$, respectively, fermented cow's and goat's milk. Bunková *et al*. (2013) found the same concentration of amine at day 10 of storage, which half of the fermented milks contained up to 10 $mg.kg^{-1}$, and in two yoghurt were found to contain slightly more than 25 $mg.kg^{-1}$, at the end of their shelf-life period.

In relation to the contents of putrescine a similar decline trend was observed in both fermented milks, as illustrated in Figures 3 and 4. Novella-Rodríguez *et al*. (2002) found in goat's cheese an increase of putrescine at the beginning of ripening, followed by a slight decrease, this decreasing behavior was verified in fermented milks of this study. The reduction of the content of these biogenic amines could be explained by the fact that some lactic acid bacteria are able to degrade biogenic amines by means of an enzymatic pathway regulated by oxidase enzyme (Dapkevicius *et al*. 2000; Tosukhowong *et al*. 2011).

In respect of cadaverine, the mean concentration of this amine remained constant during storage, 29.09 $mg.kg^{-1}$ for cow's fermented milk and 22.07 $mg.kg^{-1}$ for goat's fermented milk. These results may be related to the ability of lactic acid bacteria to produce small amounts of cadaverine (Lorencová *et al*. 2012). Furthermore, Lorencova *et al*. (2012) reported 17 strains of lactobacilli bacteria produced concentrations of cadaverine below 10 $mg.L^{-1}$ in dairy products. Most of the isolates *S. thermophilus* of Gezginc et al. (2013) study demonstrated an ability to produce cadaverine in the range from 1 to 100 $mg.L^{-1}$. It should be noted that the presence of putrescine and cadaverine in food could be indirectly a problem for the consumer. These amines could potentiate the toxic effects of others biogenic amines, as tyramine and histamine, by inhibiting the detoxifying enzymes (Silla 1996;Flick et al. 2001).

The behavior of histamine (Figures 3 and 4) was different in fermented cow's and goat's milk. The first one showed a constant concentration of histamine (average of 17.97 $mg.kg^{-1}$), while in the second one, histamine's level dropped during the storage (from 99.06 $mg.kg^{-1}$ to 53.85 $mg.kg^{-1}$). However, the initial concentration of histamine in fermented goat's milk was high, approximately 100 $mg.kg^{-1}$. Meanwhile, the difference between the concentrations of histamine in both fermented milks may be explained due to higher concentration of histidine in goat's milk (Ceballos *et al*. 2009). The presence of histamine in food represents a public health concern due to its physiological and toxicological effects for the consumer (Latorre-Moratalla *et al*. 2007; Svaro-Gajic, 2009).

In both fermented milks, the spermidine behavior was similar. This fact was constant until the fifth day and then dropped. Despite the fact that this amine presented the same behavior in both fermented milks during storage period, the concentration of spermidine was higher in cow's fermented milk (82.07 $mg.kg^{-1}$) compared with goat's fermented milk (34.85 $mg.kg^{-1}$). Recent studies have demonstrated that strains of *Lactobacillus plantarum* are capable of degrading certain biogenic amines, such as putrescine, spermidine and histamine (Tosukhowong *et al*. 2011). Thus, other strains may also present the same potential, which explains the decrease of putrescine, spermidine and histamine throughout the storage period in this study.

A limitation of this study was the period of biogenic amines analysis (ten days) at storage. However, this short period is very important duo to the highest starter culture viability time (Kongo *et al*., 2006). Bunková *et al*. (2013) found at the end of their shelf-life period of some fermented milk that the total amount of biogenic amines was lower than 30 $mg.kg^{-1}$. However, further research along the storage period of probiotic yogurts should be performed aiming to monitor the biogenic amines formed in a qualitative and quantitative level.

Consumer test

Table 1 shows the results of the overall acceptance test. The fermented cow's milk presented value of 5.575 whilst the fermented goat's milk presented 2.925. It is possible to observe that fermented cow's milk presented higher acceptance scores as compared with goat's fermented milk ($p<0.05$), so the overall sensory scores for fermented goat's milk remained low. The goat's milk was described in the United Kingdom (UK) as "strong, smelly, salty or sweet" (Mowlem 2005). Then the characteristic ''goaty'' taste of goat's milk is unacceptable to many consumers (Slacanac *et al*. 2010). These intrinsic sensory characteristics are related to the presence of short chain fatty acids, such as caproic, caprylic and capric acids

(Ceballos *et al*. 2009), and therefore the fermented goat's milk presented the lowest overall acceptance.

The result of this study, in relation of the lower acceptance of fermented goat's milk is in accordance with previous studies (Martín-Diana *et al.* 2003; Mowlem 2005; Slacanac *et al*. 2010), demonstrating the difficulty of producing a goat product with adequate acceptance and suggesting the need of development of strategies for goat chain to improve the sensory performance of the products. Ranadheera *et al*. (2012) describe that one possible alternative to increase the sensory acceptance of fermented goat's milk could be the addition of fruit juice and/or pulp to the fermented product. However, the addition of fruit juice in probiotic goat's milk yogurt should be carefully evaluated due the presence of inhibitory compounds at the pulp can decrease the viability of this probiotic strain (Ranadheera et al., 2010). And this addition can result in an increase in pH and syneresis and decrease in viscosity (Ranadheera et al., 2012).

CONCLUSIONS

Even as a first assessment, our findings suggest that the processing of probiotic fermented goat's and cow's milks can contribute for the formation of biogenic amines, particularly tyramine, during fermentation. As there are no legal maximum tolerable levels for any biogenic amine in fermented milks in Brazil. The tyramine could be used as quality index for these fermented milks, because the amount of this biogenic amine was an outstanding factor in these fermentd milks. Our findings also confirms the greater acceptance of fermented cow's milk as compared to fermented goat's milk, possibly due to intrinsic factors of goat's milk, suggesting that continuous studies should be performed to optimize the goat's milk products in order to increase their acceptance by consumers.

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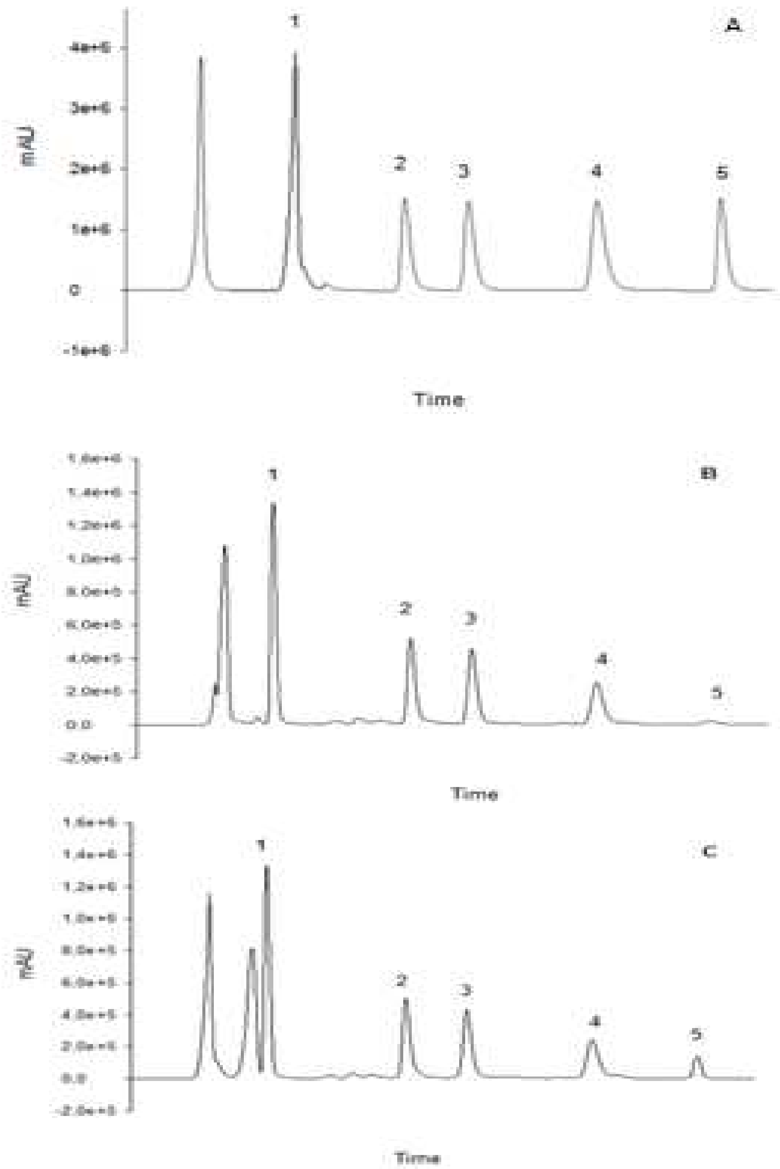

Figure 1. HPLC chromatograms relative to: (A) Standard solution of five biogenic amines; (B) Cow's fermented milk sample; (C) Goat's fermented milk sample. Biogenic amines and retention times, respectively: 1. tyramine (2.85); 2. putrescine (4.67); 3. cadaverine (5.71); 4. spermidine (7.85); 5. histamine (12.59).

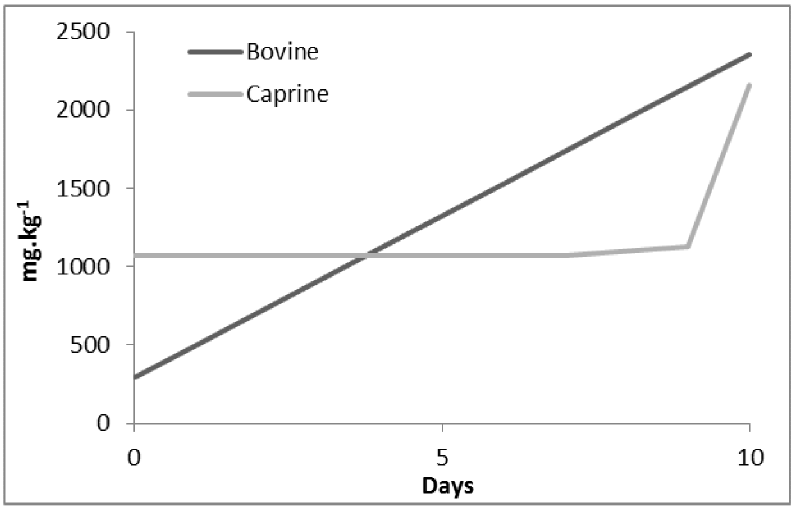

Figure 2. Behavior of tyramine found in both fermented milk (bovine and caprine) during storage.

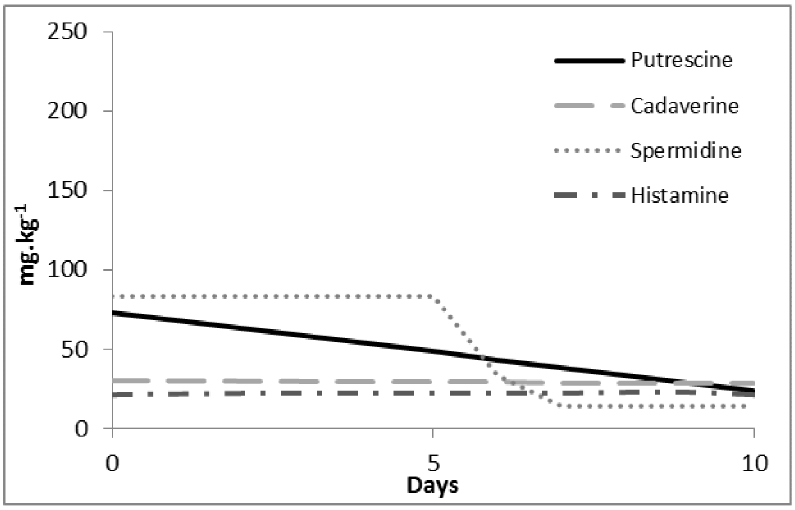

Figure 3. Behaviour of biogenic amines (putrescine, cadaverine, spermidine and histamine) found in bovine fermented milk during 10 days of storage.

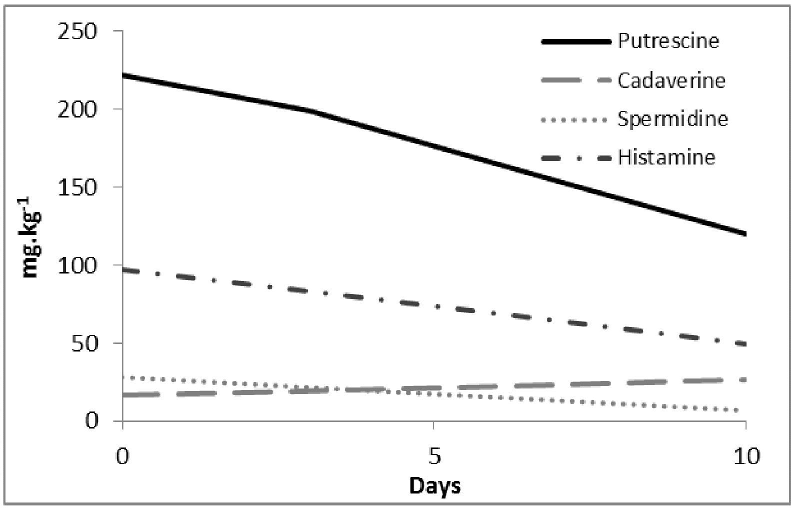

Figure 4. Behaviour of biogenic amines (putrescine, cadaverine, spermidine and histamine) found in caprine fermented milk during 10 days of storage.

Table 1. Consumer test of probiotic fermented milk

Fermented milk	Overall acceptance
Bovine fermented milk	5.575[a]
Caprine fermented milk	2.925[b]

* Mean data from 40 consumers and based on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like no dislike, 9 = like extremely). [a-b] Mean values in the same colunm not followed by the same letters are significantly different ($p < 0.05$).

3.2 ARTIGO 2: CHANGES ON EXPECTED TASTE PERCEPTION OF PROBIOTIC AND CONVENTIONAL YOGURT MADE FROM GOAT MILK AFTER RAPIDLY REPEATED EXPOSURE. SUBMITTED TO JOURNAL OF DAIRY SCIENCE (PAPER II)

Changes on expected taste perception of probiotic and conventional yogurt made from goat milk after rapidly repeated exposure

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Interpretive summary

Changes on expected taste perception of probiotic and conventional yogurt made from goat milk after rapidly repeated exposure. By Costa et al. Goat's milk is a food of high biological value. However, this matrix has a high concentration of short chain fatty acids, such as caprylic, capric and caproic acids, which influence negatively the acceptance of its derivatives for unusual consumers. In this study, there was used a different sensory strategy, a rapidly repeated exposure, which was sufficient to increase significantly the familiarity. However, it was not enough to increase significantly the acceptance of these consumers for goat's milk yogurt.

RUNNING HEAD: Repeated exposure of probiotic goat's milk yogurt

HIGHLIGHTS: Repeated exposures of goat's milk yogurt

Acceptation of probiotic caprine's milk yogurt

ABSTRACT

Goat's milk yogurt is an excellent source of fatty acids, protein and minerals, however, it is not well accepted by unusual consumers, due to the typical flavor derived from caprylic, capric and caprylic acids in this milk and dairy products. Currently, the repeated exposure test has been used to increase the consumption of some foods. This methodology has been used to increase children's willingness to eat food in some settings and has also been used to reduce sodium soup. For this reason, the aim of this study was to investigate whether repeated exposures may increase acceptance of both goat's milk yogurt and probiotic goat's milk yogurt. In a pre-exposure session, a total of 45 panelists (28 females and 17 males) evaluated the expected taste perception and the perceived liking after tasting the three yogurts. After, they were divided randomly into three groups for six days of rapidly repeated exposure session, and each panelist only consumed yogurt which would be exposed. The day after exposure session, all panelists returned to participate to the post-exposure session and they were asked to evaluate acceptance, familiarity and ‘‘goaty taste” characteristic of each yogurt. In relation to expected liking before tasting. The results showed higher expectations for cow's milk yogurt than goat's milk yogurts, which proved that the consumers were not familiar with the goat's milk yogurts. In the perceived liking of the yogurts, only cow's milk yogurt presented high acceptance and familiarity rates, confirming that panelists were used to consume this product. About the rapidly repeated exposure, six days were enough to increase significantly the familiarity of consumers for goat's milk yogurts and the probiotic one. However, it was not sufficient to increase significantly the acceptance of them. Nonetheless, there was a correlation between the exposure sessions and the increase of acceptance EXP1

and EXP2. Thus, it might be suggested that the increasing of exposure sessions could be a strategy to increase the acceptance of the product.

Keywords: caprine milk, *Lactobacillus acidophilus,* familiarity, acceptance.

INTRODUCTION

Goat's milk is an excellent source of fatty acids, protein and minerals. The importance of goat's milk as a functional food is due to its high digestibility and nutritional value, as well as its therapeutic and dietary characteristics. Goat's milk has a smaller size fat globule and high proportion of short- and medium-chain saturated fatty acids, such as butyric, caproic, caprylic and capric, and long-chain mono- and polyunsaturated fatty acids, which is easily absorbed and more digestible than cow's milk (Park et al., 2007). Moreover, it is hypoallergenic, being commonly consumed by individuals who are allergic to cow's milk, as children and elderly people (Albenzio et al., 2012). For this reason, goat's milk is widely used for processing fermented milks and others dairy products. However, its higher concentration of caproic, caprylic and capric acids, which are responsable for its distinctive taste, making this product not well accepted by consumers (Mayer and Fiechter, 2012; Park et al., 2007). Therefore goat's milk yogurt compared to cow`s milk yogurt was inferior concerning overall preference and sensory attributes (Eissa et al., 2010; Masamba and Ali, 2013) due to unpleasant "goaty" taste perceived by consumers even in goat's milk yogurt fruit in low concentrations (Senaka Ranadheera et al., 2012).

Fermented dairy products, especially yogurt, are commonly used as food vehicles to deliver probiotics to consumers. Probiotics are live microorganisms, which when consumed in adequate amounts, confer a health benefit on the host (Sanders, 2009). Health benefits attributed to probiotics included improving intestinal health by the regulation of microbiota, the stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, and reducing the risk of certain diseases (Oelschlaeger, 2010). Also, the addition of probiotic bacteria occurs in dairy industries due to sensory advantages, as well as there are a variety of products that can be formulated with them (Vinderola, Bailo and Reinheimer, 2000). The *Lactobacillus acidophilus* LA-5 may produce flavor compounds such as acetaldehyde that is recognized as an important flavor component in yogurt. The *L. acidophilus* may increase total content of acetaldehyde and it can influence the final product flavor (Ekinci and Gurel, 2008; Guler-Akin and Akin, 2007). Moreover, probiotic goat's milk yogurt may become more acceptable and appealing (Senaka Ranadheera et al., 2012).

Expectations play an important role on the perception of a product before it is tasted. These may positively or negatively influence the product flavor (Deliza and Macfie, 1996). The expectations imply a psychological anticipation that something will occur or be experienced. In general terms, we can define an expectation as a belief that an object possesses a particular attribute or that a behavior would result in a particular consequence. Hence, operationally, we might define it in terms of perceived probability or anticipated magnitude for these attributes or consequences (Cardello and MacFie, 2007). It can be analyzed if the consumers' expectations are met by the product's actual performance, and if not, how the expectations affect the consumers' perceived product performance (Cardello and Sawyer, 1992). Expectations of a product might also influence long-term consumers satisfaction. The hedonic expectations are generated from the emotional and cognitive processes, which lead to anticipation of how much the product is liked or disliked prior to consumption. Ares et al. (2010) obtained a different perception of chocolate desserts, which suggested the importance of consumer expectations on perception and hedonic reaction toward food products. Another study demonstrated that high expectations influenced the liking of local apple juices (Stolzenbach et al., 2013).

Repeated exposure methodology has been recognized as a useful strategy to increase liking of determined class of foods in a specific group or in more general context, for all population (Williams et al., 2008). This approach has also been used in several and different situation in sensory and consumer science. Williams et al. (2008) made an experiment with six children during ten to fifteen days of exposures. Study in which sixty-three infants or children were repeatedly exposed to a specific food, during eight days (Liem and Graaf, 2004) successfully increased liking for that product. Anzman-Frasca et al. (2012) showed that simple familiarization procedures can be used to promote increase vegetable liking and intake among forty-seven young children. In addition Lakkakula et al. (2010) demonstrated fourteen days of repeated exposures to poorly liked vegetables twice weekly over a period of four weeks increased liking for most of these items by elementary school children. Currently this method has been used on reduced sodium soup, performing just eight with 37 consumers (Methven et al., 2012), 46 participants (Liem et al., 2012) and sport drinks using 128 consumers (Kinnear et al., 2011).

The aim of this study was to assess expectation of unusual consumers towards goat’s milk yogurts (probiotic and conventional), and to investigate whether repeated exposures can be used to increase acceptance of this product. The results can be relevant for goat milk industries which intent to achieve more consumers for their products.

MATERIALS AND METHODS

Experimental design

The experimental design of the study is represented in Figure 1. For this experiment, 45 participants were selected. Firstly in pre-exposure session, participants underwent an expected taste perception, in which the expectation of yogurts was evaluated. After, participants received, individually, each yogurt (cows, goats and goat probiotic) and analyzed the perceived liking after tasting. On the following step, the participants were divided into three groups: 1) control, cow's milk yogurt; 2) EXP 1, goat's milk yogurt; and 3) EXP 2, probiotic goat's milk yogurt. The participants had to consume the product for six consecutive days, evaluating the acceptance and familiarity. Finally, the 45 participants returned to the session post-exposure and analyzed the three yogurts, as: characteristic flavor derived from goat's products, acceptance and familiarity.

Yogurt Processing

To produce the yogurts, thirteen liters of UHT cow's whole milk (Macuco®, Rio de Janeiro, Brazil) and twenty five liters of UHT goat's whole milk (Caprilat®, Paraná, Brazil) were used, as well as sugar (5%, v/v), in each yogurt. There were added thermophilic yogurt cultures YF-L903 (Chr Hansen, Valinhos, Brazil) to cow's milk yogurt and goat's milk yogurt at a concentration of 1% (v/v). There was inoculated a probiotic culture of *Lactobacillus acidophilus* LA-5® (Chr Hansen, Valinhos, Brazil) at a concentration of 5% (v/v) to probiotic goat's milk yogurt, further thermophilic yogurt culture YF-L903 1% (v/v).

For fermentation process, the samples remained in the oven at 40 ± 2°C for 8 hours, and fermentation was interrupted when the pH (AOAC 2005) reached 4.5. Then, the product was packaged in 500 mL plastic pots and stored at 4 ± 2°C for one day, until use.

Bacterial Analysis

Enumeration of *Streptococcus thermophilus* was performed on M17 agar, and the count of *Lactobacillus delbrueckii* subsp. *bulgaricus* on Agar de Man, Rogosa and Sharpe (MRS), according to Codex 243-2003 Stand for fermented milk to characterize the fermented product as yogurt (Codex Alimentarius, 2010). Enumeration of the probiotic bacteria (*Lactobacillus acidophilus* LA-5®) was performed on MRS agar supplemented with 0.15% (v/v) bile salts at 37°C for 72 hours under aerobic conditions, as described elsewhere (Cruz et al. 2012; 2013), because there are a specified minimum concentration of 6-7 log to classify the product as probiotic (Bedane et al. 2013). All bacterial counts were performed in triplicate.

Sensory evaluation

For the sensory evaluation the participants were recruited from the Fluminense Federal University. The consumers who did not regularly eat yogurt (i.e. at least once a week), did not like yogurt or had allergies related to any of the ingredients used in the experiment, were excluded from the sensory test. A total of 45 panelists ranging from 20 to 60 years of age (28 females – mean age 48.3 ± 13.6 years; 17 males – mean age 44.5 ± 15.2 years) participate in the study. The gender and age were not related to any of the outcome variables.

Pre-exposure session

Expected Taste Perception. This methodology was adapted from Liem et al. (2012). During a pre-exposure session, consumers were asked to rate their expectations based on their opinion for good yogurt flavor. At this stage the participants had no contact with the product. In order to assess expectations concerning the acceptance and familiarity of goat's milk yogurts and cow's milk yogurts, panelists answered three questions about each treatment: (1) expected acceptance: "What is your expectance taste about this sample”, (2) expected familiarity: “How do you expect to be familiar with this product?”, (3) tasting desire: “How much do you like the typical flavor of goat's milk products?”. The responses were measured on a 9 point-scale, which nine is the best score and one is the worst.

Perceived Liking after Tasting. This methodology was adapted from Liem et al. (2012). After filled the questions about expectation for yogurts, the participants individually tasted each yogurt. The sensory analysis was performed in individual booths, and the three samples (20 mL) were coded with three-digit codes and presented monadically, balanced according to a randomized complete block design (MacFie et al. 1989). The participants were asked to classify how the product actually tasted (hereafter referred to as ‘‘actual’’ taste). The main outcome variables were: acceptance and familiarity of the samples, based on a 9 point hedonic scale: like extremely/ extremely familiar = 9, like very much/ very much familiar = 8, like moderately/ moderately familiar = 7, like slightly/ slightly familiar = 6, neither like nor dislike/ neither familiar nor unfamiliar = 5, dislike slightly/ slightly unfamiliar = 4, dislike moderately/ moderately unfamiliar = 3, dislike very much/ very much unfamiliar = 2, dislike extremely/ extremely unfamiliar = 1.

Subsequently, panelists were randomly divided into three exposure groups, each containing 15 individuals. Over the next six successive working days (Exposures 1–6), all groups received samples of 700mL cow's milk yogurt (control), goat's milk yogurt (exposure group 1 – EXP1) or probiotic goat's milk yogurt (exposure group 2 – EXP2), depending on their group allocation.

Repeated Exposure - Exposure Session

This methodology was adapted from Methven et al. (2012). In each exposure session, the Exposure group 1 (EXP 1; N =15) and the Exposure group 2 (EXP 2; N =15) received, goat's milk yogurt samples (100 ml) and probiotic goat's milk yogurt samples (100 ml), respectively, whereas a Control group (CNTL; N =15) received cow's milk yogurt samples (100 ml). All participants were asked to classify the overall acceptance and familiarity of the samples, for each day. Participants were asked to refrain from eating for at least 1 h prior to the session, and in all cases the exposure took place prior to lunch.

Post-exposure session

After the exposure session, the panelists returned to the laboratory to participate to the final session. The sensory analysis was performed in individual staterooms, and the samples (20 mL) were coded with three-digit codes and presented monadically, balanced according to a randomized complete block design (Macfie et al., 1989). On the day following their sixth exposure, all panelists were asked to evaluate acceptance, familiarity and characteristic ‘‘goaty taste” (Slacanac et al., 2010) of each yogurt sample, using a 9 point hedonic scale. The control group did not evaluate the attribute “goaty taste”.

Statistical Analysis

The results of the first and final sessions were subjected to one-way analysis of variance (ANOVA), considering sample as source of variation. The results of the exposure sessions were subjected to two-way analysis of variance, considering samples and consumers as sources of variation. All ANOVA were subjected to Tukey's test at $P < 0.05$ using XLSTAT version 2013.2.03 (Addinsoft, Paris, France). The ratings of familiarity of the exposure session were also subjected to Pearson's correlation. Data from the final session were analyzed separately for each exposure group (Table 2), whereas acceptance and familiarity data from 45 panelists were grouped for comparison with the first session (Table 3).

RESULTS AND DISCUSSION

Probiotic viable count

After fermentation, bacteria counts of cow's milk yogurt were 9.27 and 8.25 log CFU g^{-1}, while goat's milk yogurt presented 9.31 and 8.20 log CFU g^{-1} for *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, respectively. The bacterial counts characterized the fermented milk elaborated as yogurt according to Codex Alimentarius (2010). For the probiotic goat's milk yogurt, these values were 8.99, 7.83 and 9.49 log CFU g^{-1} for *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus*, respectively. The count of probiotic strain, *L. acidophilus*, was above of the recommended, 6-7 log CFU g^{-1}, for phisiological benefits (Bedane et al. 2013).

The addition of probiotic bacteria has sensory advantages, as well as, the expanding variety of dairy products (Vinderola, et al., 2000; Castro et al., 2013a,b). Besides that the *Lactobacillus acidophilus* LA-5 may produce flavor compounds, such as acetaldehyde, which is recognized as an important flavor component in yogurt (Ekinci and Gurel, 2008; Guler-Akin and Akin, 2007). Moreover, probiotic goat's milk yogurt could become more acceptable and appealing (Senaka Ranadheera et al., 2012). In this study, despite the additional probiotic flavor, there was no increasing in acceptance by consumers (Table 1 and 2).

Expected Taste Perception and Perceived Liking after Tasting

The results of expected taste perception and perceived liking of the yogurts are shown in Table 1. No significant effects of age (20 to 60 years old) or gender (28 females – mean age 48.3 ± 13.6 years; 17 males – mean age 44.5 ± 15.2 years) were observed on liking and consumption, data from all participants were grouped for all analyses, due to lack of information from the application of this type of sensory strategy in dairy products, data were compared to other non-dairy matrices such as juice and soup.

In relation to expected liking before tasting, the control group (cow's milk yogurt) achieved good scores, 7.889 for question one (F=31.887, P <0.001) and 8.156 for question two (F=32.597, P <0.001), which can be explained by the high familiarity of consumers for with the product. These results are in accordance with Stolzenbach et al. (2013), who obtained high expectations for familiar local apple juices. The question three, "How much do you like the typical flavor of goat's milk products?", did not apply to the control group. The EXP1 and EXP2 groups were indifferent for both questions one and three scoring "will not like/will not dislike", which proved that the consumers were not familiar with these products (goat's milk yogurt and probiotic goat's milk yogurt).

The perceived liking of the yogurts evaluated the panelists' acceptance and familiarity with the product. The control group presented high acceptance and familiarity ratings (F=33.943, *P* <0.001; F=32.234, *P*<0.001, respectively), which confirmed that the panelists were used to consume cow's milk yogurt. The same was observed by Methven et al. (2012), who found that high salt soups were overall more familiar prior to exposure. In this study, the acceptance and familiarity ratings of EXP1 and EXP2 groups showed a similar pattern, presenting scores of 4.51 and 5.00, respectively. These results were different from the results found by Ares, Barreiro, Deliza et al. (2010), who obtained higher overall liking for desserts in which consumers had expected "off-flavor". In our study, the consumers were indifferent to goat's products for both expectation and acceptance, maybe due to the initial non-familiarity with these products. Indeed, there is a low consumption of goat milk product by Brazilian people, being the products restricted to a selected social class with more financial resources, which can be acquire these products in special establishments and some markets.

Repeated Exposure

The results of repeated exposure are shown in Figures 1 and 2, which exhibit the mean ratings for acceptance and familiarity of the yogurts, respectively.

An increase in acceptance was observed for EXP1 and EXP2 groups, but not for CNTL group (Fig. 1). Although this increase was not statistically significant (F=20.261, *P* <0.001), and it was not enough for the products to be accepted. Moreover, both groups EXP1 (R=0.990, *P* <0.05) and EXP2 (R=0.992, *P* <0.05) presented a positive correlation of the acceptance increasing and the following days of exposition. Thus, it can be assumed that increasing exposure time may increase the acceptance of goat's milk yogurts. According to Cardello et al. (2000), food acceptance is determined by the perception and liking of the attributes taste, flavor and texture. For children, the acceptance of a new product is related to its appearance, taste and familiarity (Burgess et al., 2006). In the present study, some modifications could also be done to increase the acceptance of goat’s milk yogurt as described by Senaka Ranadheera et al. (2012), who added fruit pulp at the formulation of product.

However, the ratings of familiarity showed a significant increase (Fig. 2) for both goat’s milk yogurts (EXP1 and EXP2 groups). Relative to the first session, this change was evident at the second exposure. A strong positive correlation between familiarity and exposure days was observed for all groups (CNTL R=0.980; EXP1 R=0.991 and EXP2 R=0.998; all P <0.05). Nevertheless, a significant increase in familiarity was not observed for the CNTL group during exposure days, which was expected since one of the criteria for participating to the experiment was regularly eat yogurt (i.e. at least once a week).

Post-exposure session

The results of the final session including acceptance, familiarity and characteristic ‘‘goaty’’ taste are expressed in Table 2. Data were analyzed separately for each exposure group. Table 3 shows the comparison between acceptance and familiarity ratings for the first and final sessions, in which acceptance and familiarity data from 45 panelists were grouped.

In relation to acceptance, there was no significant difference for all treatments (F=1.395, P =0.259), even when separating data for each exposure group (Table 2 and Table 3) or comparing the final session with the initial one (Table 3). Although not statistically significant, the control group showed better acceptance of the product, scoring the hedonic term “like moderately”, while both EXP 1 and EXP 2 groups scored “neither liked / nor disliked”. This fact shows that six sessions of exposure were not sufficient to increase the acceptance of goat’s milk yogurts. However, there was a correlation between the increased acceptance and the exposure days, thus a greater exposure period could increase the acceptance of goat's yogurt.

In children, the acceptance of a new product is related to its appearance, taste and familiarity (Burgess et al., 2006). In addition, Holmer et al. (2012) found that flavor was the main determinant factor of the overall liking, followed by texture, odor and appearance. Children preferences can be modified when they are repeatedly exposed to novel foods. Establishing the number of exposures required for preference learning depends on the sensory properties of the exposure food, for example its initial acceptance. Sometimes 10 or more exposures are needed for preference learning takes place (Liem and Graaf, 2004), varying according to the tested product and target audience. This study was used only six days because it is a rapidly repeated exposure. Once there is not information from the application of this methodology in dairy products and, the fact that, it was directed to adults.

The ratings of familiarity resulted in a significant overall increase of familiarity for both EXP 1 and EXP 2 groups, but not for the CNTL group. The result of CNTL group was not surprisingly, because it demonstrated the familiarity of the panelists for cow’s milk yogurt, which was also observed by Methven et al. (2012). Table 2, shows a significant increase in the ratings of familiarity for the goat’s milk yogurts exposure group, regardless of the addition of probiotics. As for the conventional goat's milk yogurt, the means scores were 5.333, 6.867 and 7.533, respectively for CNTL, EXP 1 and EXP 2. For the probiotic goat’s milk yogurt, the means scores were 5.267, 6.733 and 7.333, respectively for CNTL, EXP 1 and EXP 2. These results show that six days of exposure were sufficient for increasing the consumers' familiarity with the product.

As for characteristic ‘‘goaty’’ taste, the mean scores obtained for the goat’s milk yogurt were 4.867 (CNTL), 5.000 (EXP 1) and 5.533 (EXP2), while for the probiotic goat’s milk yogurt these values were 4.267, 5.067 and 5.200, respectively. Thus, the panelists from both goat’s milk yogurts exposure groups (EXP 1 and EXP2) and control group (CNTL) were indifferent to this attribute, as shown in Table 2. This distinctive taste is related to the presence of short chain fatty acids (caproic, caprylic and capric), which are in higher concentration in goat milk matrix (Mayer and Fiechter, 2012; Park et al., 2007). Surprisingly, this fact differs from literature (Martín-Diana et al., 2003; Mowlem 2005; Slacanac *et al*. 2010), once unusual consumers of these products tend to dislike this characteristic flavor.

The lower acceptance of fermented goat’s milk for unusual consumers is in accordance with some studies (Martin-Diana et al., 2003; Mowlem, 2005; Slacanac et al., 2010), which demonstrated the need for new strategies to attract this type of consumers. Besides the repeated exposure, other two strategies can be used to improve the sensory performance of the goat's yogurts. The first would be to develop these products with skim milk. Once the "goaty taste" present in dairy goat derivatives from short and medium chain fatty acids in goat's fat milk (Park et al., 2007). Senaka Ranadheera et al. (2012) verified that sensory scores for fruit in probiotic goat’s milk yogurt were low. However, their findings indicated that through the improvement of flavor with fruits, probiotic goat’s milk yogurt could become more acceptable and appealing. Therefore, the second strategy is the addition of fruit juice and/or pulp would be an alternative to increase the sensory acceptance of fermented goat’s milk.

The home use test is an effective way to mimetize actual consumption of food products. However, it is not possible to ensure that respondents consume the full sample each day during the period of the study. In addition, population of the Brazil does not consumes goat milk products in large quantities as African and Asian, mainly in Middle East, where most products are traditionally made locally and elsewhere, and some have been industrially produced (Tamime et al., 2011). Therefore, consumers of the present study are not adapted to the peculiar “goaty” taste of that product.

However, our researcher presented interesting findings for the dairy industry, especially for goat milk processors. Even with just six sessions, which in a first view can be it can be few sessions, it is consistent and similar with recent studies published elsewhere. Indeed, the improvement of the liking for a regular food in a reduced-energy version and a snack and soup with intense sensory characteristics were reported using five sessions (O’Sullivan et al., 2010; Weijzen et al., 2008). Recently just eight sessions were used to improve the acceptance and familiarity of a low sodium soup (Methven, et al., 2012) and several types of fruits and vegetables for children (Osborne et al., 2012).

Overall, our study demonstrated with rapidly repeated exposure methodology can be used to improve acceptance and familiarity of probiotic fermented goat milk. This result has not been published before. Further study should cover more consumers and more repeated exposure. The use of consumer profiling techniques (Cruz et al., 2013, Santos et al., 2013) as well as the a complete sensory profiling using quantitative descriptive analysis (Albenzio et al., 2013; Cadena et al., 2012; Kaaki et al, 2012) as well as the investigation of the non sensory factors as packaging design (Kim et al., 2013; Lim et al., 2011) are welcome too.

CONCLUSIONS

Expectations for cow’s milk yogurt were high in comparison with goat’s milk yogurts. The indifferent expectations to goat’s products may be related to the non-familiarity with these products. For exposures sessions, although six days were sufficient to consumers' familiarity with goat’s milk yogurts, there was not enough to increase the acceptance of the products. Despite the additional probiotic flavor, there was no increasing in acceptance by consumers. However, the probiotic value was inserted to the goat yogurt. Increasing exposure sessions during the application of repeated exposure methodology can be a future strategy to increase the acceptance, since there was observed positive correlation between these parameters.

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Table 1. Mean expected and perceived liking for yogurts (cow's milk yogurt, goat's milk yogurt and probiotic goat's milk yogurt).

Treatments	Expected liking before tasting			Perceived liking after tasting	
	1ª Question	2ª Question	3ª Question	Acceptance	Familiarity
Cow's milk yogurt	7.889[a]	8.156[a]	-	7.622[a]	7.644[a]
Goat's milk yogurt	5.289[b]	3.778[b]	5.067[b]	5.000[b]	4.911[b]
Probiotic goat's milk yogurt	4.956[b]	3.200[b]	5.089[b]	4.511[b]	4.311[b]

(1ª Question) "What is your expectance taste about this sample?", (2ª Question) "How do you expect to be familiar with this product?", (3ª Question) "How much do you like the typical flavor of goat's milk products?". Averages in the same column with different letters (a–b) differs $P < 0.05$.

Table 2. Means separated by each group of final session acceptance, familiarity and characteristic ''goaty'' taste of the yogurts.

Exposure groups	**Category**	**Acceptance**	**Familiarity**	**Characteristic ''goaty'' taste**
Cow milk yogurt	Control	7.467[a]	7.933[a]	-
	Exposure 1	6.933[a]	8.200[a]	-
	Exposure 2	7.267[a]	7.600[a]	-
Goat milk yogurt	Control	5.467[a]	5.333[a]	4.867[a]
	Exposure 1	5.267[a]	6.867[a,b]	5.000[a]
	Exposure 2	5.733[a]	7.533[b]	5.533[a]
Probiotic goat milk yogurt	Control	4.867[a]	5.267[a]	4.267[a]
	Exposure 1	5.333[a]	6.733[a,b]	5.067[a]
	Exposure 2	5.933[a]	7.333[b]	5.200[a]

Averages in the same column with different letters (a–b) differs $P < 0.05$.

Table 3. Comparison between first and final sessions.

Treatments	Acceptance		Familiarity	
	First session	Final session	First session	Final session
Cow's milk yogurt	7.622[a]	7.222[a]	7.644[a]	7.911[a]
Goat's milk yogurt	5.000[a]	5.489[a]	4.911[a]	6.711[b]
Probiotic goat's milk yogurt	4.511[a]	5.377[a]	4.311[a]	6.444[b]

Averages in the same line with different letters (a–b) differs $P < 0.05$.

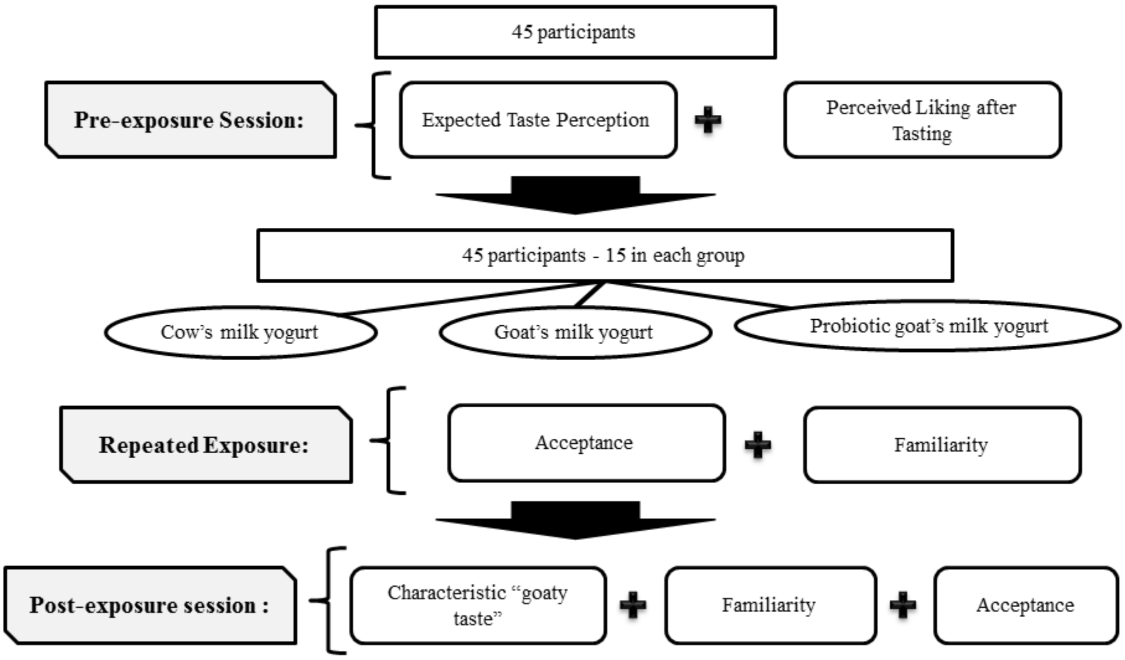

Figure 1. Schematic experimental procedure illustrating the steps involved in the study.

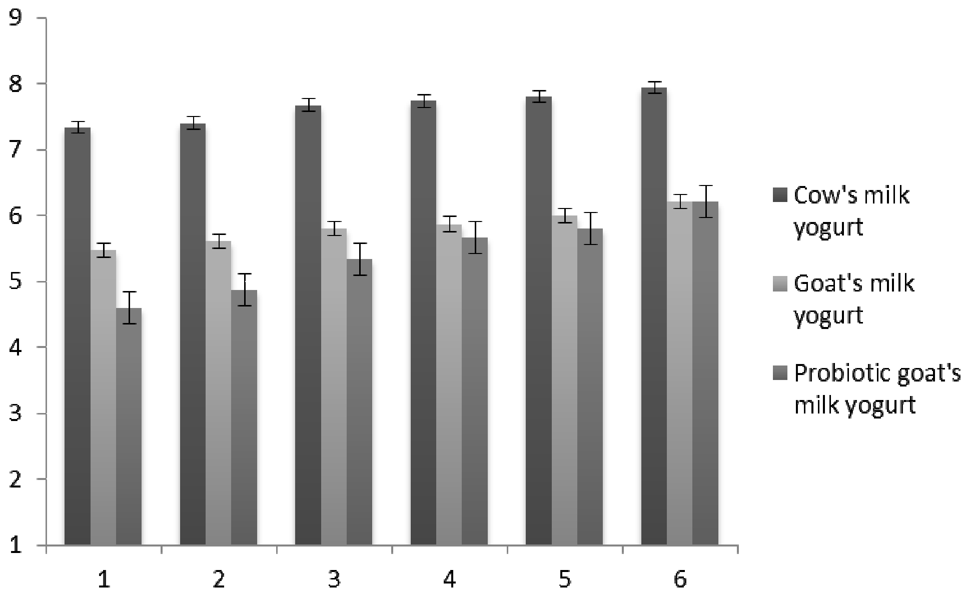

Figure 2. Means and standards deviation of acceptance for the yogurts during the repeated exposure in the study.

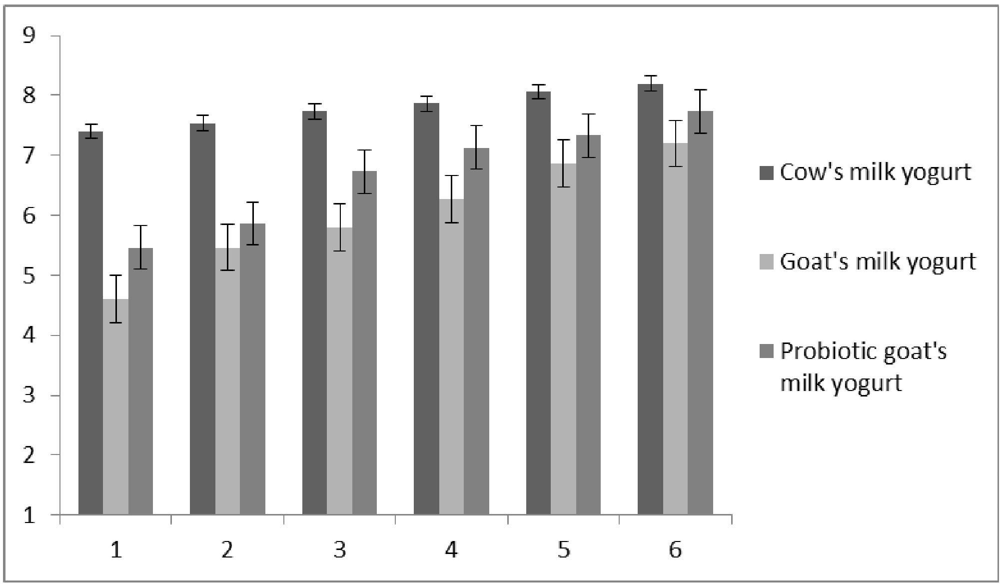

Figure 3. Means and standards deviation of familiarity for the yogurts during the repeated exposure in the study.

4 CONSIDERAÇÕES FINAIS

Em relação aos resultados obtidos nesta dissertação pode-se concluir que houve produção de todas as aminas biogênicas estudadas nos leites fermentados probióticos bovino e caprino. Em ambos os leites fermentados, a tiramina foi à amina biogênica que apresentou maiores concentrações, além de produção constante durante todo o período de estudo. Desta forma, esta amina pode ser empregada como indicadora do índice de qualidade para leites fermentados destas espécies.

Este estudo comprovou a menor aceitação do leite fermentado caprino frente a consumidores não habituais destes produtos. O segundo experimento realizando uma rápida exposição repetida, com o intuito de aumentar a aceitação destes produtos frente a consumidores não habituais, demonstrou que esta metodologia foi eficiente para a familiarização dos consumidores com o produto, no entanto o tempo de exposição deve ser maior visando aumentar também a aceitação.

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6 ANEXOS

6.1 COMPROVANTE DE SUBMISSÃO ARTIGO 1

International Journal of Dairy Technology SDT Society of Dairy Technology

Probiotic fermented cow's and goat's milks: determination of biogenic amines and sensory acceptance

Journal:	*International Journal of Dairy Technology*
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Keywords:	Goat's milk, Cow's milk, Probiotic yogurt, Biogenic amines

6.2 COMPROVANTE DE SUBMISSÃO ARTIGO 2

Journal of Dairy Science

Changes on expected taste perception of probiotic and conventional yogurt made from goat milk after rapidly repeated exposure

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Key Words:	Goat's milk, Probiotic yogurt , Repeated exposure , Biotechnology