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ELABORAÇÃO DE NOVOS IOGURTES FUNCIONAIS CAPRINOS

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DEVELOPMENT OF A NEW FUNCTIONAL GOAT MILK YOGURTS

Tese 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 Doutor.

Orientador: Prof. Dr. CARLOS ADAM CONTE JÚNIOR

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" Algo só é impossível até que alguém duvide e resolva provar ao contrário."

Albert Einst

RESUMO

O iogurte é um produto fermentado, no qual ocorre a fermentação da lactose e consequente produção de ácidos orgânicos, como o ácido lático. O iogurte de leite de cabra, quando comparado aos iogurtes das 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. Visando aumentar a aceitação dos iogurtes elaborados com leite de cabra, pode-se utilizar algumas estratégias tecnológicas, como a adição de polpa de fruta de sabor forte, como o cupuaçu, e o desnate do leite, além de estratégia sensorial, como o uso do efeito da informação de saúde dos compostos antioxidantes presentes na polpa de cupuaçu. Contudo, o desnate do leite interfere nas características físico-químicas, reológicas e de textura, sendo necessário o uso de substitutos da gordura, como carboidratos e proteínas. Ademais, uma alternativa para monitorar o processo fermentativo, além do pH e da acidez, é a análise dos carboidratos e ácidos orgânicos, o qual pode ser realizada porcromatografia líquida de alta eficiência (CLAE). A CLAE é uma técnica amplamente utilizada na análise destes compostos, permitindo avaliar o perfil e a concentração destes durante o processo fermentativo. Contudo, poucos trabalhos avaliam o desempenho desses ensaios bioanalíticos. Por estes motivos, utilizou-se diferentes percentuais de polpa de cupuaçu no processamento de iogurtes caprinos e o efeito da informação de saúde, objetivando o aumento da aceitação destes produtos lácteos. Além disso, foi pesquisado a influência da polpa de cupuaçu, probiótico e prebiótico na cor, pH, viscosidade aparente e textura, durante o período de estocagem a 4±1°C. Ademais, validou-se uma metodologia para análise simultânea dos carboidratos e ácidos orgânicos por CLAE em iogurtes de leite de cabra, estudando o comportamento destes durante a fermentação. Avaliou-se também a influência da adição de inulina, maltodextrina, proteína do soro de leite e leite em pó desnatado nas características físico-químicas, cor, viscosidade aparente e textura de iogurtes caprinos de cupuaçu com teor reduzido de gordura. Os resultados do primeiro experimento (**Artigo 1**) indicam que a adição de 10% de polpa de cupuaçu e o efeito da informação de saúde associado ao consumo de compostos antioxidantes podem ser utilizadas como estratégias tecnológica e sensorial para aumentar a aceitação dos iogurtes caprinos. No segundo experimento (**Artigo 2**) foi confirmado que a polpa de cupuaçu melhora a viscosidade dos iogurtes caprinos, apresentando potencial tecnológico superior a inulina. O terceiro experimento (**Artigo 3**) foi um artigo de revisão, no qual discutiu-se os principais métodos cromatográficos utilizados na análise de carboidratos e ácidos orgânicos em produtos de origem animal, servindo de base para o artigo subsequente de validação de método. No quarto experimento (**Artigo 4**) validou-se um método CLAE-DAD-IR para a determinação simultânea de lactose, glicose, galactose e ácidos cítrico, láctico e fórmico em iogurtes leite de cabra, o qual foi aplicado com sucesso no monitoramento do processo fermentativo iogurtes de leite de cabra acrescido de probiótico, prebiótico e polpa de cupuaçu. No quinto experimento (**Artigo 5**) o objetivo foi investigar as alterações físico-químicas, cor, viscosidade aparente e textura em iogurtes de leite de cabra adicionados de polpa de cupuaçu com substituição de gordura por inulina, maltodextrina, proteína do soro do leite e leite em pó desnatado.

Palavras chave: Polpa de cupuaçu. *Lactobacillus acidophilus* LA-5®. Inulina. Validação. Informação de saúde.

ABSTRACT

Yogurt is a fermented product, in which there is the lactose fermentation and subsequent production of organic acids, such as lactic acid. The goat milk yogurt compared to other dairy yogurts has characteristic flavor and aroma due to the high concentration of fatty acids (caprylic, capric and caproic). which influence negatively the acceptance of the derivatives front goat milk by unusual consumers. To increase the acceptance of yogurt prepared with goat's milk, it can use technology strategies, such as addition of fruit pulp and skim milk, and sensory, as the effect of health information. However, skim milk may interfere with the physicochemical characteristics, rheological and texture. To monitor the fermentation process, the analysis of organic acids and carbohydrates can use as quality index, such the pH and acidity. The high-performance liquid chromatography (HPLC) is widely used technique to analyze these compounds. However, few studies have evaluated the performance of this type of bioanalytical assay. For these reasons, different percentages of cupuassu pulp were added in technological processing of goat milk yoghurt, as well as the effect of the health information was studied to improve the acceptance of these products. Furthermore, it was investigated the influence of cupuassu pulp, probiotic and prebiotic in color, pH, apparent viscosity and texture during the storage period at 4 ± 2 ° C. Moreover, a methodology for simultaneous analysis of organic acids and carbohydrates by HPLC in goat's milk yogurt was validate to study the behavior of these compounds during the fermentation period. It also assessed the influence of the addition of inulin, maltodextrin, whey protein and skimmed powder milk on the physicochemical characteristics, apparent viscosity and texture of goat yogurts with cupuassu reduced fat content. The results of the first experiment (**Article 1**) indicates that the addition of 10% cupuassu pulp can be used as technological strategy to increase the acceptance of goat milk yogurts, as well as the effect of the antioxidants health information. In the second experiment (**Article 2**) cupuassu pulp was confirmed to improve the apparent viscosity of goat milk yogurts more than inulin. The third experiment (**Article 3**) was a review article to discuss the main chromatographic methods used in the analysis of carbohydrates and organic acids from food of animal origin, providing the basis for the subsequent article. The results of the fourth experiment (**Article 4**) demonstrated a HPLC-DAD-IR method specific, linear, accurate, precise and robust, validated for the simultaneous determination of lactose, glucose, galactose and citric, lactic and formic acids on goat milk yogurt, which has been successfully applied in monitoring the fermentation process in goat milk yogurts added probiotic, prebiotic and cupuassu pulp. In the fifth experiment (**Article 5**) the objective was to investigate changes in physicochemical, apparent viscosity and texture of cupuassu goat milk yogurts reduced fat with the addition of inulin, maltodextrin, whey protein and energy.

Keywords: Cupuassu pulp. *Lactobacillus acidophilus* LA-5®. Inulin. Validation. Healthy claim.

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1 INTRODUÇÃO

O consumo de leite de cabra está associado a diferentes efeitos funcionais, como participar da manutenção da saúde, reduzir doenças crônicas articulares e ter efeitos benéficos nas funções fisiológicas. Além disso, o leite de cabra quando comparado ao leite de vaca apresenta maior digestibilidade, alcalinidade e hipoalergenicidade (PARK 1994; PARK et al. 2007). Por estes motivos, o leite caprino apresenta elevado potencial na elaboração de diferentes produtos lácteos funcionais, como iogurtes, sendo considerado um excelente substituto ao leite bovino na alimentação humana. Contudo, 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 que interfere negativamente na textura. Outro entrave está relacionado com a aceitação dos derivados lácteos caprino frente aos consumidores não habituais (COSTA et al. 2014; 2015), devido a seu sabor e aroma característicos oriundos da elevada concentração de ácidos graxos de cadeia curta (ALBENZIO; SANTILLO 2011). A adição de cultura probiótica, ingrediente prebiótico, e/ou polpa de frutas fibrosas, como o cupuaçu, podem ser uma estratégia tecnológica empregada na elaboração de iogurtes pela indústria láctea caprina (COSTA et al. 2015b).

A polpa de cupuaçu (*Theobroma grandiflorum*) contém teores elevados de sacarose, bem como glicose e frutose (ROGEZ et al. 2004), e é uma fonte potencial de fibras alimentares (SALGADO et al. 2011). Estas características físico-químicas são favoráveis para o desenvolvimento e sobrevivência das bactérias ácido láctico presentes no iogurte, indicando que a esta polpa pode ser um ingrediente interessante na elaboração desse tipo de produto (COSTA et al. 2015). Os probióticos são microrganismos vivos que, quando ingeridos regularmente em quantidades adequadas promovem benefícios de saúde para o hospedeiro (SANDERS, 2009). O *Lactobacillus acidophilus* LA-5 é reconhecido como uma cepa probiótica, que também pode produzir metabolitos, tais como ácido lático e acetaldeído (EKINCI & GUREL 2008), os quais influenciam positivamente as características de iogurtes (GEZGINC et al. 2015). Os prebióticos, como a inulina são ingredientes alimentares não digeríveis que estimulam seletivamente o crescimento de bactérias benéficas, o que resulta na

produção de metabolitos desejáveis, ou que favorecem a competição contra bactérias patogênicas (GIBSON et al. 2004). Portanto, a inulina também pode promover o crescimento de probióticos nos iogurtes de leite de cabra (OLIVEIRA et al. 2012). Ademais, este prebiótico é muito utilizada devido as propriedades reológicas, podendo ser empregada como substituta da gordura.

Durante o processo fermentativo, ocorre maior atividade microbiana das bactérias ácido láticas, o que ocasiona na hidrólise da lactose e, consequentemente, a liberação de glicose e galactose e produção de ácidos orgânicos, os quais os tipos e concentrações são dependentes da via metabólica dos microrganismos (homofermentativos ou heterofermentativos) e dos substratos presentes (lactose, glicose, sacarose). A fermentação do carboidrato e produção de ácidos orgânicos são importantes indicadores de atividade bacteriana em produtos fermentados, como os iogurtes. Estes compostos contribuem para o desenvolvimento das características sensoriais, sabor e aroma, deste tipo de produto. Desta forma, as concentrações de carboidratos e ácidos orgânicos podem ser utilizados, tais como o pH e acidez, no monitoramento do processo fermentativo (GONZÁLEZ DE LLANO et al. 1996; COSTA & CONTE-JUNIOR 2015), sendo a cromatografia líquida de alta eficiência (CLAE) uma técnica amplamente utilizada (COSTA & CONTE-JUNIOR 2015), para esta finalidade. Portanto, métodos analíticos rápidos, simples e precisos como a CLAE são desejáveis para indústria de laticínios, especialmente para análise do processo fermentativo de iogurte. Para isso, a validação dos métodos de CLAE é um passo importante para garantir o desempenho de um ensaio bioanalítico.

Outrossim, para melhorar o sabor dos iogurtes de leite de cabra com polpa de cupuaçu, pode-se realizar o desnate do leite, visto que o sabor e aroma típicos dos derivados lácteos caprinos estão relacionados a elevada concentração de ácidos gráxos de cadeia média e curta, tais como os ácidos capróico, caprilico, cáprico (CEBALLOS et al., 2009). No entanto, o desnate do leite pode influenciar negativamente nas propriedades físico-químicas, viscosidade aparente e textura do iogurte. Alternativas para minimizar os efeitos da redução do teor de gordura incluem a adição de substitutos de gordura, como inulina (CRISPÍN-ISIDRO et al. 2015) e maltodextrina (HADNAD et al, 2014.); ou

o aumento dos sólidos totais, como proteína do soro (GAUCHE et al., 2009) e leite em pó desnatado (DAMIN, ALCÂNTARA, NUNES, & OLIVEIRA, 2009).

Por estas razões, objetivou-se neste estudo determinar o melhor percentual de polpa de cupuaçu na fabricação de iogurtes a partir de leite de cabra e do efeito da informação do consumo de compostos antioxidantes na aceitação dos iogurtes de cabra. A partir desta concentração, elaborar novos iogurtes funcionais caprinos acrescidos de cultura probiótica, ingrediente prebiótico e polpa de cupuaçu, avaliando a interferência destes ingredientes na cor, pH, viscosidade aparente e textura, durante o período de estocagem a 4±1°C (0, 7, 14, 21 e 28 dias) dos iogurtes. Além disso, validou-se uma metodologia por CLAE para análise simultânea dos carboidratos e ácidos orgânicos em iogurtes de leite de cabra, estabelecendo o comportamento destes compostos (carboidratos e ácidos orgânicos) produzidos durante o período de fermentação (30 em 30 minutos). Por fim, investigou-se a influência da adição de inulina, maltodextrina, proteína de soro de leite e leite em pó desnatado nas análises físico-químicas, cor, viscosidade aparente e textura de iogurtes caprinos adicionados de polpa de cupuaçu com teor reduzido de gordura.

2 REVISÃO DE LITERATURA

2.1 LEITES FERMENTADOS

Os leites fermentados são "os 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). Estes compreendem uma série de produtos lácteos, dentre eles: 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, não sendo necessariamente o único ácido orgânico produzido (CARNEIRO et al., 2012).

Os leites fermentados são derivados lácteos que apresentam 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 está 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).

O iogurte é o leite fermentado 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).

2.1.1 Iogurte de Leite de Cabra

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). O iogurte elaborado com leite de vaca é denominado apenas de iogurte, enquanto o produto fabricado com leite de outras espécies deve identificar no rótulo a denominação iogurte de, e a espécie correspondente, exemplo iogurte de leite de cabra. O iogurte elaborado a partir do leite de cabra difere em algumas propriedades importantes, além do sabor e aroma, do iogurte elaborado com leite de vaca, dentre elas pode-se citar a firmeza do coágulo, que tende a ser mais suave e menos viscoso no iogurte caprino e a coloração mais branca.

Em relação ao coágulo do iogurte, as discrepâncias estão diretamente relacionadas com as diferenças físico-químicas e reológicas entre estes leites, como o tamanho e a estrutura da micela de caseína, bem como os arranjos dos grupos fosfatos, e o tamanho dos glóbulos de gordura (BOVZANIĆ; TRATNIK; MARIĆ, 1998; KARADEMIR et al., 2002). As micelas de caseína do leite de cabra diferem do leite de vaca em diâmetro, hidratação e mineralização; sendo no leite caprino menores, com maior concentração de cálcio, fósforo inorgânico, menos solvatada e menos estável ao calor quando comparadas a bovina. A composição das frações da caseína no leite de cabra é diferente do leite de vaca, principalmente com relação à fração α-caseína (ALBENZIO et al., 2012). A composição proteica no leite caprino é influenciada por polimorfismos genéticos dos genes αS1-, αS2-, β- e κ-caseína (PARK et al., 2007). Essas diferenças na micela de caseína do leite caprino propiciam maior retenção de água, o que reflete na menor sinérese do iogurte caprino (HAENLEIN, 2004).

Quanto a coloração, o iogurte natural de leite de cabra apresenta coloração branca, a qual é decorrente da ausência de β-caroteno, devido a um processo fisiológico das cabras, no qual há a conversão desta substância em vitamina A (PARK et al., 2007).

2.2 CUPUAÇU

O cupuaçu (*Theobroma grandiflorum*) é um fruto da família Sterculiaceae. Suas características físicas são forma oval, casca marrom, dura, e sua polpa é branca amarelada. A origem do cupuaçu é o sul e o sudeste da Amazônia, sendo, no Brasil, nativo dos estados do Maranhão e Pará. Este é um dos frutos mais populares no mercado amazônico (GENOVESE; LANNES, 2009). Seu sabor é considerado agradável, intenso e agridoce, além de exótico. Entretanto, devido ao sabor forte característico desta fruta, a polpa de cupuaçu não é normalmente consumida pura, mas como ingrediente, utilizado na fabricação de bebidas, como o “vinho do cupuaçu” e o suco, em sorvetes, em licores, em geleias, em conservas e em doces (BASTOS et al., 2002; YANG et al., 2003).

A polpa do cupuaçu é constituída de elevados teores de vitamina C, compostos fenólicos, pectina, açúcares redutores, ferro, cálcio e fósforo, além de uma elevada acidez natural (RIBEIRO et al., 1992). Segundo Porte et al., (2010) os três principais aminoácidos encontrados nas polpas in natura (sem aquecimento) de cupuaçu foram: ácido aspártico, ácido glutâmico e alanina. Na polpa de cupuaçu aquecida, a treonina, a prolina, a isoleucina e o ácido aspártico foram os únicos aminoácidos que tiveram seus teores igualmente reduzidos em todos os tratamentos com diferentes valores de pH. Na maioria dos aminoácidos houve maiores perdas no pH alcalino, o que é interessante, visto que o iogurte é um produto ácido. A sacarose é o principal carboidrato do cupuaçu, sendo que a glicose e a frutose estão presentes em teores bastante próximos. (PORTE et al., 2010; ROGEZ et al., 2004).

Franco; Shibamoto (2000) determinaram, por cromatografia gasosa, vinte e um compostos voláteis do cupuaçu. E determinaram que os compostos químicos predominantes nas amostras de cupuaçu foram os ésteres, e destes, os principais foram o butanoato de etila e hexanoato de etila, seguidos pelo ácido hexadecanóico. Por isto, estes autores concluíram que os ésteres são

importantes para o aroma do cupuaçu. Quijano; Pino (2007), também analisando os compostos voláteis do cupuaçu, identificaram um total de 24 ésteres, 8 álcoois, 4 carbonilas, 4 ácidos, 2 lactonas e um fenol. Destes, os principais compostos relacionados ao aroma foram butanoato de etila, hexanoato de etila e linalol (ALVES; JENNINGS, 1979; BOULANGER; CROUZET, 2000; FRANCO; SHIBAMOTO, 2000; QUIJANO; PINO, 2007).

A polpa fresca de cupuaçu apresenta um alto teor de ácido ascórbico, no entanto, parte desta vitamina é perdida durante o processamento (PUGLIESE et al., 2013), provavelmente devido a reações oxidativas durante o tratamento térmico. Além disso, também apresenta uma quantidade considerável de fenólicos totais, e elevada atividade antioxidante quando comparada a polpa de morango (DA SILVA PINTO; LAJOLO; GENOVESE, 2008). O cupuaçu apresentou uma elevada quantidade de compostos fenólicos, especialmente nas sementes, e de ácido ascórbico em polpas. Esses compostos resultaram em uma alta atividade antioxidante in vitro. (PUGLIESE et al., 2013).

Além disso, a polpa de cupuaçu tem uma composição química particular, rica em fibras, contendo uma quantidade considerável de amido, bem como polissacarídeos de pectina (VRIESMANN et al., 2009), sendo fonte de fibra dietética, principalmente fibra solúvel (SALGADO et al., 2011). As fibras dietéticas ou alimentares são polissacarídeos que não são digeridos pelas enzimas do sistema digestivo humano. As principais fontes de fibras alimentares são os cereais integrais, leguminosas, frutas e hortaliças (LAIRON et al., 2005). Estas são divididas em fibras solúveis e insolúveis. As solúveis, viscosas ou facilmente fermentáveis no cólon, compostas pelas pectinas, gomas, mucilagens, beta-glucanas, psillium e algumas hemiceluloses, formam géis na presença de água. As insolúveis têm ação no aumento de volume do bolo fecal, mas com limitada fermentação no cólon, como a celulose, algumas hemiceluloses e a lignina, formam misturas de baixa viscosidade (ANDERSON et al., 2009; LAIRON et al., 2005). As fibras solúveis e insolúveis têm apresentado, quando ingeridas, efeitos fisiológicos diferenciados. O efeito hipocoleterolêmico das fibras é atribuído à sua fração solúvel, porém, a taxa de redução do colesterol pode variar com o tipo e a quantidade de fibra solúvel consumida (BELL et al., 1990; BROWN et al., 1999).

Estas fibras têm apresentado, quando ingeridas, efeitos fisiológicos diferenciados. Desta forma, a ingestão adequada de fibras alimentares tem sido recomendada por diversas agências governamentais de saúde pública, como uma forma de manter e aumentar a saúde e o bem-estar. Alguns estudos epidemiológicos têm apoiado este consumo, por demonstrar uma relação inversa entre o consumo de fibra dietética e o risco de algumas doenças crônicas (SLAVIN, 2008). Estudos sugerem que as fibras dietéticas protegem contra doença cardiovascular (ESHAK et al., 2010; HARRIS; KRIS-ETHERTON, 2010; MELLEN; WALSH; HERRINGTON, 2008), obesidade (TUCKER; THOMAS, 2009), e diabetes tipo 2 (KALINE et al., 2007; KRANZ et al., 2012). Neste contexto, a fibra dietética tem sido considerada essencial para a saúde digestiva. Os efeitos positivos da fibra alimentar estão relacionados, em parte, ao fato de que uma parcela da fermentação de seus componentes ocorre no intestino grosso, o que produz impacto sobre a velocidade do trânsito intestinal, sobre o pH do cólon e sobre a produção de subprodutos com importante função fisiológica (DEVRIES, 2003). Ademais, o iogurte de cabra acrescido de polpa de cupuaçu pode ser uma boa fonte de fibras, trazendo implicações positivas para a saúde de quem os consome, bem como pode proporcionar uma melhor textura deste produto lácteo caprino.

2.3 PROBIÓTICO E PREBIÓTICO

Os probióticos são micro-organismos vivos, que administrados em quantidades adequadas conferem benefícios à saúde do hospedeiro (FAO, 2001). 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).

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. No entanto, 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.

As bactérias probióticas só apresentam efeitos biológicos no ambiente intestinal se atingirem um número mínimo. No entanto, a dose de probióticos necessária, varia segundo a cepa envolvida e o tipo de produto elaborado. Por este motivo, é difícil estabelecer uma dose aplicavél para todos os probióticos, o ideal seria estabelecer uma dosagem para cada linhagem baseada em estudos clínicos em humanos. Contudo, diversos autores consideram, no caso do consumo de 100 g de produtos lácteos diário, a faixa de 10^7 a 10^9 UFC g-1 de micro-organismos probióticos viáveis uma concentração recomendável (VINDEROLA & RENHEIMER, 2000; SHAN 2000; TAMINE et al.,2005). Especificamente em relação ao Brasil, a Comissão Tecnocientífica de Assessoramento em Alimentos Funcionais e Novos Alimentos, instituída junto à Câmara Técnica de Alimentos (BRASIL, 1999), têm avaliado os produtos com alegações de propriedades funcionais e/ou de saúde aprovados no país. A recomendação brasileira para alimentos probióticos é com base na porção diária do alimento de micro-organismos viáveis que devem ser ingeridos para efeitos funcionais, sendo o mínimo estipulado de 10^8 a 10^9 UFC dia^{-1}.

Os prebióticos são substâncias seletivamente fermentáveis que permitem modificações específicas na composição e/ou na atividade da microbiota gastrointestinal, resultando em benefícios ao bem estar e à saúde do hospedeiro

(ROBERFROID, 2007; WANG, 2009). A inulina e os fruto-oligossacarídeos são os principais prebióticos utilizados pela indústria de alimentos e os mais estudados (SIRÓ et al., 2008), e ainda são os únicos nos quais a alegação de efeito sobre a composição da microbiota intestinal é permitida no Brasil. A inulina pertence a uma classe de carboidratos denominados frutanos, que são considerados ingredientes funcionais, uma vez que exercem influência sobre os processos fisiológicos e bioquímicos no organismo, resultando em melhoria da saúde e redução no risco de ocorrência de diversas enfermidades (ROBERFROID, 2007).

Quanto à dose prebiótica, alguns autores afirmaram que de 5 g/dia de inulina, oligofrutose ou FOS pode ser considerada suficiente para alterar beneficamente a microbiota do cólon, no entanto este valor pode chegar a 8 g/dia em casos específicos (MANNING & GIBSON, 2004; GIBSON, 2007; KOLIDA, MEYER & GIBSON, 2007). Deve-se levar em consideração que a administração conjunta de prebióticos e probióticos (simbióticos) possui efeito superior ao quando são administrados separadamente (DENIPOTE et al., 2010). Segundo a legislação brasileira, para um alimento ser considerado fonte de fibra deve apresentar de fruto-oligossacarídeos (FOS) e de inulina 3 g para alimentos sólidos e de 1,5 g para alimentos líquidos (ANVISA, 2008).

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). Desta forma, esta ciência 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 (BRASIL, 2008). Sendo a análise sensorial muito importante, uma vez que fornece subsídios fundamentais para a produção e comercialização de produtos, entre eles os alimentos, conseguindo caracterizar as preferências e exigências dos consumidores (SILVA; DUARTE; CAVALCANTI-MATA, 2010).

Dentre os diversos testes, o teste de aceitação, que é um teste afetivo, é uma importante ferramenta, por acessar 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). Outra metodologia muito utilizada é “Just About Right”, que possui uma abordagem direta para a mensuração de um nível adequado e desvio de níveis ideais de um atributo específico em um determinado produto. Esta análise permite que os julgadores avaliem diretamente os desvios do ideal, geralmente com escalas hedônicas de cinco a sete pontos, sendo o termo “ideal” o ponto médio da escala (CHAMBERS; BAKER, 1996). Ademais, os resultados obtidos através dos métodos utilizando escalas hedônicas e JAR podem ser correlacionados com a finalidade de fornecer informações direcionadas para a reformulação ou otimização de produtos (LAWLESS; HEYMANN, 1998). Além disso, esta metodologia sensorial está relacionada com a maior discriminação dos atributos. Por esta razão, o JAR é muito popular no caso de desenvolvimento de novos produtos, e muitas vezes é utilizado em conjunto com questões que quantificam a presença e / ou intensidade de um determinado atributo (JAEGER et al. 2015).

Muitos aspectos podem influenciar os consumidores na escolha do alimento, sejam eles atributos intrínsecos, os quais são inerentes aos alimentos, ou extrínsecos, que englobam preço, tipo de alimento, origem, produção e informação nutricional. Estes são usados para formar expectativas sobre o produto. Numerosos estudos têm sido realizados sobre o efeito da informação sobre a preferência dos alimentos, voltados principalmente para a informação de rastreabilidade e processamento de alimentos, os quais demonstram que os consumidores têm uma maior preferência por informações simples e bem conhecidas, tais como alimentos funcionais (BITZIOS et al, 2011; LÄHTEENMÄKI et al, 2010). Contudo, até o presente nenhum estudo avaliou o efeito da informação de saúde relacionado ao consumo de compostos antioxidantes.

2.5 MÉTODOS INSTRUMENTAIS: COR e TEXTURA

A cor dos alimentos é o principal critério de indicação de qualidade, bem como está diretamente relacionado com a aceitação destes produtos frente aos consumidores. Nas medidas instrumentais da cor de materiais opacos, a reflexão da luz sobre o objeto é detectada em escala de três elementos *L**, *a** e *b** (sistema Hunter Lab e CIELAB). O *L** representa à luminosidade, no qual a escala colorimétrica varia entre 0 e 100, cujo o zero corresponde a coloração preta e o 100, a coloração branca. O *a** e o *b** referem-se às coordenadas de cromaticidade. As escalas colorimétricas dos parâmetros *a** e *b** são caracterizadas pela coloração vermelho e amarelo, quando os valores são positivos, e verde e azul, quando os valores são negativos, sendo respectivamente + *a** = vermelho, - *a** = verde, + *b** = amarelo, - *b** = azul (CIE, 2004).

Segundo a Associação Brasileira de Normas Técnicas – ABNT, a textura é definida como todas as propriedades reológicas e estruturais (geométricas e de superfície) de um alimento, perceptíveis pelos receptores mecânicos, táteis e eventualmente pelos receptores visuais e auditivos (ABNT, 1993). Diversos métodos instrumentais têm sido desenvolvidos para determinar as propriedades de textura dos alimentos, estes avaliam propriedades mecânicas a partir de forças aplicadas ao alimento tais como compressão, cisalhamento, corte e tensão (BOURNE, 2002). A Análise do Perfil de Textura (TPA) instrumental aplica sucessivas forças deformantes, numa simulação da ação de compressão e corte dos dentes durante a mastigação (LI, CARPENTER & CHENEY, 1998). Desta forma, durante o teste é realizada uma primeira compressão seguida por um relaxamento e uma segunda compressão, obtendo-se ao final um gráfico (força versus tempo), do qual calculam-se os parâmetros de textura. Este tipo de análise é muito utilizada em alimentos sólidos, no entanto, também pode ser utilizada em alimentos viscosos, como os iogurtes (BURITI et al., 2014; COSTA et al., 2015; ESPÍRITO SANTO et al., 2012; ILIČIĆ et al., 2014).

2.6 CARBOIDRATOS E ÁCIDOS ORGÂNICOS

Os carboidratos são estruturalmente classificados como monossacarídeos, oligossacarídeos e polissacarídeos. Os monossacarídeos e alguns oligossacarídeos têm um sabor doce. Os polissacarídeos, em

combinação com proteínas, lípidos e ácidos nucleicos, desempenham importante papel nos sistemas metabólicos dos animais. Os carboidratos são fonte de energia, e fornecem sabor, estrutura e textura dos alimentos (MANTHEY & XU, 2009). Os ácidos orgânicos são compostos orgânicos com propriedades ácidas que contêm átomos de carbono. Estes não são geralmente considerados nutrientes, mas conferem sabor característico ao alimento. Portanto, os ácidos orgânicos estão entre os principais compostos relacionados com o sabor, em conjunto com os açúcares, gordura e compostos voláteis (URBACH, 1997). Estas substâncias ocorrem naturalmente numa série de alimentos, principalmente em produtos fermentados, como por exemplo os leites fermentados, como resultado da hidrólise do carboidrato devido ao metabolismo bioquímico e da atividade microbiana (LEROY & DE VUYST 2004).

Os ácidos orgânicos são muito utilizados como aditivos alimentares e conservantes para prevenir a degradação e prolongar a vida comercial de alimentos (CHEN et al., 2006; JURADO-S'ANCHEZ et al., 2011), pois atuam principalmente como acidulantes, inibindo o crescimento bacteriano por reduzir o pH dos alimentos (HINTON, 2006; CONTE-JUNIOR et al., 2010). O ácido em estado não dissociado é capaz de penetrar na célula microbiana, o qual não é capaz de tolerar uma grande alteração no seu pH interno (ADAMS & HALL, 1988; GOOSEN et al., 2011).

A lactose é o principal carboidrato no leite de toda as espécies mamíferas, por exemplo, cabras, ovelhas, vacas e búfalas. O teor de lactose no leite é relativamente constante, mas pode sofrer variações entre os diferentes produtos lácteos. A lactose é um dissacarídeo composto por uma molécula glicose e uma molécula galactose, a qual é sintetizada na glândula mamária a partir da glicose sanguínea. O leite, pode apresentar pequenas quantidades de glicose e galactose livres (PARK 1994; HAENLEIN 2004). Outros carboidratos também são encontrados em pequenas concentrações no leite, como os oligossacarídeos, os glicopeptidos, e as glicoproteínas, embora em quantidades muito pequenas (PARK et al., 2007).

Os ácidos orgânicos do leite variam entre 0,12% a 0,21%, ou cerca de 1,2% da matéria seca. O ácido cítrico é o ácido orgânico predominante no leite, estando presente sob a forma de citrato (WALSTRA et al., 2000). Durante o armazenamento, o ácido cítrico desaparece rapidamente como resultado do

crescimento bacteriano, enquanto os ácidos lático e acético são produzidos a partir da degradação da lactose. Outros ácidos também são produzidos a partir da hidrólise da lactose, do ácido cítrico e da gordura. O leite também contém compostos ácidos nitrogenados, tais como o ácido orótico e ácido hipúrico. A concentração de ácido orótico é influenciada principalmente pela dieta e estágio de lactação (TORMO & IZCO 2004).

Durante a fermentação do leite, as bactérias ácido láticas (BAL) utilizam no seu metabolismo a lactose e sintetizam ácidos orgânicos (COSTA et al., 2013). O primeiro passo é a hidrólise da lactose em seus monossacarídeos devido a ação da β-galactosidase, para a maioria das espécies de bactérias, ou por fosfo-β-galactosidase. No leite fermentado, em geral, a produção de alguns ácidos orgânicos, tais como ácidos lático, fórmico, acético, succínico é o resultado da atividade metabólica das culturas *starter* (AMMOR et al., 2006). Estes ácidos contribuem para o sabor do leite fermentado, especialmente o ácido lático, o qual é importante na fabricação de vários derivados lácteos. O ácido lático confere um sabor acentuado, ácido e refrescante para os iogurtes e outros leites fermentados. Durante a fermentação, há um aumento apreciável no nível de alguns ácidos orgânicos, tais como ácidos lático e cítrico. As concentrações de ácidos orgânicos, em qualquer tipo de derivado lácteo depende de diversos fatores como as culturas utilizadas (*starter* e probiótica), tipo de leite e temperatura e tempo de incubação (AKALIN et al., 1997).

A determinação dos teores de carboidratos e ácidos orgânicos em produtos lácteos fermentados faz-se importante, uma vez que estes compostos contribuem para o sabor, textura e propriedades aromáticas destes alimentos (TORMO & IZCO 2004; FARAJZADEH & ASSADI 2009; KRITSUNANKUL et al., 2009). As proporções presentes de carboidratos e ácidos orgânicos podem afetar as características químicas e sensoriais da matriz alimentar (incluindo pH, acidez total, e estabilidade microbiana) e podem fornecer informações sobre as propriedades nutricionais dos alimentos e para otimizações nos processos tecnológicos (CHINNICI et al., 2005). A determinação quantitativa de carboidratos e ácidos orgânicos, também é importante para monitorar o crescimento e a atividade microbiana (IZCO et al., 2002). Neste sentido, a cromatografia líquida de alta eficiência (CLAE) tem sido amplamente utilizado para analisar carboidratos e ácidos orgânicos não-volátil (MURTAZA et al., 2012;

TEROL et al., 2012; LEITE et al., 2013; WANG et al., 2013; ZHOU et al., 2014; GAZE et al., 2015), enquanto a cromatografia gasosa (CG) é usada para determinar ácidos orgânicos voláteis em matrizes complexas (YANG & CHOONG, 2001; ALJADI & YUSOFF 2003; SPAZIANI et al., 2009; SUZZI et al., 2014).

2.7 VALIDAÇÃO EM MÉTODOS CROMATOGRÁFICOS

A validação de um método cromatográfico, de uma forma geral, visa garantir, através de estudos experimentais, que o método em análise atenda às exigências das aplicações analíticas, assegurando a confiabilidade dos resultados (ANVISA). Pode-se distinguir dois tipos de validação: validação no laboratório (“in house validation”) e validação completa (“full validation”). A primeira consiste das etapas de validação dentro de um único laboratório, seja para validar um método novo que tenha sido desenvolvido localmente ou para verificar que um método adotado está bem aplicado. A validação no laboratório é utilizada nas etapas preliminares do desenvolvimento de uma metodologia e na publicação de artigos para revistas científicas, em que são avaliadas todas as características de desempenho da validação da metodologia, porém sem verificar a reprodutibilidade. A segunda envolve todas as características de desempenho e um estudo interlaboratorial que é utilizado para verificar como a metodologia se comporta com uma determinada matriz em vários laboratórios, estabelecendo a reprodutibilidade da metodologia e a incerteza expandida associada à metodologia como um todo (RIBANI et al. 2004).

Os parâmetros analíticos para validação de métodos têm sido definidos em diferentes grupos de trabalho de organizações nacionais e internacionais. No entanto, algumas definições são diferentes entre as diversas organizações (RIBANI et al. 2004). Contudo, alguns parâmetros analíticos são normalmente encontrados para validação de métodos de separação, nos quais podemos citar: seletividade; linearidade; precisão; limite de detecção; limite de quantificação e recuperação (Anvisa, Inmetro, FDA, ICH, AOAC, Comunidade européia).

2.7.1 Seletividade

A seletividade é o primeiro passo no desenvolvimento e validação de um método cromatográfico, e deve ser reavaliada continuamente durante a validação e também durante o uso do método. A seletividade representa a capacidade do método de avaliar, de forma inequívoca, as substâncias em exame na presença de componentes que podem interferir com a sua determinação em uma amostra complexa. Esta considera o grau de interferência de espécies como outro ingrediente ativo, excipientes, impurezas e produtos de degradação, bem como outros compostos de propriedades similares que possam estar, porventura, presentes. A seletividade garante que o pico de resposta seja exclusivamente do composto de interesse. Se a seletividade não for assegurada, a linearidade, a exatidão e a precisão estarão seriamente comprometidas. A seletividade pode ser obtida de várias maneiras. Pode ser avaliada por comparação de matriz isenta da substância de interesse e a matriz adicionada com esta substância (padrão); através da avaliação com detectores modernos (arranjo de diodos, espectrômetro de massas), que comparam o espectro do pico obtido na separação com o de um padrão e utiliza-se; pelo método de adição padrão (RIBANI et al., 2004).

2.7.2 Linearidade

A linearidade corresponde à capacidade do método em fornecer resultados diretamente proporcionais à concentração da substância em exame, dentro de uma determinada faixa de aplicação (ANVISA). A correlação entre o sinal medido (área ou altura do pico) e a massa ou concentração da espécie pode ser expressa mediante a equação da reta ($x = ay + b$) chamada curva analítica. Além dos coeficientes de regressão a e b, também é possível calcular, a partir dos pontos experimentais, o coeficiente de correlação r. Este parâmetro permite uma estimativa da qualidade da curva obtida, pois quanto mais próximo de 1,0, menor a dispersão do conjunto de pontos experimentais e menor a incerteza dos coeficientes de regressão estimados. Um coeficiente de correlação maior que 0,999 é considerado como evidência de um ajuste ideal dos dados para a linha de regressão (RIBANI et al., 2004). As diretrizes da ICH e da ANVISA especificam um mínimo de cinco níveis de concentração, juntamente com certos mínimos de variação especificados. O GARP também sugere cinco

concentrações que devem ser injetadas em ordem crescente de concentração, no mínimo três vezes cada, com estimativa do desvio padrão relativo (RSD) entre as injeções inferior a 5%. A IUPAC recomenda seis ou mais níveis de concentração.

2.7.3 Precisão

A precisão representa a dispersão de resultados entre ensaios independentes, repetidos de uma mesma amostra, amostras semelhantes ou padrões, sob condições definidas. A precisão em validação de métodos é considerada em três níveis diferentes: repetitividade; precisão intermediária; reprodutibilidade. A repetitividade (“repeatability”) representa a concordância entre os resultados de medições sucessivas de um mesmo método, efetuadas sob as mesmas condições de medição, chamadas condições de repetitividade: mesmo procedimento; mesmo analista; mesmo instrumento usado sob as mesmas condições; mesmo local; repetições em um curto intervalo de tempo. A precisão intermediária indica o efeito das variações dentro do laboratório devido a eventos como diferentes dias ou diferentes analistas ou diferentes equipamentos ou uma combinação destes fatores. A reprodutibilidade é o grau de concordância entre os resultados das medições de uma mesma amostra, efetuadas sob condições variadas (mudança de operador, local, equipamentos, etc.). A reprodutibilidade refere-se aos resultados dos estudos de colaboração entre laboratórios (RIBANI et al., 2004).

2.7.4 Limite de detecção e quantificação

O limite de detecção (LD) é a menor quantidade de analito presente na amostra que pode ser verdadeiramente distinguida de zero. O LD do equipamento é definido como a concentração do analito que produz um sinal de três a cinco vezes a razão sinal/ruído do equipamento. Enquanto o LD do método é a concentração mínima de uma substância medida e declarada com 95% ou 99% de confiança de que a concentração do analito é maior que zero (INMETRO).

O limite de quantificação (LQ) é quantidade igual ou maior que o ponto de concentração mais baixo na curva de calibração (AOAC), sendo a característica de desempenho que define a habilidade de um processo quantificar um analito adequadamente. Desta forma, LQ é a concentração mais baixa de um analito que pode ser determinada com precisão aceitável (repetitividade) e exatidão, nas condições declaradas do teste, representando a menor concentração da substância em exame que pode ser medida, utilizando um determinado procedimento experimental (RIBANI et al. 2004).

O LD pode ser calculado de três maneiras diferentes: método visual, método relação sinal-ruído, método baseado em parâmetros da curva analítica. Enquanto o LQ pode ser calculado pelo método relação sinal-ruído, método baseado em parâmetros da curva analítica, sendo o segundo o mais confiável (RIBANI et al. 2004).

2.7.5 Recuperação

A recuperação (ou fator de recuperação), R, é definida como a proporção da quantidade da substância de interesse, presente ou adicionada na porção analítica do material teste, que é extraída e passível de ser quantificada. A recuperação do analito pode ser estimada pela análise de amostras adicionadas com quantidades conhecidas do mesmo analito (RIBANI et al., 2004).

2.7.6 Robustez

A robustez de um método (“robustness”) mensura a sensibilidade que este apresenta frente a pequenas variações, como variações na temperatura, fluxo, tempo de derivatização, e outras. Um determinado método é considerado robusto quando não sofre alteração devido a modificação pequena e deliberada em seus parâmetros. A robustez de um método cromatográfico é avaliada, por exemplo, pela variação de parâmetros como a concentração do solvente orgânico, pH e força iônica da fase móvel. As mudanças introduzidas refletem as alterações que podem ocorrer quando um método é transferido para outros laboratórios, analistas ou equipamentos. Contudo, muitas vezes este parâmetro

é negligenciado na validação de métodos bioanalítico (KARAGEORGOU & SAMANIDOU, 2014).

2.8 SUBSTITUTOS DA GORDURA

A formulação de alimentos em geral com pouca ou nenhuma gordura, sem alteração de sabor, textura e estabilidade durante o armazenamento é um desafio para a indústria, devido às complexas funções dos lipídeos. As indústrias de alimentos, visando a produção de alimentos de baixa caloria, utilizam substitutos de gordura, mantendo a qualidade e minimizando as possíveis alterações. Para tal, deve-se levar em conta que os substitutos da gordura devem desempenhar funções equivalentes às do produto original, em termos funcionais e sensoriais. O uso dessas substâncias vai depender das características e do conteúdo de gordura inicial dos alimentos, e do nível de substituição desejada. A escolha do substituto a ser utilizado é ainda guiada pelo custo, qualidade e inocuidade (SINGHAL et al. 1991). Os substitutos de gordura englobam os carboidratos, como a inulina e a maltodextrina, e as proteínas, como as proteínas do soro e o leite em pó desnatado (MONTEIRO et al. 2006; PINHEIRO & PENNA 2008).

Os Padrões de Identidade e Qualidade (PIQ) dos Produtos Lácteos estabelecem os substitutos de gordura que podem ser utilizados como aditivos nestes produtos. Para os leites fermentados, os substitutos que tem uso autorizado incluem as gomas (carragena, alfarroba, jataí, garrofi n, caroba, guar, tragacanto, arábica, acácia, xantana, karaya, sterculia, caráia, gelan, konjac), celulose microcristalina, metilcelulose, hidroxipropilcelulose, metiletilcelulose, carboximetilcelulose sódica, pectinas, pectina amidada. A legislação brasileira preconiza a adição de 5g de substituto de gordura/kg de produto (BRASIL, 2000).

Para a produção de iogurtes sem gordura, um substituto de gordura é indicado tanto para estabilização da textura, quanto para conferir a sensação de saciedade. Tamime et al. (1994) estudaram as propriedades reológicas de nove tipos de iogurtes com diferentes substitutos de gordura, e encontraram similaridade nas propriedades reológicas de todos os iogurtes, concluindo que os substitutos de gordura não afetam as propriedades reológicas dos iogurtes. Isto

demonstra que estes substitutos podem ser utilizados em iogurtes de baixas calorias. O uso de diferentes substitutos de gordura (à base de proteínas e de carboidratos) podem proporcionar a diminuição da sinérese, enquanto aumenta a viscosidade.

2.8.1 Derivados de carboidratos

A maioria dos substitutos de gordura pertence a esta categoria. Os substitutos de gordura derivados de carboidratos são os amidos modificados, dextrinas, maltodextrinas, gomas, pectina, celulose, inulina e polidextrose. Estes substitutos podem ser empregados da mistura de vários carboidratos para conferir a textura adequada. São usados principalmente como agentes espessantes e estabilizantes e empregados em uma grande variedade de alimentos, como produtos lácteos, sobremesas congeladas, salsichas, molhos para saladas, carnes processadas, assados, margarinas e doces (AMERICAN DIETETIC ASSOCIATION REPORTS, 2005).

A inulina é um carboidrato polidisperso, constituído de subunidades de frutose (2 a 150), ligadas entre si e a uma glicose terminal. É uma fibra solúvel, fermentável e não digerível pela α-amilase e por enzimas hidrolíticas, como a sacarase e a maltase, desta forma não é absorvida na parte superior do trato gastrintestinal, fornecendo substrato para as bactérias do intestino grosso (CARABIN & FLAMM, 1999). Este composto é muito utilizado na indústria alimentícia com o intuito de obter produtos com menor teor de gordura. Em altas concentrações, a inulina tem propriedade de formação de gel quando misturada à água ou leite, resultando em estrutura cremosa que pode ser incorporada em alimentos para substituir até 100% da gordura (FRANCK, 2002). A produção comercial da inulina ocorre a partir da extração de raízes de chicória (*Cichorium intybus*). É comercializada na forma de pó branco, sem odor, de sabor neutro e alta pureza. Não contém glúten, gordura, proteína e ácido fítico, podendo apresentar apenas pequenas quantidades de alguns minerais e sais (ROBERFROID, 2005). Atualmente, é empregada em diversos produtos, como, por exemplo, produtos lácteos e de panificação, bebidas, cereais, entre outros (MEYER et al., 2011).

Cruz et al., (2010) demonstraram que a inulina pode substituir a gordura em produtos lácteos, sem alterar as propriedades sensoriais dos mesmos. Fato este que é corroborado por Pimentel, Garcia & Prudencio (2012) que utilizaram a inulina de cadeia longa como substituto de gordura na elaboração de iogurtes naturais desnatados obtendo características texturais (firmeza, coesividade, adesividade e gomosidade) e sensoriais (aceitabilidade) semelhantes aos iogurtes integrais. Além disso, Oliveira et al. (2011) demonstraram que a suplementação de leite desnatado com inulina, mesmo em baixas concentrações, estimula significativamente o crescimento e viabilidade de *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* e *Bifidobacterium lactis* em leite fermentado desnatado.

As maltodextrinas são biopolímeros originados da hidrólise parcial do amido e são classificadas pelo seu grau de hidrólise, expresso em dextrose equivalente (DE), que é a porcentagem de açúcares redutores calculados como glicose em relação ao peso seco do amido" (COUTINHO, 2007). Em geral, são carboidratos de baixa densidade, totalmente solúveis em água e não possuem aroma de amido (KEARSLEY et al., 1995). Desta forma, A maltodextrina é um polímero de D-glicose, produzida por hidrólise ácida ou enzimática de amido de milho. Quando utilizada como substituto de gordura, a relação água:maltodextrina é de 3:1, produzindo um gel cujo valor calórico é de 1kcal/g ou menos (SOBCZYNSKA; SETZER, 1991; THOMAS; ATWELL, 1999).

2.8.2 Derivados de proteínas

Os substitutos de gordura à base de proteínas são, em sua maioria, produtos convencionalmente utilizados e de segurança estabelecida, e derivados principalmente do leite, ovos e soja. São utilizados em produtos lácteos, doces, sobremesas geladas, manteigas espalháveis, bolos e cobertura para bolos e molhos para salada.

As proteínas do soro proporcionam numerosas vantagens funcionais ao serem usadas em alimentos: são muito nutritivas, criam viscosidade devido à sua capacidade de reter água, formam géis, emulsificam, retêm e incorporam gordura, facilitam o batimento, formação de espuma e aeração, realçam a cor, o sabor, a textura, além de vários. Os isolados protéicos de soro (IPS) são

concentrados com teor de proteínas acima de 90% que possuem excelentes propriedades de geleificação, aeração, emulsificação, retenção de água e incorporação de gordura 30. As principais aplicações de IPS incluem produtos lácteos, de panificação e de confeitaria, “snacks”, aperitivos e carnes processadas. Desta forma, as proteínas do soro apresentam propriedades funcionais variadas nos sistemas alimentares, incluindo a gelificação, espessamento e capacidade de retenção de água (BRYANT & MCCLEMENTS 1998).

No soro do leite estão presentes, um grupo heterogêneo de proteínas que permanecem solúveis após a precipitação das caseínas, sendo caracterizados por mutações genéticas que normalmente se traduzem em substituição de um ou mais resíduos de aminoácido na sua sequência peptídica original (HERNÁNDEZ et al., 2008). Essas frações são representadas por proteínas globulares, sendo elas: β-lactoglobulina (β-Lg), α-lactoalbumina (α-La), albumina do soro bovino (BSA), imunoglobulinas (Ig), lactoferrina, lactoperoxidase, glicomacropeptídeos (GMP), proteose-peptona, entre outras (HARAGUCHI et al., 2006; YÜKSEL & ERDEM, 2009; SOUSA et al., 2012). As proteínas do soro em maior concentração são a β-Lg e α-La, elas constituem de 70 a 80% das proteínas totais do soro (SAARELA, 2007).

Guzmán-González et al. (1999) estudaram a substituição do leite desnatado por produtos lácteos secos, como concentrados de proteína de soro de leite, concentrados de proteínas do leite e leite em pó desnatado, obtendo iogurtes com diferente composição mineral e proteína. Foi observado que estes componentes são decisivos para o processo de gelificação e no tipo de gel obtido. Concluindo que iogurtes preparados com concentrados de proteínas do leite e leite em pó desnatado, exibem maiores valores de viscosidade e sinérese do que os iogurtes preparados com concentrados de proteína de soro de leite.

3 DESENVOLVIMENTO

3.1 ARTIGO I: CONSUMER PERCEPTIONS, HEALTHY INFORMATION AND INSTRUMENTAL PARAMETERS OF CUPUASSU (*Theobroma grandiflorum*) GOAT MILK YOGURTS SUBMETIDO PARA REVISTA INTERNATIONAL DAIRY JOURNAL

Consumer perceptions, health information and instrumental parameters of cupuassu (*Theobroma grandiflorum*) in goat milk yogurt

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ABSTRACT

The objective of this study was to investigate consumers' perceptions of new goat milk yogurt manufactured with cupuassu pulp, including the effect of antioxidant health information on consumer acceptance and purchase intention. A positive expectation regarding linking and familiarity to goat's milk products and products with cupuassu pulp were obtained. Based on PCA, PLSR, JAR and penalty analysis, the addition of cupuassu

pulp improved some sensory attributes of the goat milk yogurt such as cupuassu aroma, cupuassu flavor, yellow color, consistency and viscosity, which positively influenced product acceptance. In addition, antioxidant health information increased the acceptance and purchase intention of cupuassu goat milk yogurts. Taking into account the parameters investigated in this study, the optimal formulation was goat milk yogurt containing 10% cupuassu pulp. Our results suggest that cupuassu pulp can be considered a potential ingredient in goat milk yogurt.

Keywords: Novel product, Expectations, Familiarity, Liking, Antioxidant, Health claims.

1.1. Introduction

The functional dairy market has been increasing due to high demand from health conscious consumers interested in products with physiological benefits, basic nutritional functions and/or decreasing of the risk of chronic disease (Kraus, 2015). In this current scenario, the dairy industry faces great challenges in developing innovative products with useful functional properties, convenience and appropriate sensory quality (Khan et al., 2013). Therefore, the milk products are in evidence and represents a potential target for the functional foods market because of its great product diversity, such as yogurt, which is widely consumed throughout the world (Costa & Conte Junior, 2013).

Although there is an expansive volume of cow milk derivatives consumed, the demand for goat milk products has been growing due to problems with allergies and individuals with special food needs (Ribeiro & Ribeiro 2010). In addition, goat milk products are used consistently as an excellent cow milk substitute in children and elderly nutrition (Costa et al., 2013; Park et al., 2007). Nevertheless, goat milk yogurt has a low

acceptability compared to cow's milk yogurt (Costa et al., 2015a) and is not accepted by non-habitual consumers (Costa et al., 2014). Moreover, goat milk leads to weak curd formation in yogurt due to a lack of αs-1 casein, which makes it difficult to manufacture goat milk yogurt with the appropriate texture. Considering the economic importance and particular nutritional attributes of goat milk products (Li & Guo, 2006), studies that investigate the production of an acceptable yogurt by improving its sensory and instrumental properties (Senaka Ranadheera et al., 2012; Costa et al., 2015b) must be encouraged.

The health-beneficial properties and the potential for use in the exotic tropical fruits food industry in Brazil are well documented in the literature (Bezerra et al., 2015; Costa et al., 2013; Illupapalayam et al., 2014; Paz et al., 2015). However, to the best of our knowledge there is no information available about using cupuassu (*Theobroma grandiflorum*) in the development of goat milk yogurt. Cupuassu is a Brazilian Amazonian fruit belonging to the same family (Sterculiaceae) as cacao (*Theobroma cacao*). This fruit is mainly marketed in pulp form and can be used as an ingredient in producing ice cream, juice, liquors, jellies and yogurts (Vriesmann et al., 2009). Cupuassu is composed of a large amount of starch, pectin polysaccharides (Vriesmann et al., 2009) and dietary fiber, mainly in the form of insoluble fiber (Salgado et al., 2011), which can improve the texture parameters of dairy products compared to other fruit pulps (Costa et al., 2015b). Furthermore, cupuassu pulp contains high ascorbic acid and total phenolic contents, resulting in a greater antioxidant activity compared to fruit pulps that are more commonly used for yogurt production such as strawberry pulp (Silva Pinto et al., 2008; Pugliese et al., 2013).

Moreover, cupuassu is a natural source of antioxidants, which has been widely studied because of the negative impact of synthetic antioxidants on human health (Lobo

et al., 2010). Antioxidant consumption has been associated with decreased levels of oxidative damage to lymphocytic DNA leading to minimized risks for pathologies induced by oxidative stress such as cancer, Alzheimer's and Parkinson's diseases (Zhao, 2009). Studies have reported that information referring to nutrition and health claims may have a positive influence on the decisions of consumers and in improving the overall acceptability and purchase intention of several products such as yogurts, milk and vanilla soybean beverages (Annunziata & Vecchio, 2013; Fernqvist & Ekelund, 2014; Lampila et al., 2009; Vidigal et al., 2011; Villegas et al., 2008). Moreover, some factors, such as attitudes and health interest, may play a role in the consumer liking foods with health claims (Fernqvist & Ekelund, 2014).

In this context, the aim of the present study was to investigate the consumer's perceptions of a new product based on goat milk yogurt and cupuassu pulp. To achieve this aim, the work was divided into three parts, concerning the following: (i) consumer expectations (expected liking and expected familiarity); (ii) consumer acceptance and JAR attributes as well as relationships between their sensory evaluations and the physicochemical parameters; and (iii) impact of health information on the acceptance and flavor of cupuassu goat milk yogurt. As previously described, we hypothesized that cupuassu pulp can improve the sensory attributes of goat milk yogurt. Moreover, health claims can enhance consumer acceptance and purchase intention. Therefore, the cupuassu flavor was assessed to determine the effect of healthy information in this specific parameter considering that flavor is the most important attribute in product evaluation.

2. Materials and methods

2.1. Study design

The sensory evaluation consisted of three studies (Figure 1). Study I: predictions of expected liking and expected familiarity using questionnaire procedures with 300 participants (no consumption). Study II: product optimization by overall acceptability, purchase intention and Just-about-right scaling with 160 participants. In addition, physicochemical parameters were defined in order to correlate with sensory data. Study III: effect of antioxidant health information (blind and informed groups) on overall acceptability, cupuassu flavor and purchase intention for the cupuassu goat milk yogurts (n = 160 participants). The yogurts manufacturing was identical for all studies, which were presented in randomized blocks in a sequential monadic way. Physicochemical analyses (pH, total phenolic content, instrumental color, apparent viscosity and texture) was repeated twice (n = 2). These analyses were performed in the same period of study II, which conducted over two weeks.

2.2. Samples

Four goat milk yogurt samples were formulated utilizing different cupuassu pulp ratios. Twenty-three liters of goat milk yogurt was produced as describe by Costa *et al.* (2015b) with modifications. Thermophilic yogurt cultures (1% v/v; YF-L903; Chr. Hansen, Valinhos, Brazil) were added into UHT goat whole milk (86 – 96% v/v; Cappry's®, Rio Grande do Sul, Brazil) for the fermentation process in a drying oven (42–44 °C) until the pH reached 4.6. The samples were maintained between 3–5 °C for 24 h. Thereafter, yogurts were formulated by the mixing of two ingredients as follow: sugar (3% w/v) and cupuassu pulp (0 – 10% w/v). Four treatments of goat milk yogurt samples were prepared: natural (NY), 5% cupuassu pulp (CY5), 7.5% cupuassu pulp (CY7.5) and

10% cupuassu pulp (CY10). After the mixture of ingredients, samples were stored and refrigerated at 3–5 °C for 2 h until analysis.

2.3. Consumer testing

Participants were recruited from the Food Science and Technology Department and the Nutrition Department (Universidade Federal Fluminense, Brazil). For the questionnaire session, participants were randomly recruited. For the food tasting sessions, all participants provided written informed consent and were selected as participants with a frequency of yogurts consumption (at least once a week), and as non-sensitive consumers such as individuals free of lactose intolerance and allergies to milk and its derivatives.

In addition, samples (20 mL) were served in individual glasses in a monadic sequential order using a balanced block design in standard sensory booths. All samples were served in plastic containers (35 mm diameter) between 7–9 ºC and coded with random 3 digit codes. For sensory evaluation of aroma, all samples were served with lids closed and participants were instructed to remove the lid only at the moment of the aroma analysis to avoid volatile loss. Cream crackers without salt and filtered water at controlled room temperature (23–25 °C) were used to cleanse the palate between samples.

2.4. Study I – Expectations

This study was carried out according to Costa et al. (2014) with modifications. Participants (n = 300, 228 female, 72 male) with ages ranging from 18 to 65 years old (M = 25.73, SD = 6.76) were instructed to answer a questionnaire about expected liking and expected familiarity regarding goat milk yogurt, cupuassu pulp and the specific product

(cupuassu pulp added to goat milk yogurt). This study was performed over two weeks. The consumers who infrequently eat yogurt (i.e., at least once per week) were excluded from the sensory test. Three questions were used to score participants' expectations regarding product acceptance:

(i) What is your expected liking about the flavor of goat milk products?

(ii) What is your expected liking about the flavor of cupuassu pulp?

(iii) What is your expected liking about the flavor of goat' milk yogurt with cupuassu pulp added?

These questions were answered on a 9-point category scale (1 = would extremely dislike to 9 = would extremely like). In addition, participants rated their expected familiarity on a 9-point scale anchored at both extremes (1 = not at all familiar to 9 = extremely familiar) by using two questions:

(i) What is your familiarity with goat milk products?

(ii) What is your familiarity with products containing cupuassu pulp?

2.5. Study II – Sensory and physicochemical analyses

In study II, the sensory tests were assessed by overall acceptability, purchase intention and Just-about-right scaling. In addition, physicochemical analyses (pH, total phenolic content, instrumental color, apparent viscosity and texture) were determined to correlate them with sensory data.

2.5.1. Acceptance test and purchase intention

Test sessions were performed between Monday and Friday over two weeks. One-hundred and sixty participants (122 females, 38 males) ranging from 19 to 63 years old (M = 25.99, SD = 6.90) were recruited for study II.

The participants evaluated appearance, color, aroma, flavor, texture, and overall acceptability of each sample based on 9-point category scale (1 = extremely dislike to 9 = extremely like). Additionally, aroma (acid, alcoholic, caprine, and cupuassu), taste (sweet and acid), flavor (caprine, and cupuassu), color (blank and yellow) and texture (consistency and viscosity) were evaluated using a five-point Just-About-Right (JAR) scale anchored at both extremes (1 = not enough to 5 = too much), with a central point at 3 (ideal) (Li et al., 2014). Participants also scored their purchase intention on a five-point scale ranging from 1 = certainly wouldn't buy to 5 = certainly would buy.

2.5.2. Physicochemical analyses

Physicochemical analyses of pH, total phenolic content, instrumental color, apparent viscosity and texture parameters were determined in Study II to characterize the new goat milk yogurt as well as to correlate these parameters with sensory attribute data. The pH values were measured according to AOAC methods (AOAC, 2012), using a digital pHmeter Model PG1800 (Cap Lab®, São Paulo, Brazil).

Total phenolics content (TPC) was performed using a Folin–Ciocalteu method (Singleton & Rossi, 1965) with modifications. Briefly, 1 mL of sample was transferred to a 100 mL volumetric flask and its volume adjusted with Milli-Q water. Subsequently, the volumetric flask was placed in an ultrasound bath for 10 min. After an overnight 12 h (2–4 °C) incubation, samples were again placed in an ultrasound bath for 10 min and filtered through a 0.45 µm membrane. Folin-Ciocalteu phenol reagent (2.5 mL) was added to the

samples (500 µL) followed by vortexing for 1 min. Thereafter, 2 mL Na_2CO_3 solution (7.5%) was added and mixed by vortexing for 1 min. All samples were kept in the dark for 2 h before absorbance reading at 760nm on a UV-1800 Spectrophotometer (Shimadzu, Tokyo, Japan). The results were expressed as milligrams of Gallic acid equivalents (GAE) per liter of yogurt (mg GAE/L). Both pH and TPC analyses were performed in triplicate.

Instrumental color parameters were determined by reflectance using a Minolta CM-600D Spectrophotometer (Minolta Camera Co., Osaka, Japan). The colorimeter was previously calibrated with illuminant D65 and a 2° standard observer (Costa et al., 2015b). Yogurt samples (50 mL) at between 4–6 °C were stirred and filled in an aluminum cylinder (55 mm outside diameter), with the surface optically flattened and the sensor was mounted directly on the top of the cylinder to avoid light noise from the environment. The L^* regarding lightness (from black to white, 0 – 100), a^* (from green to red, $-a^* - +a^*$) and b^* (from blue to yellow, $-b^* - +b^*$) values were determined.

For apparent viscosity analyses, yogurts samples (100 mL) between 4–6 °C were stirred and placed on glass with a 65 mm outside diameter. This parameter was evaluated using a Q860M21 microprocessor- controlled rotational viscometer (Quimis, São Paulo, Brazil) equipped with rotor No. 2 and a speed of 60 rpm (Costa et al., 2015b). The results were expressed in mPa.s.

Texture analysis was performed according Costa et al. (2015b) using a TA.XT plus texture analyzer (Stable Micro Systems Ltd., Surrey, England) equipped with a 50 kg.f load cell. The texture parameters analyzed were firmness, consistency and cohesiveness. The samples (100 mL) were compressed at 10% of original height with a back extrusion cell (A/BE) disc (36 mm diameter; 30 mm distance; 0.001/ms speed), under temperature-controlled conditions (4–6 °C). The assays were carried out in a 50

mm diameter standard size back extrusion container, and the disc was placed in a central position over the sample container.

All instrumental measurements were performed in triplicate.

2.6. Study III – Effect of health information

Study III was carried out over two days. Participants (n = 160, 90 females, 70 males) ranging from 19 to 60 years old (M = 28.85, SD = 8.62) were divided into two groups to evaluate the impact of health information on the overall acceptability, cupuassu flavor and purchase intention of the cupuassu goat milk yogurts (CY5, CY7.5 and CY10). The participants assessed cupuassu flavor, overall acceptability (9-point scale ranging from 1 = extremely dislike to 9 = extremely like) and purchase intention (5-point scale ranging from 1 = certainly wouldn't buy to 5 = certainly would buy). Moreover, a dummy sample was served as the first sample to participants with the aim of eliminating the first sample effect (Lawless & Heymann 2010). This sample contained an identical volume of each sample (CY5, CY7.5 and CY10). The results from the dummy sample were not considered in the statistical analyses (Kim and Hong 2015). Eighty participants received the samples without information (blind group), while the following information was provided to the other 80 participants (informed group):

You will taste a new health yogurt, which was prepared with goat milk and cupuassu pulp. The cupuassu pulp contains high ascorbic acid levels and phenolic contents, which are natural antioxidants compounds. The consumption of both ascorbic acid and phenolic compounds is associated with beneficial health effects such as delayed aging and prevention of degenerative pathologies i.e. Alzheimer's disease.

After score testing, a question was asked to ensure that participants read the information: Why is the consumption of antioxidants important? The results from participants who provided wrong response or not answered were not used for any data analyses.

2.7. Statistical analyses

Physicochemical parameters (pH, total phenolic content, and instrumental color, apparent viscosity and texture) as well as sensory parameters (acceptance, JAR, purchase intention, and health information) were analyzed by one-way ANOVA with post-hoc Tukey tests at the 95% confidence level ($p < 0.05$). For sensory data, samples were considered as a fixed source of variation and consumer as a random effect. Principal component analysis (PCA) was performed to verify the parameters that were influenced by the cupuassu pulp. Partial least squares regression (PLSR) was used to verify if the determinant parameters contributed positively or negatively to the overall acceptability of the cupuassu yogurts made from goat milk. Penalty analysis was carried out on JAR data to identify decreases in the overall acceptability wherein consumers rated the attributes at "not enough" or "too much". Pearson's correlation was performed to correlate the physicochemical and sensory data ($P < 0.05$). All statistical analyses were performed using XLSTAT version 2012.6.08 software (Addinsoft^{TM}, Paris, France).

3. Results

3.1. Study I – Expected liking and expected familiarity

The percentage of answers related to each question about expectations (expected liking and expected familiarity) for each item of the 9-point hedonic scale is exhibited in Figures 2A and 2B, respectively. Regarding expected liking, most of the responses scored between 'would like slightly' and 'would like very much' (6–8) totaling 65%, 67% and 69% for goat milk products, cupuassu pulp and cupuassu goat milk yogurt respectively (Figure 2A). However, the expected liking [$F_{(0.10)} = 53.220$, $P < 0.001$] related to cupuassu pulp flavor was lower than the expected liking concerning goat milk products and cupuassu goat milk yogurt flavor (Table 1).

The expected familiarity for goat's milk products was rated between 'neither would like' nor would dislike' – 'would like moderately' (5–7) by 48% of the participants, whereas 57% of the consumers scored the expected familiarity between 'slightly familiar' and 'very much familiar' (6–8) for products containing cupuassu pulp (Figure 2B). Regarding mean values, the participants demonstrated greater familiarity [$F_{(0.04)} = 27.775$, $P < 0.001$] with products containing cupuassu pulp than with goat milk products (Table 1).

3.2. Study II – Sensory and physicochemical analyses

3.2.1. Acceptance test and purchase intention

Table 2 presents the data on consumer acceptance test on NY, CY5, CY7.5 and CY10 goat milk yogurt. No difference ($P > 0.05$) was observed in appearance, color, aroma, flavor, texture, overall acceptability and purchase intention for all treatments (NY, CY5, CY7.5 and CY10). Nevertheless, appearance, color and aroma attributes were positively scored. Appearance and color were rated between like slightly and like

moderately (6–7) whereas aroma was scored between neither like nor dislike and like slightly (5–6). Regarding purchase intention, all treatments were rated between ‘probably would not buy’ and ‘may/may not buy’ (2–3).

3.2.2. JAR profile and penalty analysis

The responses for the JAR questions on a 5-point hedonic scale are presented in Table 3. No difference ($P > 0.05$) was observed in caprine aroma, blank color, acid and sweet taste. In general, the cupuassu increment resulted in a greater perception of acid, alcoholic and cupuassu aroma, cupuassu flavor, yellow color, consistency and viscosity. In addition, cupuassu pulp (10%) decreased the perception of caprine flavor in yogurt.

Penalty analysis was used with JAR scores to identify a potential formulation for the improvement of goat milk yogurt proposed to increase consumer acceptance (Table 4). The parameters with a > 0.5 penalty score and > 20% occurrence were considered detrimental attributes for overall acceptability. Blank and yellow colored attributes were not penalized in any treatment. However, all treatments were penalized by too much of a caprine aroma, acid taste, and caprine flavor as well as not enough sweet taste, consistency and viscosity. The yogurt samples containing cupuassu pulp (CY5, CY7.5 and CY10) were penalized by too much of an acid aroma and alcoholic aroma but not enough cupuassu aroma and cupuassu flavor. Nevertheless, all attributes evaluated in this experiment were close to ideal (JAR ranging from 2.07 to 3.57) in all treatments.

3.2.3. Physicochemical analyses

In study II, the physicochemical parameters were also defined with the aim of characterizing the new yogurt and of correlating these parameters with sensory attributes. The physicochemical results (pH, TPC, color, apparent viscosity and texture) are exhibited in Table 5. The cupuassu increment did not affect the firmness, consistency or cohesiveness ($P > 0.05$); however, other physicochemical parameters such as pH, TPC, lightness, redness, yellowness, and apparent viscosity were potentially affected by the addition of cupuassu ($P < 0.05$). High cupuassu levels (CY10) demonstrated greater apparent viscosity than natural goat milk yogurt (NY). Moreover, the addition of cupuassu pulp demonstrated lower pH values and lightness but greater TPC, redness and yellowness compared to NY ($P < 0.05$). In addition, these parameters exhibited a significant interaction with the cupuassu level wherein pH and lightness decreased with increasing cupuassu levels while TPC, redness and yellowness increased with increasing cupuassu increments ($P < 0.05$).

3.2.4. PCA and PLSR

The two principal components explained 89.39% of the observed variance (Figure 3). The first component (PC1) was predominant and contributed a higher percentage of explained variance (70.41%) than the second component (PC2) (18.98%). PC1 separated all treatments (NY, CY5, CY7.5 and CY10) into two groups based on sensory properties (aroma, taste, flavor, color, texture) and physicochemical parameters (pH, TPC, color and apparent viscosity). The cupuassu goat milk yogurts (CY5, CY7.5 and CY10) can be identified by greater acid, alcoholic and cupuassu aromas, acid taste, cupuassu flavor, yellow color, consistency and viscosity, TPC, redness, yellowness and apparent viscosity. Nonetheless, cupuassu treatments were characterized by lower caprine aroma, caprine

flavor, black of color, pH and lightness. In addition, PC1 and PC2 demonstrated that acid taste, cupuassu aroma, cupuassu flavor, yellow color, consistency, TPC, redness and yellowness were more pronounced in CY10.

PLSR was used to determine the sensory attributes and physicochemical parameters, which contributed to overall acceptability (Figure 4). The PLSR model (Q2 = 0.818) explained 95.0% of the overall acceptability by consumers (Y-axis) and 99.4% of the hedonic scores and physicochemical parameters (X-axis). The sensory and physicochemical parameters were considered relevant when their respective "Variable Important to the Projection" value was >1.0 (Wold *et al.* 2001). Aroma, flavor, texture, cupuassu aroma, acid taste, cupuassu flavor, yellow color, consistency, viscosity, TPC, redness, yellowness, and apparent viscosity positively contributed to the overall acceptability of the cupuassu goat milk yogurt. On the other hand, pH and lightness were considered detrimental parameters to overall acceptability. Regarding Pearson's correlation, coefficients of a strong association were observed amongst sensory attributes and physicochemical parameters. The most important correlations were between flavor and caprine flavor (r = -0.850), cupuassu flavor and caprine flavor (r = -0.907), acid taste and cupuassu flavor (r = 0.847), acid taste and pH (r = -0.827), yellow color and lightness (r = -0.925), yellow color and redness (r = 0.959), yellow color and yellowness (r = 0.977), sensory consistency and apparent viscosity (r = 0.922), and sensory viscosity and apparent viscosity (r = 0.919).

3.3. Study III – Antioxidant health information

The hedonic evaluation of yogurt samples (CY5, CY7.5 and CY10) in the two different experimental conditions (blind and informed) was performed to investigate the

influence of antioxidant health information on consumers' acceptance concerning overall acceptability, cupuassu flavor and purchase intention (Table 6). The healthy information increased the overall acceptability [$F_{(0.08)} = 14.95$, $P < 0.001$; $F_{(0.17)} = 32.44$, $P < 0.001$; $F_{(0.13)} = 23.33$, $P < 0.001$], cupuassu flavor [$F_{(0.08)} = 14.95$, $P < 0.001$; $F_{(0.13)} = 24.79$, $P < 0.001$; $F_{(0.11)} = 19.95$, $P < 0.001$], and purchase intention [$F_{(0.13)} = 24.10$, $P < 0.001$; $F_{(0.15)} = 28.20$, $P < 0.001$; $F_{(0.18)} = 34.38$, $P < 0.001$] in all treatments (CY5, CY7.5 and CY10).

4. Discussion

In the present manuscript, our main interest was to study the acceptance of goat milk yogurts manufactured with different levels of cupuassu pulp based on evaluation of expectations, consumer perception and the effect of health information.

For food consumption, a positive expectation plays an important role, suggesting that it can improve the perception of a traditional product, even before it is tasted. In addition, different levels of product familiarity strongly influence the perception of traditional products by consumers (Hong et al., 2014). The results of study I indicate that the most of participants exhibited a positive expectation for both expected liking and expected familiarity with respect to goat milk products and products with cupuassu pulp. In addition, for expected familiarity, consumers were more familiar with products containing cupuassu pulp than with goat milk products. However, the relationship between expectations and real sensory perception is likely very critical in the case of a novel product (Tuorila et al., 1998), which explains our findings. Despite the low score on the expected liking of cupuassu pulp flavor and less familiarity with goat milk products, no influence was observed in the acceptance of cupuassu goat milk yogurt (CY5, CY7.5

and CY10), indicating the potential of using cupuassu pulp as a functional ingredient in products derived from goat milk.

Although the addition of cupuassu did not affected ($P > 0.05$) the acceptance attributes (appearance, color, aroma, flavor, texture and overall acceptability) and purchase intention, cupuassu influenced ($P < 0.05$) some JAR attributes such as aroma, flavor, color, consistency and viscosity. According to Jaeger et al. (2015), JAR questions can improve the consumers' discrimination of samples based on hedonic scores once this type of questionnaire increases consumers' engagement. This fact may explain the differences found between formulations of JAR attributes related to acid, alcoholic and cupuassu aroma, cupuassu and caprine flavor, yellow color, consistency and viscosity. Combining the data from JAR profiles and penalty analysis made it possible to identify the parameters, for each treatment (NY, CY5, CY7.5 and CY10) that can be improved to increase consumer acceptance. Based on PCA, PLSR, JAR and penalty analysis, the cupuassu addition demonstrated great potential for improving some of the penalized attributes, such as caprine and cupuassu aroma, acid taste, caprine and cupuassu flavor, yellow color, consistency, and viscosity, which positively contributed to overall acceptability. To the best of our knowledge, there are no sensory studies regarding the addition of cupuassu pulp to goat milk yogurt, therefore, this study presently contributes to the scientific community with unpublished data. However, previous studies (Mangia et al., 2014; Senaka Ranadheera et al., 2012) confirm that the addition of fruit juice and syrup can improve the sensory characteristics of yogurt formulations made from goat's milk.

Regarding physicochemical analyses, all parameters except texture were influenced ($P < 0.05$) by the addition of cupuassu pulp, except the texture. Cupuassu formulations were characterized by greater TPC, redness, yellowness and apparent

viscosity, whereas they had lower pH and lightness than NY ($P < 0.05$). Costa et al. (2015b) observed the same behavior on the instrumental physicochemical parameters of cupuassu goat milk yogurt. The gradual decrease in pH of goat milk yogurt when cupuassu pulp was added can be related to the pH of cupuassu pulp, which averages 3.4 (Rogez *et al.* 2004). The cupuassu pulp is rich in phenolic compounds (Pugliese et al., 2013). Therefore, the addition of this fruit promoted an increase in TPC content of the final product, possibly resulting in better antioxidant activity of the cupuassu goat milk yogurt, mainly in the CY10. The white color of goat milk yogurt is due to absence of β-carotene in goat milk, which is converted to vitamin A (Park et al., 2007). Thus, the different color values (L^*, a^* and b^*) of cupuassu goat milk yogurts can be assigned to the distinctive color of the cupuassu pulp, which presents with a light yellow color (Silva & Silva, 1999). Furthermore, the addition of cupuassu pulp into goat milk yogurts increased the apparent viscosity. This is due to the particular chemical composition of this fruit pulp that is rich in fibers and contains a considerable amount of starch as well as pectin polysaccharides (Vriesmann et al., 2009). However, texture parameters (firmness, consistency and cohesiveness) were not influenced by cupuassu pulp increments. This fact can be justified, once yogurt texture is highly dependent on the type of culture, total solid and protein contents of the product (Costa et al., 2015b; Oliveira et al., 2001), which were the same composition in all treatments.

Cupuassu pulp presents a specific chemical composition (Rogez et al., 2004) resulting in significant sensory changes, which were detected by PCA and JAR data in our study. Pearson's correlation indicated that cupuassu pulp addition masked the caprine flavor, positively influencing the flavor of the new product. These results are corroborated by Senaka Ranadheera et al. (2012), which confirm that the addition of fruit pulp can mask the goat milk taste and improve the texture of goat milk yogurt. Once the flavor of

goat milk is not accepted by non-habitual consumers (Costa et al., 2014) and decreases the acceptance of natural goat milk yogurt (Costa et al., 2015a). Similarly, to our findings, Senaka Ranadheera et al. (2012) and Mangia et al. (2014) observed that the addition of commercial fruit juice and myrtle berry juice masked the caprine flavor of the goat milk yogurt. Thus, the addition of cupuassu pulp can be considered as a new strategy to improve the acceptance of goat milk yogurts. In addition, ANOVA analyses revealed that the cupuassu pulp addition increased the redness and yellowness while decreasing the lightness, which resulted in a more pronounced yellow color that positively contributed to overall acceptability by PLSR. In the same regard, increased lightness can be detrimental to the acceptance of cupuassu goat milk yogurts. Moreover, the cupuassu pulp increased the apparent viscosity, possibly leading to an increase in the sensory perception of the consistency and viscosity by ANOVA analysis, which was positive for the overall linking taking into account the PLSR data. Food acceptance depends on the interaction between the food and the consumer. In this context, food characteristics such as flavor and physical structure can influence on consumers' decisions to accept or reject a food. Nonetheless, the evidence regarding the influence of the food color on consumers' perception is ambiguous; while some studies have demonstrated a significant effect of the color on product acceptance, other experiments have not observed any effect of this parameter (Spence *et al.* 2010). Our results indicate that cupuassu pulp potentially affected the flavor, color and texture of the goat milk yogurt, thereby improving the acceptance of the final product.

Yogurt consumption is recommended worldwide as part of a healthy diet, which makes this product a healthy food (Costa et al., 2013). Some studies show that consumers have a stronger preference for simple and well-known claims, such as probiotic and functional food (Bitzios et al., 2011; Lähteenmäki et al., 2010). We tested the effect of an

unfamiliar claim (benefits of antioxidants), which, in this study, was related to the addition of cupuassu pulp. This pulp contains high ascorbic acid and total phenolic contents that promote antioxidant activity (Pugliese et al., 2013). We found that antioxidant health information increased ($P < 0.05$) the acceptance of yogurt, indicating another advantage for the addition of cupuassu pulp.

5. Conclusions

The present study focused on consumer perceptions of a new yogurt manufactured with goat milk and cupuassu pulp, in addition to the influence of healthy information on the overall acceptability of cupuassu goat milk yogurts. Our results indicate that expectations did not affect consumer acceptance. Nevertheless, the goat milk yogurts formulated with cupuassu pulp presented a favorable acceptance. Cupuassu pulp demonstrated great potential as an ingredient in goat milk yogurt, as this fruit pulp improved the sensory attributes of this dairy product. In addition, the antioxidant health information increased the acceptance of cupuassu goat milk yogurts, which can be used to enhance some attributes such as acid and alcoholic aroma. Furthermore, the goat milk yogurts containing 10% cupuassu pulp was considered the optimal formulation based on the parameters investigated in this study. However, further studies are needed to improve the aroma of cupuassu goat milk yogurt.

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TABLE 1. Scores regarding questions about expected liking and expected familiarity in a 9-point hedonic scale.

Expected liking*			**Expected familiarity#**	
Question 1	**Question 2**	**Question 3**	**Question 1**	**Question 2**
6.05±1.87[a]	4.60±2.29[b]	5.99±1.75[a]	5.60±2.23[b]	6.46±1.84[a]

Values were expressed as mean ± standard deviation.

[a-b] Different lower case letters in the same line represent significant differences ($p < 0.05$).

[*] Question 1: "What is your expected liking about the flavor of goat milk products?"; Question 2: "What is your expected liking about the flavor of cupuassu pulp?"; Question 3: "What is your expected liking about the flavor of goat milk yogurt with cupuassu pulp added?"

[#] Question 1: "What is your familiarity with goat milk products?"; Question 2: "What is your familiarity with products added of cupuassu pulp?"

TABLE 2. Mean liking scores[1] for the different formulations of cupuassu yogurts made from goat milk.

Treatments	Attributes[1]						
	Appearance	**Color**	**Aroma**	**Flavor**	**Texture**	**Overall Acceptability**	**Purchase Intention**
NY	6.42±1.85[a]	6.91±1.64[a]	5.74±1.89[a]	4.45±2.14[a]	4.85±2.06[a]	4.82±2.00[a]	2.29±1.09[a]
CY5	6.47±1.73[a]	7.02±1.49[a]	6.13±1.93[a]	4.72±2.26[a]	5.26±2.04[a]	4.90±2.09[a]	2.40±1.18[a]
C7.5	6.27±1.80[a]	6.73±1.61[a]	6.14±2.10[a]	4.80±2.50[a]	5.37±2.14[a]	5.03±2.28[a]	2.49±1.23[a]
CY10	6.46±1.81[a]	6.86±1.46[a]	6.17±2.21[a]	4.77±2.35[a]	5.42±1.96[a]	5.09±2.17[a]	2.55±1.19[a]

NY – natural goat milk yogurt; CY5 – cupuassu goat milk yogurt with 5.0% of pulp; CY7.5 – cupuassu goat milk yogurt with 7.5% of pulp; CY10 – cupuassu goat milk yogurt with 10.0% of pulp.

Values were expressed as mean ± standard deviation.

[a] Different lower case letters in the same column represent significant differences ($p < 0.05$).

[1] Purchase intention was evaluated in a structured 5-point hedonic scale whereas the other attributes were evaluated in a 9-point hedonic scale.

TABLE 3. Just-About-Right profile scores[1] for the different formulations evaluated.

Treatments	Aroma			
	Acid	**Alcoholic**	**Caprine**	**Cupuassu**
NY	3.08±0.85[b]	2.89±0.76[b]	3.19±0.84[a]	2.33±0.91[b]
CY5	3.41±0.80[a]	3.30±0.87[a]	3.14±1.05[a]	2.73±1.01[a]
C7.5	3.34±0.80[a]	3.24±0.84[a]	3.08±0.93[a]	2.82±0.96[a]
CY10	3.32±0.86[ab]	3.28±0.91[a]	3.07±0.99[a]	2.88±1.06[a]
Treatments	**Taste**		**Flavor**	
	Acid	**Sweet**	**Caprine**	**Cupuassu**
NY	3.35±0.91[a]	2.25±0.95[a]	3.40±0.98[a]	2.20±0.96[b]
CY5	3.44±0.99[a]	2.27±0.88[a]	3.17±1.09[ab]	2.68±1.05[a]
C7.5	3.42±0.97[a]	2.20±0.87[a]	3.24±1.00[ab]	2.71±1.00[a]
CY10	3.57±0.99[a]	2.40±0.94[a]	3.13±0.96[b]	2.87±0.98[a]
Treatments	**Color**		**Texture**	
	Blank	**Yellow**	**Consistency**	**Viscosity**
NY	3.14±0.63[a]	2.68±0.74[b]	2.07±0.89[b]	2.19±0.94[b]
CY5	2.97±0.61[a]	2.87±0.77[ab]	2.20±0.85[ab]	2.33±0.91[ab]
C7.5	2.98±0.59[a]	2.96±0.71[a]	2.35±0.87[a]	2.47±0.96[a]
CY10	2.98±0.68[a]	2.97±0.80[a]	2.41±0.86[a]	2.41±0.83[ab]

NY – natural goat milk yogurt; CY5 – cupuassu goat milk yogurt with 5.0% of pulp; CY7.5 – cupuassu goat milk yogurt with 7.5% of pulp; CY10 – cupuassu goat milk yogurt with 10.0% of pulp.

Values were expressed as mean ± standard deviation.

[a-b] Different lower case letters in the same column represent significant differences ($p < 0.05$).

[1] All JAR attributes were evaluated in a structured 5-point hedonic scale.

TABLE 4. Consumer penalty analysis of the JAR diagnostic attributes (percentage of consumers and mean decreases).

Treatments	Aroma							
	Acid		Alcoholic		Caprine		Cupuassu	
	Not enough	Too much	Not enough	Too much	Not enough	Too much	Not enough	Too much
NY	-	-	-	-	-	28.13* (1.06)#	55.00* (0.51)#	-
CY5	-	37.50* (0.92)#	-	33.75* (0.87)#	-	24.38* (1.36)#	38.13* (1.36)#	-
C7.5	-	33.75* (1.84)#	-	29.38* (0.85)#	-	25.63* (0.67)#	33.13* (0.62)#	-
CY10	-	32.50* (1.78)#	-	34.38* (0.97)#	-	25.01* (0.87)#	30.63* (1.63)#	-
Treatments	**Taste**				**Flavor**			
	Acid		**Sweet**		**Caprine**		**Cupuassu**	
	Not enough	**Too much**	**Not enough**	**Too much**	**Not enough**	**Too much**	**Not enough**	**Too much**
NY	-	41.25* (1.23)#	60.00* (1.74)#	-	-	41.88* (1.23)#	61.25* (0.87)#	-
CY5	-	46.88* (0.84)#	56.88* (1.40)#	-	-	31.88* (1.25)#	43.75* (1.29)#	-
C7.5	-	43.13* (1.93)#	58.13* (1.78)#	-	-	35.00* (1.24)#	40.00* (1.53)#	-
CY10	-	48.13* (1.72)#	50.63* (1.54)#	-	-	30.00* (1.18)#	30.00* (1.64)#	-
Treatments	**Color**				**Texture**			
	Blank		**Yellow**		**Consistency**		**Viscosity**	
	Not enough	**Too much**	**Not enough**	**Too much**	**Not enough**	**Too much**	**Not enough**	**Too much**
NY	-	-	-	-	73.75* (0.83)#	-	65.63* (0.92)#	-
CY5	-	-	-	-	65.00* (1.41)#	-	58.13* (1.34)#	-
C7.5	-	-	-	-	52.50 * (1.38)#	-	55.00* (1.03)#	-
CY10	-	-	-	-	48.75* (1.21)#	-	49.31* (0.78)#	-

NY – natural goat milk yogurt; CY5 – cupuassu goat milk yogurt with 5.0% of pulp; CY7.5 – cupuassu goat milk yogurt with 7.5% of pulp; CY10 – cupuassu goat milk yogurt with 10.0% of pulp.

* The percentage of consumers who found treatments to be not enough or too much for JAR aroma, taste, flavor, color and texture.

The number in parentheses is the change in mean compared to the consumer response score to overall acceptability.

(-) indicates that less than 20% of consumers chose that JAR category.

TABLE 5. Physicochemical parameters of cupuassu yogurts made from goat milk.

Parameters	Treatments			
	NY	**CY5**	**CY7.5**	**CY10**
pH	4.65±0.01[a]	4.47±0.01[b]	4.44±0.01[c]	4.39±0.01[d]
TPC (mg de EAG/L)	127.45±0.30[d]	154.89±0.52[c]	176.89±0.31[b]	197.17±0.27[a]
L*	89.18±0.02[a]	88.81±0.02[b]	88.65±0.01[c]	88.33±0.01[d]
a*	0.69±0.01[d]	0.93±0.01[c]	1.15±0.01[b]	1.27±0.01[a]
b*	7.52±0.01[d]	8.47±0.01[c]	9.10±0.01[b]	9.46±0.01[a]
Apparent viscosity (mPa.s)	157.60±9.62[b]	172.37±4.50[ab]	175.10±7.55[ab]	177.33±5.93[a]
Firmness (g)	20.94±0.48[a]	21.09±0.66[a]	21.00±1.13[a]	20.70±0.28[a]
Consistency (g.sec)	124.17±5.55[a]	126.45±6.02[a]	125.21±7.25[a]	121.55±1.57[a]
Cohesiveness (g)	-30.75±1.85[a]	-29.17±1.02[a]	-30.12±0.42[a]	-31.11±1.18[a]

NY – natural goat milk yogurt; CY5 – cupuassu goat milk yogurt with 5.0% of pulp; CY7.5 – cupuassu goat milk yogurt with 7.5% of pulp; CY10 – cupuassu goat milk yogurt with 10.0% of pulp.

Values were expressed as mean ± standard deviation.

[a-d] Different lower case letters in the same line represent significant differences ($p < 0.05$); n = 2. TPC - total phenolics content; *L**- lightness; *a** - redness; and *b** - yellowness.

TABLE 6. Mean liking scores[1] for the three treatments containing cupuassu pulp by the blind and informed groups (n = 160).

Attributes	CY5		CY7.5		CY10	
	Blind*	Informed#	Blind*	Informed#	Blind*	Informed#
Overall acceptability	5.41±1.88[a]	6.51±1.71[b]	4.84±2.01[a]	6.52±1.73[b]	5.22±2.11[a]	6.67±1.66[b]
Cupuassu flavor	5.52±1.74[a]	6.58±1.74[b]	5.07±1.85[a]	6.44±1.60[b]	5.27±1.86[a]	6.66±2.07[b]
Purchase intention	2.62±1.11[a]	3.50±1.15[b]	2.32±1.06[a]	3.22±1.08[b]	2.57±1.13[a]	3.56±0.99[b]

CY5 – cupuassu goat milk yogurt with 5.0% of pulp; CY7.5 – cupuassu goat milk yogurt with 7.5% of pulp; CY10 – cupuassu goat milk yogurt with 10.0% of pulp.

Values were expressed as mean ± standard deviation.

[a-b] Different lower case letters in the same line represent significant differences ($p < 0.001$).

[1] Overall acceptability and cupuassu flavor attributes were evaluated in a structured 9-point hedonic scale while purchase intention was evaluated in a structured 5-point hedonic scale.

* Participants who received the samples without information; # Participants who received the samples with healthy information.

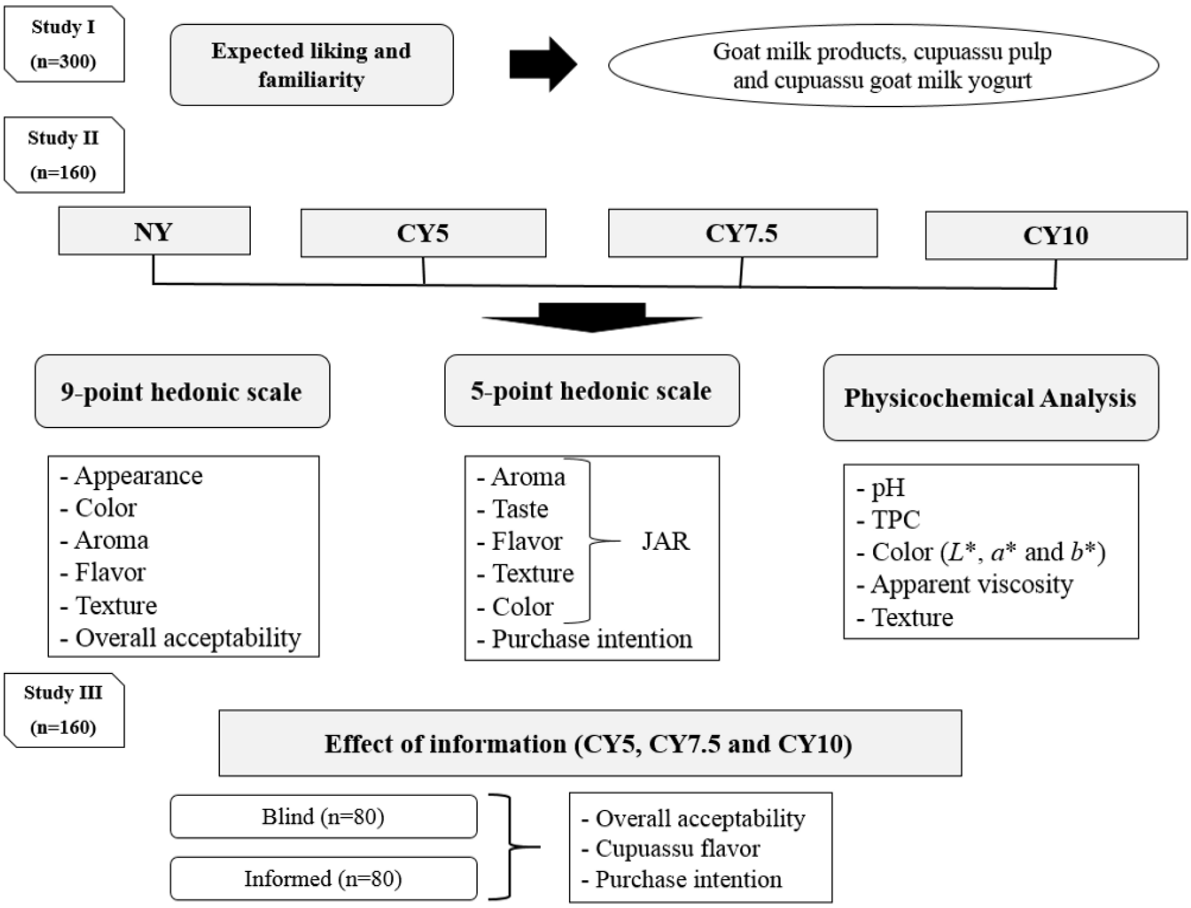

Fig. 1. Study design illustrating the sensory and physicochemical parameters investigated in this experiment. NY – natural goat milk yogurt; CY5 –goat milk yogurt with 5.0% of cupuassu pulp; CY7.5 –goat milk yogurt with 7.5% of cupuassu pulp; CY10 –goat milk yogurt with 10.0% of cupuassu pulp; TPC – total phenolic content; L*- lightness; a* - redness; and b* - yellowness.

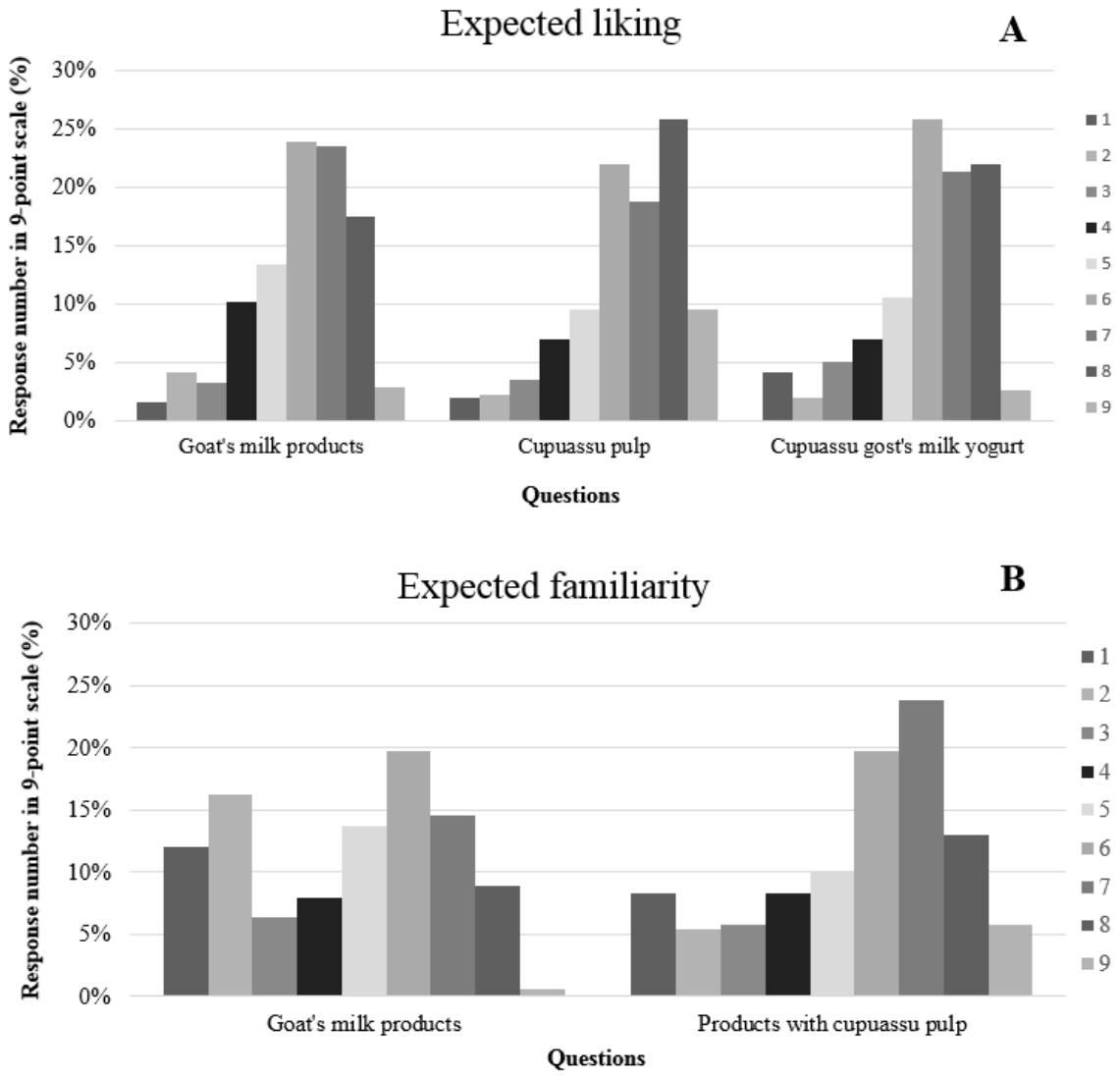

Fig. 2. The percentage of participants' answer about the expected liking (A = goat milk products, cupuassu pulp and cupuassu goat milk yogurt) and expected familiarity (B = goat milk products and products with cupuassu pulp) in a 9-point hedonic scale (1 = would dislike extremely to 9 = would like extremely).

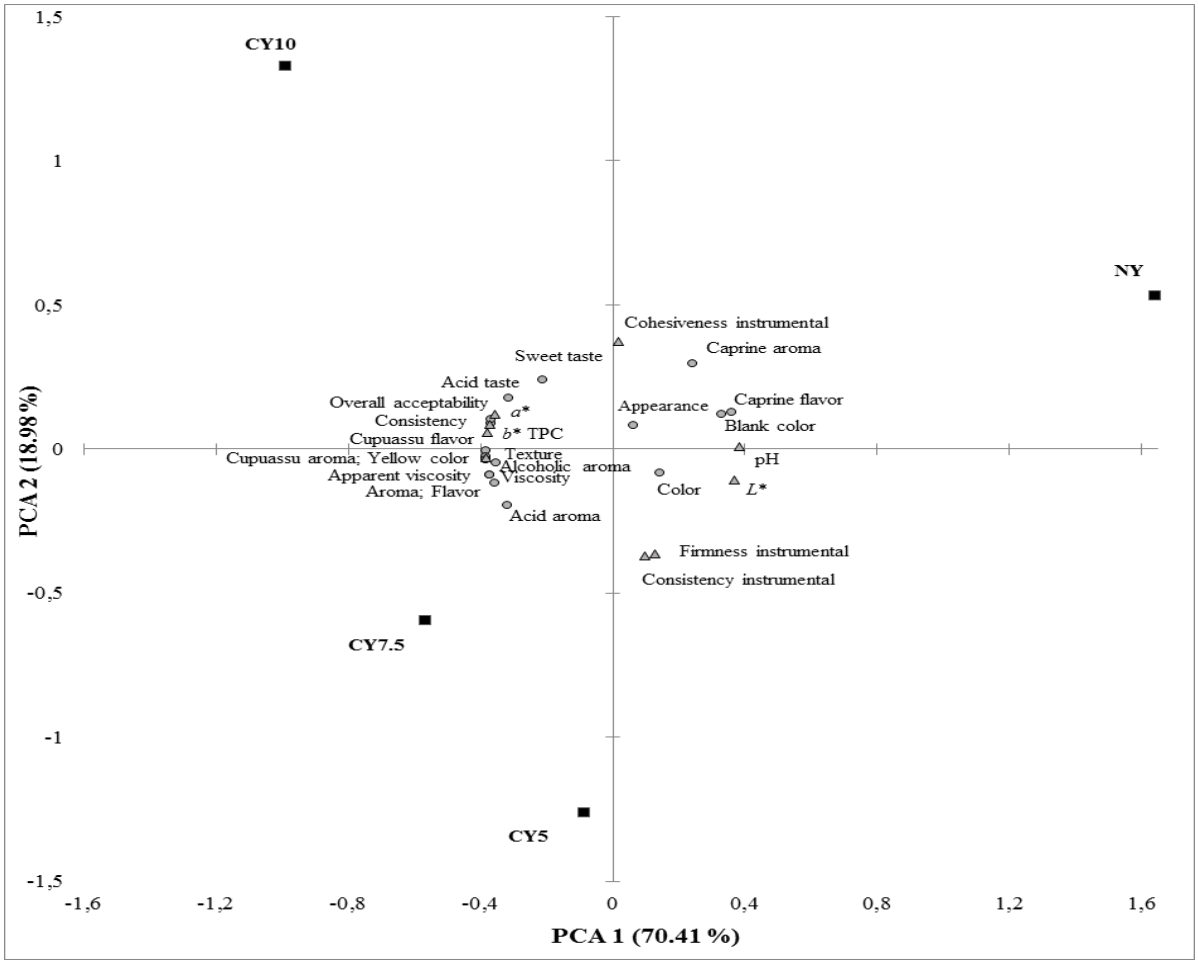

Fig. 3. Physicochemical and sensory data of different formulations of cupuassu yogurts manufacture from goat milk in the plane defined by two principal components. NY – natural goat milk yogurt; CY5 –goat milk yogurt with 5.0% of cupuassu pulp; CY7.5 – goat milk yogurt with 7.5% of cupuassu pulp; CY10 –goat milk yogurt with 10.0% of cupuassu pulp; TPC – total phenolic content; L*- lightness; a* - redness; and b* - yellowness.

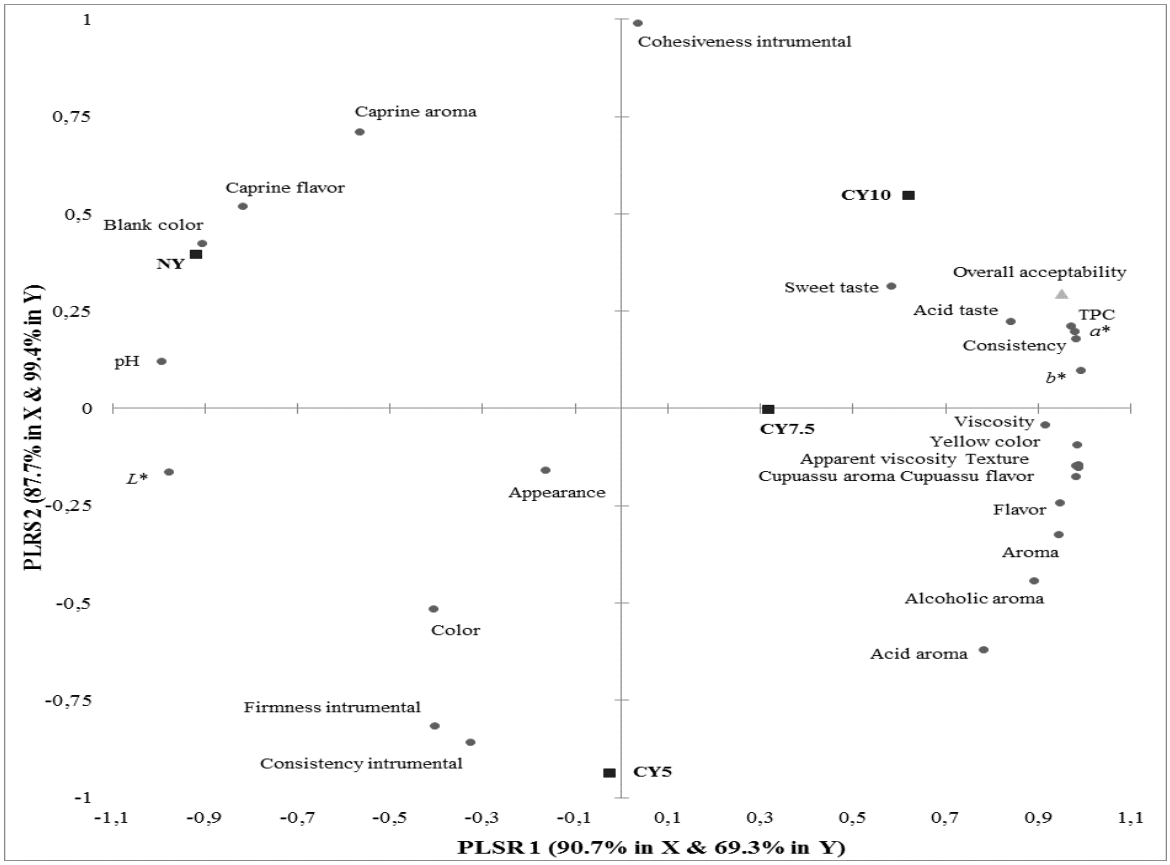

Fig. 4. Partial least square regression (PLSR) for sensory attributes and physicochemical parameters of different formulations of cupuassu yogurts made from goat milk. PLSR 1 = physicochemical and sensory parameters; PLSR 2 = overall acceptability. NY – natural goat milk yogurt; CY5 –goat milk yogurt with 5.0% of cupuassu pulp; CY7.5 –goat milk yogurt with 7.5% of cupuassu pulp; CY10 –goat milk yogurt with 10.0% of cupuassu pulp; TPC – total phenolics content; L*- lightness; a* - redness; and b* - yellowness.

3.2 ARTIGO II: CUPUASSU (*Theobroma grandiflorum*) PULP, PROBIOTIC, AND PREBIOTIC: INFLUENCE ON COLOR, APPARENT VISCOSITY, AND TEXTURE OF GOAT MILK YOGURTS PUBLICADO NA REVISTA JOURNAL OF DAIRY SCIENCE

INTERPRETIVE SUMMARY

Cupuassu (*Theobroma grandiflorum*) pulp, probiotic and prebiotic: influence on color, apparent viscosity and texture of goat's milk yogurts. By Costa et al. Cupuassu is a fruit native to the Brazilian Amazon, and it has a characteristic aroma, flavor and texture. Goat's milk is a food of high biological value. However, compared to others yogurts, such as cow and sheep yogurts, goat's milk yogurts do not naturally have an appropriate consistency, which complicates the production and acceptance of this product. In this study, different ingredients (cupuassu pulp, probiotic and prebiotic) were used to improve increase the texture of goat's milk yogurt. The different treatments were evaluated for color, pH, apparent viscosity and texture in order to assess their potential value as additives.

RUNNING HEAD: Cupuassu potential in probiotic goat yogurts

Cupuassu (*Theobroma grandiflorum*) pulp, probiotic and prebiotic: influence on color, apparent viscosity and texture of goat's milk yogurts

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ABSTRACT

Cupuassu is an acidic fruit that has a characteristic aroma, flavor and texture; its fiber rich pulp can provide a different consistency than other fruit pulps. Goat's milk is an excellent source of amino acids, fatty acids and minerals, and is widely used for processing fermented milks, such as yogurt. However, compared to cow's milk yogurts it is difficult to make goat's milk yogurts with a good consistency. Therefore, it is necessary to use certain technological strategies. This study was carried out to investigate the possibility of adding cupuassu pulp, probiotic (*Lactobacillus acidophilus* LA-5) and prebiotic (inulin) to improve the texture of goat's milk yogurt. A total of six treatments were performed: natural (N), probiotic (Pro), prebiotic (Pre), symbiotic (S), cupuassu (C) and probiotic with cupuassu (PC). The viability of probiotic in yogurts (Pro, S and PC) was evaluated. In addition, instrumental analyses such as color, pH, apparent viscosity and texture were performed in all treatments. The probiotic bacteria remained viable ($\geq$ 7 log CFU.mL^{-1}) throughout the 28 days of refrigerated storage. The lightness (L^*) was affected initially by addition of all ingredients (cupuassu pulp, probiotic and prebiotic). The addition of cupuassu pulp (C and PC) increased ($P < 0.05$) the L^* during the period of storage. All yogurt samples underwent gradual decreases in pH until 7-14 days of storage. Apparent viscosity and firmness decreased ($P < 0.05$) in the PC yogurt. The consistency was highest ($P < 0.05$) in the yogurts with added prebiotic (Pre and S). The cohesiveness remained constant in all yogurts. We conclude that cupuassu pulp addition could improve the texture of goat's milk yogurts.

Keywords: Instrumental analysis, *Lactobacillus acidophilus* LA5, inulin, consistency, caprine milk.

INTRODUCTION

Cupuassu (*Theobroma grandiflorum*) is a tropical fruit, native to the Brazilian Amazon. Cupuassu has a high economic potential because of its excellent characteristics such as the aroma, flavor and texture (Faber and Yuyama, 2015). However, due to distinctive flavor, cupuassu pulp is used as ingredient in the manufacture of ice cream, juice, liquors, wines, jellies and other products, such as yogurts, rather than being consumed *in natura* (Vriesmann and Petkowicz, 2009; Salgado et al., 2013). Cupuassu is a potential source of dietary fiber, mainly soluble fiber (Salgado et al., 2011). The cupuassu pulp has a particular chemical composition, rich in fibers, and contains a considerable amount of starch as well as pectin polysaccharides (Vriesmann et al., 2009), which can provide a different texture than other fruit pulps.

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 (Park et al., 2007; Fonseca et al., 2013). It is an excellent substitute for cow's milk in the nutrition of children and elderly person (Park et al., 2007; Kapila et al., 2013). Goat's milk is widely used for processing fermented milks and other dairy products. Yogurt is the most widely produced and consumed fermented milk, and is used as a vehicle for probiotic cultures and prebiotics (Costa et al., 2013; Costa and Conte-Junior, 2013). However, compared to cow's milk yogurt, it is difficult to make goat's milk yogurt with an appropriate flavor (Costa et al., 2014) and consistency, which is mainly due to the difference in casein composition and content (Li and Guo, 2006). Therefore, it is

necessary to use certain technological strategies. One alternative is the addition of inulin or another type of fiber, such as is present in fruit pulp (Buriti et al., 2014).

Inulin is one of the most studied and widely used prebiotics, with advantageous technological and nutritional properties (Paseephol et al., 2008). Prebiotics are selectively fermented ingredients that allow specific changes in the composition and / or activity of gastrointestinal microbiota, which confers a health benefit on the host (Gibson, 2007). Depending on the concentration, inulin may increase its effect on the structure and texture of dairy products, such as yogurt. Addition of inulin can change the texture and rheological properties of dairy foods (Paseephol et al., 2008).

Probiotics are live microorganisms which when administered in adequate amounts may benefit the health benefits of the host (Sanders, 2009). The *Lactobacillus acidophilus* LA-5 strain shows viability in milk matrix, such as fermented milks (Costa et al., 2015). However, there are no reports in the literature that this probiotic can improve the texture of goat's milk yogurt. Certain strains of Lactobacillus, such as *L. delbrueckii* subsp. *Bulgaricus*, have this ability (Shihata and Shah, 2002).

In this context, the aim of the present study was to improve the texture of goat's milk yogurt by adding cupuassu pulp, probiotic and/or prebiotic. Instrumental analyses (color, pH, apparent viscosity and texture) were performed in order to evaluate the influence of these different ingredients on goat's milk yogurts.

MATERIALS AND METHODS

Goat's Milk Yogurts

The yogurt was produced using UHT whole goat's milk (Cappry's®, Rio Grande do Sul, Brazil) and thermophilic yogurt cultures (YF-L903®; Chr. Hansen, Valinhos, SP, Brazil) at a concentration of 1% (vol/vol). A total of six treatments were performed: natural (N), probiotic (Pro), prebiotic (Pre), symbiotic (S), cupuassu (C) and probiotic with cupuassu (PC). For treatments with a probiotic (Pro, S and PC), *Lactobacillus acidophilus* culture (LA-5®; Chr. Hansen, Valinhos, Brazil) was inoculated at a concentration of 5% (vol/vol) in relation to the total milk volume used to produce the probiotic. For treatments with a prebiotic (Pre and S) 5% (vol/vol) of inulin (Ingredients & Systems Biotechnology, São Paulo, SP, Brazil) was added. The inulin polymer has a degree of polymerization from 2 to 50 with an average degree of polymerization of 9. For, the treatments with cupuassu (C and PC) 10% pasteurized cupuassu pulp (Polpa de Fruta®, Macapá, AP, Brazil) was added.

The yogurt mixtures were fermented in an oven at 43 ±2°C. The fermentation was interrupted when the pH (AOAC, 2012) reached 4.5. Finally, the product was packaged in 500-mL plastic pots and stored at 4 ± 2°C for 28 days. The physicochemical analysis and probiotic viability assay were performed during the storage period (0, 7, 14, 21, 28 days). This experiment was repeated three times (n = 3) and all analyses were performed in triplicate.

Bacteriological Analysis and Survivability of Probiotic

Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* analysis were analyzed after the yogurt was prepared (day 1) to characterize the fermented product as yogurt. Enumeration of *S. thermophilus* was performed on M17 agar with lactose, which was incubated under aerobiosis at 37 °C for 2 days. The count

of *L. delbrueckii* subsp. *bulgaricus* on Agar de Man, Rogosa and Sharpe (MRS) with pH 5.4 was performed after incubation under anaerobiosis at 37 °C for 3 days (Codex Alimentarius, 2010). The probiotic (*Lactobacillus acidophilus* LA-5) was counted according to the procedures of Costa et al. (2014), during the storage period (0, 7, 14, 21, 28 days). *L. acidophilus* was grown on MRS agar supplemented with 0.15% (v/v) bile salts, and aerobically incubated at 37 °C for 2 days.

Colorimetric and pH Analysis

Color determinations were made at 5 ± 2 °C by means of a Minolta CM-600D spectrophotometer (Minolta Camera Co., Osaka, Japan). Yogurt samples (50 mL) at 5 °C were stirred and placed in an aluminum cylinder (outside diameter 55 mm), with the surface optically flat before measuring, and the sensor was mounted directly on top of the cylinder to prevent ambient light noise. The color space of the yogurts was studied, and the following color coordinates were determined: lightness (L^*, 100 = white, 0 = black), redness (a^*, + red, −green), and yellowness (b^*, + yellow, −blue). These analyses were performed in triplicate.

Samples of goat's milk yogurts were also analyzed for pH, using a digital pHmeter (pH Model PG1800, Cap Lab®, SP, Brazil) (AOAC 2012).

Apparent Viscosity and Instrumental Texture Analysis

The apparent viscosities of the yogurts samples (100 mL) were measured at 5 °C using a Quimis viscometer (Viscosímetro Rotativo Microprocessado – Q860M21, SP,

Brazil) equipped with rotor no. 3, mixing at 60 rpm. The apparent viscosity was measured in triplicate.

Texture was assessed using a texture analyzer (TA-XT.Plus, Stable Micro Systems Ltd., Surrey, UK) equipped with a 5-kg load cell, according to Iličić et al. (2014). Texture profile analysis (TPA) was used, analyzing firmness, consistency and cohesiveness. The samples were compressed at 10% of original height with a back extrusion cell (A/BE) disc (diameter 36 mm; distance 30 mm; speed 0.001/ms), at a temperature of 4°C, with 3 measurements per sample averaged for data analysis. The tests were carried out in a standard size back extrusion container (50 mm in diameter). The extrusion disc was positioned centrally over the sample container.

Statistical Analysis

The results for color, pH, apparent viscosity and texture were subjected to one-way analysis of variance (ANOVA), considering treatments and days 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 mean bacteria counts were calculated and expressed as log 10 CFU.g^{-1}.

RESULTS AND DISCUSSION

Bacteriological Analysis

The counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were evaluated to characterize the products made with yogurts, which was

analyzed only on day 1. The yogurts contained, respectively, for *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*: 11.37 and 7.30 (N); 11.34 and 7.62 (Pro); 11.44 and 10.73 (Pre); 9.10 and 7.97 (S); 9.02 and 7.9 (C); 11.16 and 11.13 (PC) log $CFU.g^{-1}$. Thus, the fermented milks produced in all treatments were considered to be yogurt, according to the Codex Alimentarius (2010).

For the probiotic yogurts *Lactobacillus acidophilus* LA-5 initial values were 11.01, 9.11 and 11.29 log CFU g^{-1} for Pro, S and PC yogurts, respectively. Figure 1 demonstrates the behavior of the probiotic in all probiotic goat's milk treatments. The viability of the probiotic bacteria decreased ($P < 0.05$) in all treatments (Pro, S and PC) during the first week of storage. The decrease of *L. acidophilus* LA-5 can be explained by three mechanisms: the depletion of some nutrients needed by this bacteria; *L. acidophilus* may have upset the desirable symbiotic relationship between the yogurt starter culture; and *L. acidophilus* in the yogurt may have initially produced higher concentrations of antimicrobials such as bacteriocins, H_2O_2, or organic acids that may have eventually inhibited more *L. acidophilus* (Olson and Aryana, 2008).

Thereafter, they were stable, and all probiotic yogurts maintained counts $\geq 10^7$ $CFU.mL^{-1}$ during 4 weeks (28 days) of storage. *L. acidophilus* LA-5® showed variable viability in the yogurts, with final counts of 9.40, 8.02 and 8.43 log CFU g^{-1} for Pro, S and PC yogurts, respectively. These counts exceeded the minimum count required to confer probiotic physiological benefits (Bedani et al., 2013; Costa et al., 2013). Regarding the lower viability of the PC yogurts, Kailasapathy et al. (2008) suggested that probiotic strains can be influenced by the pH of the fruit preparation.

pH Analysis

The pH of the goat's milk used to produce the yogurts was 6.62 (±0.03). The pH values of the yogurts are shown in Table 1. The reduction ($P < 0.05$) of milk pH after yogurt production (day 0), in all treatments, was in line with the growth of the starter culture and the probiotic bacteria. The pH of all yogurt samples decreased ($P < 0.05$) gradually until 7-14 days of storage, and then increased ($P < 0.05$) in Pre and C treatments. The high bacterial metabolic activity ferments lactose and produces lactic acid, which decreases the pH of yogurts (Gaspar et al., 2013). However, when the sugar sources are exhausted, microorganisms begin to consume proteins and start to produce other metabolites, such as biogenic amines (Costa et al., 2015), which increase the pH (Vahedi et al., 2008). This explains the pH increase ($P < 0.05$) at the end of the storage period (21 and 28 days).

Although all yogurts were cooled at pH 4.5, the pH levels of the yogurts inoculated with *L. acidophilus* (Pro, S and PC) were lower ($P < 0.05$) than the pH levels of the remaining yogurts at the end of storage. Espírito Santo et al. (2011) observed similar behavior, and suggested that the occurrence of fatty acid consumption as a carbon source after sugar depletion and fiber pectin degradation to uronic acids could explain the pH reduction.

Color Analysis

The appearance of dairy products is major importance for acceptability by consumers. In this study, the influence of adding different ingredients (cupuassu pulp, probiotic and prebiotic) on the color of the goat's milk yogurts were investigated. The color parameters L^*, a^*, and b^* showed some differences ($P < 0.05$), and these changes

in color in the six goat's milk yogurts stored at 4 °C for 28 days are presented in Table 2.

The *L** is lightness, in which 100 represents white, while zero represents the black. The *L** values were significantly affected by the addition of the cupuassu pulp probiotic and prebiotic on the initial day ($P < 0.05$). The *L** values in all yogurt samples increased ($P < 0.05$) during the 28 days of storage. The white color of goat's milk is due to the absence of β-carotene, because of a physiological process of the goats. This substance is converted into vitamin A (Park et al., 2007), which explains the high *L** values. The goat's milk yogurt sample containing cupuassu pulp (C and PC) had a lower *L** value than the others. These results suggest that the cupuassu pulp decreased the lightness values of the yogurts, which can be related to this fruit pulp color. Probably this difference could be well accepted by consumers, as it would reflect the presence of cupuassu. As for the storage period, the greatest change occurred in Pro, where the *L** value increased ($P < 0.05$) from 89.24 to 92.39. This result differs from those found by Mani-López et al. (2014), who observed no changes in color parameters during storage. This difference may related to such factors as the probiotic strain, the ingredients used, and the type of milk.

Regarding *a** (greenness-redness) initial values, treatments added with prebiotic and cupuassu pulp differed from control ($P < 0.05$). However, Pre and S had lower values, while C and PC had higher. Kim et al. (2011) achieved the same behavior, explaining it because of the high capacity of hold water, in our study by inulin. In all treatments, during storage, an increase was observed ($P < 0.05$), indicating an increase in the redness of the yogurts. Estrada et al. (2011) explained this increase through the gel stirring and acidity changes in yogurt during refrigerated storage, because they may

cause changes in tissue structure that result in leakage of natural pigments, such as carotenoids, to the yogurt matrix.

The *b** (blueness-yellowness) values was difference between all treatments, and the N treatment was less yellow than the other treatments (Pro, Pre, S, C and PC). These significantly ($P < 0.05$) greater yellowness of Pro, Pre, S, C and PC can be attributed to the addition of cupuassu pulp, probiotic and prebiotic. The *b** values decreased significantly in all yogurt during the 28 days of refrigerated storage ($P < 0.05$). These results (increase *a** and decrease *b**) mean that the reddish color was reinforced, which should be attributed to the goat's milk, since all the yogurts showed the same behavior. Statistical analyses showed that, although the pattern was the same, the treatments with and without cupuassu pulp differed ($P < 0.05$). Other studies have shown the same performance when fruit and vegetal ingredients were added to yogurt (Kim et al., 2011; Trigueros et al., 2014).

Apparent Viscosity Analysis

The effects of addition of a probiotic, a prebiotic and cupuassu pulp on the apparent viscosity of the goat's milk yogurts during storage are shown in Figure 2. On the initial day, the viscosities of the prebiotic, symbiotic, cupuassu and probiotic with cupuassu yogurts were higher than natural goat's milk ($P < 0.05$), i.e., the addition of cupuassu pulp and inulin increased the apparent viscosity. The apparent viscosity remained constant until day 7 of storage, in all goat's milk yogurts, and then decreased ($P < 0.05$). The decrease in apparent viscosity might have been caused by the whey separation with increasing storage time (Al Mijan et al., 2014). This behavior is in

agreement with the results of Wang et al. (2012), who compared the apparent viscosity of goat and cow's milk yogurts.

The development of apparent viscosity in yogurts is associated with the aggregation of casein micelles and gel formation, which is a consequence of biochemical and physicochemical changes during fermentation of milk (Gaygadzhiev et al., 2009; Singh and Kim, 2009). The apparent viscosity also increases as the pH of milk decreases, which is attributable to the additional swelling of casein micelles. At pH 5.4–5.3, the initial increase of apparent viscosity can be observed, at this stage indicating the initiation of aggregation. In the pH range of 5.1–4.6, the apparent viscosity of goat products increases (Park, 2007).

Instrumental Texture Analysis

The TPA parameters well represented the yogurt textural characteristics. Firmness, consistency and cohesiveness are commonly evaluated in determining yogurt texture. Different goat's milk yogurts were measured, as shown in Table 3.

Regarding firmness, there was no statistical difference ($P > 0.05$) between the treatments. The firmness decreased in all yogurts during 28 days of storage (Table 3). However, despite similar behavior in the different treatments, this decline was statistically significant ($P < 0.05$) only in the PC yogurt. Therefore, the addition of each ingredient (cupuassu pulp and probiotic) separately did not affect the firmness, although together, they changed this parameter. The firmness of yogurts is related to the bacteria *L. delbrueckii* subsp. *bulgaricus*. The incorporation of this microorganism into the yogurt starter culture improved the firmness, which in general, is due to the attachment

of mucogenic strains to the protein matrix via the exopolysaccharides (Shihata and Shah, 2002).

The consistency of the samples was significantly high ($P < 0.05$) in the yogurts added with prebiotic (Pre and S). Furthermore, the consistency of the symbiotic goat's milk yogurt increased significantly during the storage period (Table 3). A similar result was obtained for the yogurt' consistency with the addition of the inulin (Pimentel et al., 2012, 2013). This prebiotic helped to increase this physical property, but up to a certain concentration. The interactions between whey proteins and k-casein make the micelles less sensitive to the pH decline, increasing their solubility. Inulin is a soluble fiber, and a water-structuring agent. In addition, this prebiotic can complex with the protein aggregates, and it must be part of the structural network that is formed during fermentation and structuring of the stirred yogurt (Srisuvor et al., 2013).

The cohesiveness values indicated that if the predominance of protein in the composition of the yogurt caused the large number of casein–casein linkages broken during stress application to reform after the stress was released (Peng et al., 2009). The cohesiveness values are provide in Table 3. In this study, the cohesiveness, in all treatments, remained constant during refrigeration storage. Therefore, the addition of the cupuassu pulp, probiotic and prebiotic did not affect the cohesiveness. The cohesiveness value together with the springiness may indicate a predominance of protein in the composition of the yogurt, which led to a large promoted great number of broken casein–casein linkages during stress application, which reformed after the stress was released (Sandoval-Castilla et al., 2004). A possible explanation for the similar behavior of this parameter in all yogurts is that they have a similar percentage of milk proteins.

CONCLUSIONS

According to the results of this study, we conclude that cupuassu pulp, probiotic and inulin affect color and texture parameters of goat's milk yogurts. Furthermore, cupuassu pulp represents a potential fruit to be useful in the manufacture of goat's milk yogurts, which is an important technological strategy for the dairy goat industry.

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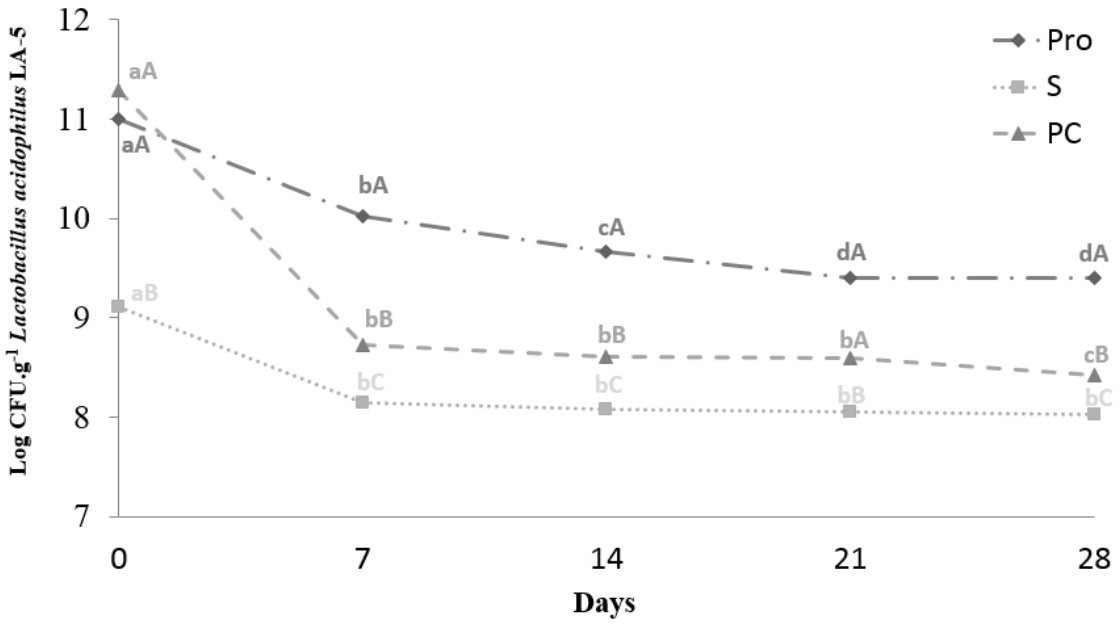

Figure 1: Counts of *Lactobacillus acidophilus* LA-5 (Log CFU.g^{-1}) in goat's milk yogurts with added probiotic (Pro), symbiotic (S) and probiotic with cupuassu (PC) goat's milk yogurts during 28 days of storage $^{A-C}$ Letters indicate significant differences among goat's milk yogurts, $P < 0.05$. $^{a-d}$ Letters indicate significant differences among storage times, $P < 0.05$.

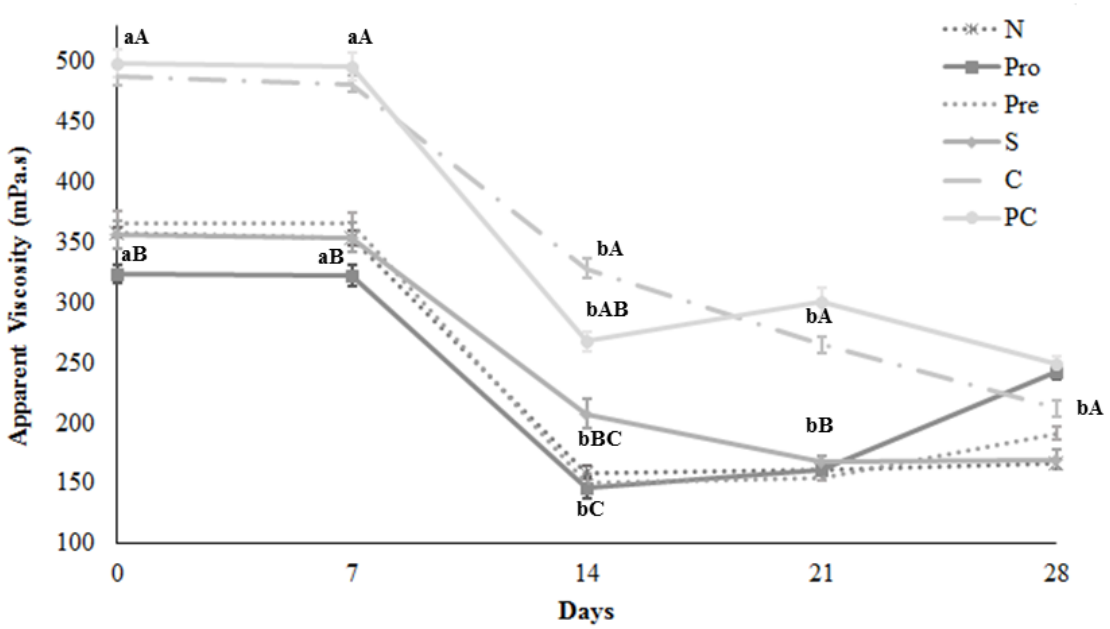

Figure 2: Apparent viscosity of the natural (N), probiotic (Pro), prebiotic (Pre), symbiotic (S), cupuassu (C) and probiotic with cupuassu (PC) goat's milk yogurts during 28 days of refrigerated storage. [A-C] Letters indicate significant differences among oat's milk yogurts, $P < 0.05$. [a-d] Letters indicate significant differences among storage times, $P < 0.05$.

Table 1: pH values (means ± standard deviation) of natural (N), probiotic (Pro), prebiotic (Pre), symbiotic (S), cupuassu (C) and probiotic with cupuassu (PC) goat's milk yogurts during the storage period (0, 7, 14, 21 and 28 days).

Treatment	Storage period (days)				
	0	**7**	**14**	**21**	**28**
N	4.57^{aA}±0.04	4.42^{cA}±0.01	4.48^{bcB}±0.01	4.51^{acA}±0.03	4.57^{aA}±0.01
Pro	4.45^{abB}±0.08	4.38^{bcB}±0.05	4.47^{aB}±0.02	4.38^{cB}±0.01	4.37^{cC}±0.03
Pre	4.55^{aA}±0.01	4.41^{bAB}±0.05	4.54^{bA}±0.08	4.51^{aA}±0.06	4.47^{abB}±0.01
S	4.42^{aB}±0.04	4.27^{cD}±0.05	4.35^{abC}±0.05	4.26^{cC}±0.01	4.34^{bC}±0.01
C	4.43^{cB}±0.02	4.35^{dC}±0.05	4.60^{aA}±0.08	4.51^{bA}±0.02	4.53^{bAB}±0.03
PC	4.50^{aAB}±0.01	4.28^{bcD}±0.01	4.24^{cD}±0.01	4.28^{bcC}±0.02	4.30^{bC}±0.01

$^{A-D}$ Values with different superscript letters within a column are significantly different, $P < 0.05$.

$^{a-d}$ Letters indicate significant differences among storage times, $P < 0.05$.

Table 2: The color values (means ± standard deviation) of goat's milk yogurt.

Properties	Treatment	Storage period (days)									
		0		7		14		21		28	
L*	**N**	90.05^{eA}	±0.01	90.22^{dA}	±0.05	90.40^{cA}	±0.02	90.71^{bA}	±0.02	92.78^{aA}	±0.02
	Pro	89.24^{dC}	±0.01	89.90^{cB}	±0.04	90.06^{cB}	±0.02	90.88^{bA}	±0.08	92.39^{aA}	±0.10
	Pre	89.41^{eB}	±0.02	89.83^{dB}	±0.01	90.13^{cB}	±0.03	90.89^{bA}	±0.18	92.43^{aA}	±0.03
	S	89.06^{dD}	±0.01	89.45^{cC}	±0.00	89.68^{cC}	±0.01	90.58^{bA}	±0.17	92.05^{aA}	±0.02
	C	87.76^{cF}	±0.01	88.44^{bD}	±0.01	87.90^{cE}	±0.04	88.33^{bB}	±0.08	90.17^{aB}	±0.13
	PC	88.09^{cE}	±0.01	88.33^{cE}	±0.01	88.07^{cD}	±0.02	88.78^{bB}	±0.23	89.70^{aB}	±0.01
a*	**N**	-1.74^{dB}	±0.02	-1.69^{dA}	±0.03	1.99^{cA}	±0.01	2.09^{bC}	±0.02	2.37^{aB}	±0.01
	Pro	-1.74^{dB}	±0.01	-1.86^{eB}	±0.04	1.85^{cA}	±0.01	2.32^{aA}	±0.03	2.20^{bD}	±0.02
	Pre	-1.78^{dC}	±0.01	-2.01^{eC}	±0.02	1.62^{cA}	±0.02	2.19^{bBC}	±0.05	2.21^{aDC}	±0.01
	S	-1.78^{deC}	±0.01	-2.04^{eC}	±0.02	1.81^{cA}	±0.04	2.35^{aA}	±0.01	2.24^{bDC}	±0.01
	C	-1.32^{dA}	±0.01	-1.68^{eA}	±0.01	1.78^{cA}	±0.07	2.27^{bAB}	±0.01	2.90^{aA}	±0.01
	PC	-1.35^{dA}	±0.01	-1.72^{eA}	±0.01	2.09^{cA}	±0.01	2.37^{aA}	±0.02	2.26^{bC}	±0.01
b*	**N**	8.23^{aD}	±0.02	8.08^{bE}	±0.05	6.89^{cE}	±0.01	4.86^{dD}	±0.05	4.55^{eD}	±0.01
	Pro	8.41^{bC}	±0.01	8.57^{aC}	±0.07	6.94^{cD}	±0.01	4.51^{eC}	±0.03	4.85^{dC}	±0.01
	Pre	8.30^{bD}	±0.03	8.40^{aD}	±0.03	7.56^{cB}	±0.03	4.92^{dDB}	±0.01	4.96^{dC}	±0.03
	S	8.50^{bB}	±0.03	8.80^{aB}	±0.04	7.09^{CC}	±0.02	4.97^{eB}	±0.02	5.22^{dB}	±0.04
	C	10.32^{aA}	±0.01	10.15^{aA}	±0.03	8.76^{bA}	±0.02	7.38^{dA}	±0.02	7.40^{cA}	±0.14
	PC	10.32^{aA}	±0.01	10.17^{bA}	±0.01	8.55^{cA}	±0.02	7.09^{dA}	±0.01	7.11^{dA}	±0.01

N, natural; Pro, probiotic; Pre, prebiotic; S, symbiotic; C, cupuassu; PC, probiotic with cupuassu.

$^{A-E}$ Letters indicate significant differences among goat's milk yogurts, $P < 0.05$.

$^{a-f}$ Letters indicate significant differences among storage times, $P < 0.05$.

[1]Measured *L**, *a**, and *b** values were used as indicators of lightness (*L**), redness (*a**), and yellowness (*b**).

Table 3: Firmness, consistency and cohesiveness values (means ± standard deviation) of goat's milk yogurts measured during the storage period (0, 7, 14, 21 and 28 days).

TPA parameter	Treatment	Storage period (days)									
		0		7		14		21		28	
Firmness (g)	N	22.28^{aB}	±0.12	21.42^{aA}	±0.20	20.81^{aA}	±0.13	21.27^{aA}	±0.18	20.70^{aAB}	±0.11
	Pro	22.32^{aB}	±0.11	21.85^{aA}	±0.06	20.52^{abA}	±0.28	19.30^{abA}	±0.62	17.57^{bB}	±0.12
	Pre	21.92^{aB}	±0.21	22.81^{aA}	±0.47	21.41^{aA}	±0.17	21.17^{aA}	±0.14	21.45^{aA}	±0.32
	S	22.14^{Ab}	±0.16	21.59^{aA}	±0.11	20.52^{aA}	±0.28	21.59^{aA}	±0.31	20.16^{aAB}	±0.02
	C	21.85^{ab}	±0.23	21.88^{aA}	±0.02	21.78^{aA}	±0.35	20.88^{aA}	±0.02	20.73^{aAB}	±0.12
	PC	26.16^{aA}	±0.52	21.45^{bA}	±0.18	21.42^{bA}	±0.17	20.73^{bA}	±0.03	20.88^{bAB}	±0.16
Consistency (gs)	N	122.86^{aAB}	±0.15	121.27^{aA}	±0.01	122.50^{aB}	±0.08	123.23^{aA}	±0.07	118.14^{aA}	±0.19
	Pro	126.92^{aB}	±0.13	121.77^{aA}	±0.20	118.69^{abB}	±0.17	103.22^{bA}	±0.19	89.07^{bA}	±0.16
	Pre	129.31^{aAB}	±0.44	126.43^{aA}	±0.30	125.98^{aAB}	±0.32	127.23^{aA}	±0.05	121.30^{aA}	±0.31
	S	120.12^{aAB}	±0.29	117.63^{aA}	±0.16	115.34^{aB}	±0.43	113.96^{aA}	±0.06	132.13^{aA}	±0.06
	C	122.09^{aAB}	±0.12	122.18^{aA}	±0.05	124.25^{aA}	±0.21	123.55^{aA}	±0.10	122.46^{aA}	±0.20
	PC	127.29^{aA}	±0.67	122.46^{aA}	±0.31	120.56^{aB}	±0.04	120.25^{aA}	±0.11	122.6^{aA}	±0.14
Cohesiveness (g)	N	-30.29^{aAB}	±0.16	-31.48^{aA}	±0.32	-30.54^{aA}	±0.25	-30.72^{aA}	±0.55	-30.97^{aA}	±0.05
	Pro	-32.45^{aB}	±0.65	-30.29^{aA}	±0.11	-31.55^{aA}	±0.10	-29.75^{aA}	±0.48	-29.83^{aA}	±0.04
	Pre	-29.75^{aAB}	±0.53	-30.58^{aA}	±0.57	-30.79^{aA}	±0.30	-31.48^{aA}	±0.50	-30.97^{aA}	±0.10
	S	-31.84^{aAB}	±0.50	-29.36^{aA}	±0.32	-31.30^{aA}	±0.50	-30.87^{aA}	±0.55	-32.05^{aA}	±0.32
	C	-29.75^{aAB}	±0.17	-30.87^{aA}	±0.50	-31.04^{aA}	±0.10	-31.08^{aA}	±0.15	-32.23^{aA}	±0.30
	PC	-29.39^{aA}	±0.54	-29.76^{aA}	±0.10	-29.86^{aA}	±0.16	-31.73^{aA}	±0.10	-28.89^{aA}	±0.16

N, natural; Pro, probiotic; Pre, prebiotic; S, symbiotic; C cupuassu; PC probiotic with cupuassu.

$^{A-B}$ Letters indicate significant differences among goat's milk yogurts, $P < 0.05$.

$^{a-b}$ Letters indicate significant differences among storage times, $P < 0.05$.

3.3 ARTIGO III: CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF CARBOHYDRATES AND ORGANIC ACIDS IN FOODS OF ANIMAL ORIGIN PUBLICADO NA REVISTA COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Chromatographic methods for determination of carbohydrates and organic acids in food of animal origin

Short version of title: Sugars and organic acids: HPLC and GC

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Abstract

Carbohydrates are ubiquitous, which range from a simple monosaccharide to a large complex polysaccharide. Furthermore, organic acids are compounds with acidic properties. Both occur naturally in a number of foods, and in fermented products. Organic acids are usually present by the hydrolysis of carbohydrates for microorganisms, such as lactic acid bacteria. They converted for carbohydrate metabolic the energy required for growth, once are not equipped with the enzymes necessary for respiration and unable to perform oxidative phosphorylation. The carbohydrates and organic acids determination in foods from animal origin is important, since they contribute to the flavor, texture and aromatic properties of these products. Their presence and relative ratio can affect the chemical and sensorial characteristics of the food matrix and can provide information on nutritional properties of food and means to optimize selected technological processes. The carbohydrates and organic acids content are also important to monitorate bacterial growth and activity. Presently several methodologies can be applied to the quantification of these compounds, such as high

performance liquid chromatography and gas chromatography. In this context, high performance liquid chromatography has been widely used for analyzing carbohydrates and non-volatile organic acids, while gas chromatography has been used to determine the volatile organic acids in complex matrixes. This article discusses about the types of carbohydrates and organic acids in different products of animal origin, and approaches the different chromatographic forms to analyze them. Thus, the objective of this article is to provide an overview of chromatographic methods (HPLC and GC) applied in carbohydrates and organic acids of food from animal origin.

Introduction

The carbohydrates are structurally classified into monosaccharides, oligosaccharides and polysaccharides. Monosaccharides and some oligosaccharides have taste sweet. Polysaccharides in combination with proteins, lipids, and nucleic acids play an important role in animal metabolic systems. In food systems, carbohydrates have function to provide flavor, structure, and texture to food (Manthey, Xu 2009).

The term “organic acid” refers to organic compounds with acidic properties, which containing carbon. These are not considered as nutrients, but they are responsible to give a characteristic taste to food. Therefore, they are one of the major contributors to the flavor, besides sugars and volatile compounds. Organic acids occur naturally in a number of foods, mainly in fermented products as result of hydrolysis, biochemical metabolism and microbial activity. Furthermore, the organic acids have been widely used for the food industry as food additives and preservatives for avoiding food deterioration and extending the shelf life of food ingredients (Chen and others 2006; Jurado-Sánchez and others 2011). Once organic acids primarily acts as acidulants and

reducing bacterial growth by lowering the pH of food products to levels that will inhibit bacterial growth (Hinton 2006; Conte-Junior and others 2010). The acid in its undissociated state is able to penetrate the microbial cell, which is not able to tolerate a major change in its internal pH (Adams, Hall, 1988; Goosen and others 2011).

The determination of carbohydrates and organic acids content in food products is important, since they contribute to the flavor, texture and aromatic properties (Tormo and Izco 2004; Farajzadeh and Assadi 2009; Kritsunankul and others, 2009). Their presence and relative ratio of carbohydrates and organic acids can affect the chemical and sensorial characteristics of the food matrix (e.g., pH, total acidity and microbial stability) and can provide information on nutritional properties of food and means to optimize selected technological processes (Chinnici and others, 2005). The quantitative determination of carbohydrates and organic acids is also important to monitorate bacterial growth and activity. In this context, high performance liquid chromatography has been widely used for analyzing carbohydrates and non-volatile organic acids, while gas chromatography has been used to determine the volatile organic acids in complex matrixes.

This review highlights the main chromatographic methods in the analysis of carbohydrates and organic acids of food from animal origin, providing an overview since the types of carbohydrates and organic acids in different products of animal origin to different methods used (HPLC and GC) to analyze these compounds.

Carbohydrates and organic acids in foods from animal origin

The type and concentration of carbohydrate will vary depending on the animal product. The monosaccharides glucose and fructose occur naturally in honey. Free

glucose is also found in animal fluids (blood, lymph and cerebrospinal fluid). The pentose monosaccharides, arabinose, xylose and ribose, and the hexoses, mannose and galactose, rarely occur free in nature, except as breakdown products during fermentation. Of the disaccharides, lactose is the most abundant in milk and derivatives, which occurs solely in mammary tissue (Ball 1990).

Organic acids are found in foods from animal origin, as result of metabolism of large molecular mass compounds, such as carbohydrates, lipids, and proteins. These acids are also found in several products as compounds added to food to carry out some hygienic or technology function. Therefore, organic acid such as lactic and acetic acids are used as direct antimicrobial activity incorporated into human foods, because of their ability to lower the pH, resulting in instability of bacterial cell membranes (Luck and Jager 1997). They can accumulate over time as consequence of production by microorganisms, due the fermentation activity of indigenous or starter cultures added (Ricke 2003; Costa and Conte-Junior, 2013).

Milk and derivatives

Lactose is the major carbohydrate in milk from different species, such as goat, sheep and cow milk. The lactose content in milk is relatively constantly, though varies among different dairy products. Lactose is a disaccharide made up of glucose and galactose molecules, which is synthesized in the mammary gland. In addition, the glucose and galactose may also be present in small free amounts (Haenlein 2004; Park 1994). Others carbohydrates than lactose found in milk are oligosaccharides, glycopeptides, glycoproteins, and nucleotide sugars, although in small amounts (Park and others 2007).

The organic acid content of milk varies in the range of 0.12%–0.21%, which is around 1.2% in dry matter. The citric acid is the predominant organic acid in milk, and is present in the form of citrate (Walstra 1999). During storage, it disappears rapidly as a result of bacterial growth. Lactic and acetic acids are degradation products of lactose. In addition to these, others acids are produced from hydrolysis of lactose, citric acid and fat. Milk also contains nitrogenous acidic compounds, as orotic acid and hippuric acid. Orotic acid is another organic acid value found in milk, and its concentration is mainly influenced by diet and stage of lactation (Tormo and Izco 2004).

During a milk fermentation process, the lactic acid bacteria (LAB) utilize lactose and synthesize organic acid by products (Costa and others, 2013. The species that can ferment lactose the first step in the metabolism is a hydrolysis to its component monosaccharides by β-galactosidase, for most species, or phospho-β-galactosidase. Therefore, in fermented milk, generally, the production of some organic acids, such as lactic, formic, acetic and succinic acids, is the result of metabolic activities of starter or probiotic cultures (Ammor and others 2006). These acids contribute to the aroma of fermented milk, as yogurt, especially lactic acid, which is very important in the formation of the typical flavor of these products. This acid gives a sharp, acidic and refreshing taste to yogurt and others fermented milks. During the manufacture of these, there is an appreciable increase in the level of some organic acids such as lactic and citric acids. The level of organic acids in this type of product is dependent on several variables such as the starter cultures, type of milk, incubation temperature and time (Akalin, Kinik and Gonc 1997).

Cheese ripening is a complex process that involves several concurrent and interlinked reaction pathways. The primary biochemical events of ripening include metabolism of lactose, lactate and citrate, lipolysis, and proteolysis. The products of

primary events such as free fatty acids, organic acids, and amino acids are further catabolized to smaller volatile and non-volatile flavor compounds (Subramanian and others 2011). For cheese ripening the decrease of the sugars and the evolution of organic acids is technological important. In fact, they directly or indirectly determine the chemical composition, as well as the sensory characteristics, hence the quality (Zeppa, Conterno and Gerbi 2001). Organic acids present in the various types of cheese ripening can vary according to the manufacturing process and cheese starter culture involved.

Table 1 shows the different HPLC methods for carbohydrates and organic acids determination in milk and derivatives.

Meat and derivatives

Meat is a major source of proteins, particularly those containing amino acids essential to human health, and a good source of iron, zinc and vitamin B_{12} (Bax and others 2013). Nevertheless, meat is not a good source of carbohydrates. In addition, they are used as energy production, which has two main alternative routes: the oxidative and glycolytic pathways. Glycolysis is a very important metabolic pathway in the postmortem period, this pathways changes glycogen, a polymer of glucose and the major energy reserve in muscle, into lactate (Choe and others 2008). Lactate formed is either converted back to pyruvate to be used oxidatively via the tricarboxylate acid cycle (Pösö and Puolanne 2005). However, the processing of meat, as sausages and frankfurters production, can increase the carbohydrate content by adding sugars, starch products and others (Costa-Lima et al., 2014).

The predominant acid in muscle tissue is lactic acid that is formed by glycolysis, followed by glycolic and succinic acids. The pyruvate that is a generated as the end product of glycolysis is converted to lactic acid by the lactic dehydrogenase, and, since the metabolic waste products cannot be removed without a blood stream, the lactic acid accumulates in the muscle. Other acids of the Krebs cycle are present in negligible amounts (Greaser 2001; Kauffman 2001). The aerobic mechanism in muscle produces energy from glycogen, which normally comprises about 1% of the muscle weight. When the muscle is contracting rapidly, its oxygen supply becomes inadequate for the support of ATP resynthesis via an aerobic metabolism. Under these conditions, the aerobic metabolism supplies energy for a short time, converting glycogen to lactic acid, especially after slaughtering. In beef muscle, 48 h after the post mortem, the glycogen level drops rapidly from the initial value, in the same period occurs the lactic acid level increases (Pearson and Young 1989).

Various microorganisms produce organic acids and alcohols by anaerobic fermentation of food substrates, which inhibiting other organisms that are concomitantly present and could spoil the food or make it toxic. Lactic acid, for example, is a frequently effective inhibitory agent used in fresh meat preservation. However, other organic acids have also been found to be responsible for discolouration and production of pungent odors (Zhou and others 2010). For example, Samelis and others (2005) evaluated combinations of nisin with or without organic acids (latic and acetic acids), as inhibitors of *Listeria monocytogenes* in sliced pork bologna. In this way, lactic and acetic acids may be present in meat, because they are used in the beef industry for the decontamination of carcasses or derived meat. The effectiveness of these acids is dependent on the concentration and temperature of the acid solution, the exposure time and application pressure, the application stage in the slaughtering process, the tissue

type, the group of microorganisms, and the level of initial concentration (Li, Kundu, Holley 2015). Therefore, a higher lactic and/or acetic acid concentration might be expected in meats treated with these acids (Sofos 2005; Carpenter, Smith and Broadbent 2011).

In fermented meat products, the production of organic acids by bacteria is undoubtedly the determining factor on which the shelf life and the safety of the final product depend. This fact is due the immediate and rapid formation of acids at the beginning of the fermentation process, and the production of sufficient amounts of organic acids allowing a pH below 5.1 (Maijala and others 1993). Regarding the type of organic acid present, several factors can interfere, including the microorganism involved in the fermentation process. The homofermentation routes produce more than 85% lactic acid as a major end product of glucose catabolism, while the hetero- or mixed acid fermentation routes give not only lactic acid (50%), but formic and acetic acids as by-products (Stiles and Holzafel 1997).

Nevertheless, few studies assess the production of organic acids in meat products. Table 2 presents the articles that quantifying the organic acids in these products.

Fish and derivatives

As in the meat, fish meat is also not a good source of carbohydrates. Moreover, the processing of fish can also increase the carbohydrate content by the same factors. Regarding the organic acids, the main constituent in fish meat is also lactic acid. During the storage of fish, some organic acids are formed, which includes formic, acetic, propionic, n-butyric and isobutyric, n-valeric and isovaleric acids (Osako and others

2005). As for meat, organic acids are also used as additives for conservation of fish and derivatives (Mejlholm, Dalgaard, 2007; Calo-Mata and others 2008; Tomé and others 2008; García-Soto and others 2014).

In fermented fish products occurs the similar process that in fermented meat, with majority production of lactic acid. Saithong and others (2010) in their study with four different treatments Thai fermented fish evaluated the production of five organic acids (lactic, acetic, butyric, propionic and gluconic acids). They observed that lactic and gluconic acids were present in all treatments, but their behavior differs depending on the treatment. Whilst butyric, succinic, acetic and propionic acids were not detected on any treatment during fermentation. There is a lack of information about organic acids in meat fish from different species and their derivate products. The different conditions of analysis published are shown in Table 2.

Honey

Honey is a natural product produced by honeybees. The honeybees collect nectars taking place in flowers of plants convert their compositions and place them into cells of combs to be matured (Codex Stan 1981). Sugars and water represent the main chemical constituents of honey (>95%), whereas proteins, flavors and aromas, pigments, vitamins, free amino acids, and numerous volatile compounds constitute the minor components. The honey carbohydrate mainly includes a complex mixture of 70% monosaccharides (glucose and fructose), 10% disaccharides, and small amount of trisaccharides and tetrasaccharides (White 1978). Due to its composition, honey can be adulterated in various ways. One method of honey adulteration is the addition of different sugar syrups (Tosun 2013), as glucose syrup. Therefore, the analysis of

carbohydrate by chromatography can be used in order to detect the change thereof by addition of others carbohydrates, such as cornstarch.

Honey acidity is mainly due to organic acids whose quantity is lower than 0.5%. Acidity contributes to the honey flavor, stability against microorganisms, enhancement of chemical reactions, and antibacterial and antioxidant activities. Gluconic acid, resulting from the action of honey's glucose oxidase on glucose, provides the major contribution to acidity and is in equilibrium with gluconolactone. Other organic acids together with inorganic anions also contribute to the acidity of honey (Cavia and others 2007). The acid level is mostly dependent on the time elapsed between the nectar collection by bees and the final honey density in the honeycomb cells. Other acids such as acetic, butyric, lactic, citric, succinic, formic, malic, maleic, and oxalic acids are also present in small amounts. Besides that there are differences in composition of organic acids in the monofloral honey varieties. Therefore, the acids can be used as internal standards in order to detect honey adulteration (Daniele, Maitre and Casabianca 2012).

The organic acids comprise a small proportion of honey (0.5%) and together with the total acidity can be used as an indicator of deterioration due to storage, aging or even to measure the purity and authenticity (Cavia and others 2007). They are also components of the honey flavour (Crane 1990; Wang and Li 2011). Some organic acids have been identified in honey, which could be useful for characterizing different honey types. For example, citric acid concentration is used as a reliable parameter for the differentiation of two main types of honey: floral and honeydew honey (Daniele, Maitre and Casabianca 2012).

Table 3 summarizes the main carbohydrates and organic acids analyzed in honey by HPLC.

Carbohydrates metabolism and organic acids production by Lactic acid bacteria

Lactic acid bacterias (LAB) are Gram-positive, microaerophilic, acidtolerant, non-spore-forming, mainly nonmotile rods orcocci. They are characterized by the production majority of L (+) and/or D (-) lactic acid from the fermentation of sugars, including lactose. The main characteristic of LAB, which renders this group of organisms ideal as a starter culture in the fermentation of food, is their ability to produce organic acids and thereby also to decrease pH in food (Røssland and others 2005). Lactic acid bacteria occur naturally in various foodstuffs, their growth is enhanced, or they are added deliberately to produce a range of fermented foods. These include fish, meat, various dairy products, cereals, fruits, vegetables, and legumes. They are a very important group of starter cultures, applied in the production of a wide range of fermented foods, they contribute to the enhancement of the characteristics of food, and they have been recognized as contributing to the microbial safety of fermented food (O'Sullivan, Ross and Hill 2002). The LAB have an important antimicrobial function, due to their production of some metabolites, such as organic acids (Messens and De Vuyst 2002).

Lactic acid bacteria are not equipped with the enzymes necessary for respiration, and they are, therefore, unable to perform oxidative phosphorylation. The energy demand is, consequently, satisfied solely through substrate-level production of adenosine triphosphate (ATP) or the equivalent of ATP. Therefore, the generation of energy required for growth is converted for carbohydrate metabolic by the starter cultures. In addition, the metabolic pathways of the lactic acid bacteria can be homolactic or heterolactic fermentation. Bacterial homolactic fermenters strains are able to convert the fermented carbohydrate into products other than lactate, and the end-

products are represented with the enzymes catalysing the reactions. During heterolactic fermentation, the fermentation process can produce simultaneously various other metabolites besides the lactic acid, such as acetic acid, fumaric acid, ethanol, malic acid, etc. However, the amount of these metabolites can have a significant influence on the downstream process and the quality of the L(+)-lactic acid produced (Wang and others 2005). Hence, not all LAB produce the same lactic acid isomer (Gravesen and others 2004). The levels and also the type of organic acids that are produced during any fermentation process are, therefore, dependent on LAB species or strains, growth conditions, and food composition (Ammor and others 2006).

HPLC analysis

The analysis of carbohydrates and organic acids in different food items such as dairy products, meat products and honey is of great interest for food industry. Once these compounds are responsible of sensory properties, deterioration and authenticity, in addition, they may also influence technology stability in theses matrixes (Rodrigues and others 2007). For this reason, different HPLC techniques have been used for the separation and identification of these compounds in different foods (Van Hees and others 1999), such as foods from animal origin. Moreover, the high performance liquid chromatography methods have gained importance in these analyses because of the speed, selectivity, sensitivity and reliability of this technology (Chen and others 2006).

Sample preparation

Sample preparation is an important procedure in chemical study of foods. The procedure of sample preparation of foods from animal origin for analysis of carbohydrates and organic acids by HPLC is considered relatively simple, mainly due to not require many steps, and different or dangerous reagents requirement. Regardless of the complexity of the matrix, the sample preparation involves three phases: (1) extraction, (2) centrifugation and (3) filtration.

The extraction step is usually performed using an acid, which can be the one mobile phase but with a higher concentration, such as sulfuric and phosphoric acids. However, for meat samples, the PCA is the most used and the most efficient. The centrifugation step may be applied or not, which depends mainly of foods from animal origin analyzed. The most authors who apply centrifugation used to a range from 6,000 to 17,000 x *g*, however, in dairy products, the use of 5000 *g* of rotation is sufficient (Gaze and others 2015). The supernatant generally is filtered through a 0.22 or 0.45-μm cellulose acetate filter, and then preparation obtained is ready and is just inject the equipment (González de Llano and Cuesta, 1996; Suárez-Luque and others 2002a; Suárez-Luque and others 2002b; Kaminaride, Stamou and Massouras 2007; Leite and others 2013; Gaze and others 2015). The use of centrifugation in the analysis of carbohydrates and organic acids in complex matrices facilitates the extraction with a purer final extract.

Separation columns

Liquid chromatography has simplified the analysis for various food constituents, including carbohydrates and organic acids. In chromatography, the selection of the stationary phase is essential in order to achieve a suitable separation. A number of

different separation mechanisms have been widely employed in different matrix, which including ion-exchange, ion-exclusion, ion-par, hydrophilic interaction and reverse-phase. Consequently, the choice of method in each case is dictated essentially by the types of analyte to be determined and their proportions as well as by the nature of food matrix (Quirós and others 2009; Churms 1996). For determination of carbohydrates and organic acids in foods from animal origin, the most usual method is ion exchange chromatography followed by reverse-phase chromatography.

For carbohydrates, analysis is also widely used hydrophilic interaction chromatography (HILIC) and ion exchange chromatography (Dvořáčková, Šnóblová and Hrdlička 2014). Although both, hydrophilic interaction and ion exchange, are effective in the separation, the first is most commonly used in the separation of mono- and oligosaccharides, while the second of mono- and disaccharides.

The ready ionization of organic acids has long been exploited for their isolation by ion-exchange chromatography, which involves the use of an ion-exchange resin as stationary phase. This separation technique is extremely used nowadays, and the column most frequently used for this purpose is the Aminex HPX-87H 300 x 7.8mm model from Biorad Laboratories (Fernandez-Garcia and Mcgregor1994; Gonzalez de Llano and Cuesta 1996; Zeppa, Conterno and Gerbi 2001; Adhikari and others 2002; Ong and others 2006; Donkor and others 2007; Kaminaride, Stamou and Massouras 2007; Ong, Henrikssonb and Shah 2007; Kaminarides and others 2009; Sriphochanart and Skolpap, 2011; Cruz and others 2012; Madureira and others 2013; Leite and others 2013). One of the main reasons for the use of this particular column is related to its length (300 mm), which provides a better separation of peaks, facilitating the simultaneous analysis of carbohydrates and organic acids.

The stationary phases most used in bonded-phase chromatography in its reversed-phase mode are based on octyl (C8 columns) and octadecyl (C18 columns) functionality. The difference between the two columns will be in the length of the carbon-chain attached to the silica surface, as for organic acid analysis to C18 column is the most used (Bevilacqua and Califano 1992; Tormo and Izco 2004; Bensmira and Jiang 2011; Murtaza and others 2012; Saithong and others 2010).

Detection

The detectors most frequently used in HPLC for analysis of carbohydrates and organic acids are the conductivity (CD), the pulsed amperometric (PAD) the refractive index (RI), the evaporative light scattering (ELSD) and the ultraviolet (UV), beyond mass spectrometric (MS). In general, the most detectors used for carbohydrates analysis are CD, PAD, RI and ELSD whilst for organic acids are RI, ELSD and UV. Nowadays, the high performance liquid chromatography has been widely used with detection mode dual UV-VIS detector and refractive index detector for analyzing carbohydrates and non-volatile organic acids in complex matrixes, in the same chromatographic run (Gaze et al., 2015).

The conductivity detectors were originally employed in ion chromatography for determination of inorganic ions, later for organic acids. However, the inherent difficulties have deterred potentials user from applying them to food analyses. Because this type of detector has low selectivity; and the solute conductivity measurements require the prior elimination of the eluent background conductivity using a conventional suppressing column or a more modern alternative such as a cation-exchange membrane. Currently, due to their limitations, this type of detector is not widely used (Blanco

Gomis 2000). However, it can be used for analysis of carbohydrates in different food matrices, such as foods from animal origin (Yoshida, Terashima, and Takahashi 1999; Wang and others 2013; Mullin and Emmons 1997).

The pulsed amperometric detector operates using a triple step potential waveform to combine amperometric detection with alternating anodic and cathodic polarization to clean and reactivate the electrode surface. This waveform exploits the surface-catalyzed oxidation of the amine group, activated by the transient formation of surface oxides on noble metals (Welch and others 1990). In alkaline solutions, which are useful for anion-exchange separation of carbohydrates, PAD is significantly more sensitive than the conductivity detector. However, the CD provides linear response to higher concentrations than those observed for PAD (Welch, Mead Jr., and Johnson 1988). The combination of these two detectors may be a strategy for better resolution of the chromatograms. Some studies using this detector for the analysis of carbohydrates in foods from animal origin (Mora and Marioli 2001; Cordella and others 2003; Hurum and Rohrer 2012).

The refractive index detector responds to a difference in the refractive index of the column effluent as it passes through the detector flow cell. For this reason, RI detection has been used very successfully for the analysis of sugars, triglycerides, and organic acids (Swartz 2010). The RI detector is a bulk-property detector that responds to all solutes, if the refractive index of the solute is sufficiently different from that of the mobile phase. These detectors are somewhat sensitive to changes in pressure, temperature, and composition of the mobile phase, this must demand strict control of the chromatographic conditions and the use of isocratic elution. However, despite its limitations RI detector has an advantage of this detectors, they can use for determining

other components interest, as carbohydrates, simultaneously in a single chromatographic analysis (Morgan and Smith 2011).

The evaporative light scattering detection works by nebulizing the column effluent, forming an aerosol that is further converted into a droplet cloud for detection by light scattering. Currently, ELSD is gaining popularity due to its ability to detect analytes on a nonselective basis. This type of detector has been applied to studies of carbohydrates (Wei and Ding 2000; Liu and others 2012; Dvořáčková, Šnóblová and Hrdlička 2014; Ma and others 2014), and lipids (Rodríguez-Alcalá and Fontecha, 2010; Imbert and others 2012; Kobayashi and others 2013).

The most widely used detectors in modern HPLC are photometers based on ultraviolet (UV) and visible light (VIS) absorption. They have a high sensitivity for many solutes, including organic acids, but samples must absorb in the UV region (Swartz 2010). These detectors are no doubt the most frequently used at present for determining organic acids in food. And they can be used for analysis of underivatized organic acids, detection at 206-220 nm, usually poses no serious problem in the determination of major organic acids (Blanco Gomis 2000; Saithong and others 2010; Sriphochanart and Skolpap 2011; Murtaza and others 2012; Madureira and others 2013; Cruz and others 2012; Leite and others 2013). Nevertheless, this detector is not used for carbohydrate analysis. These compounds absorb light at wavelengths included within the 190-200 nm range, which corresponding the zone of spectrum of many organic compounds present in foods and organic solvents (Paredes and others 2006).

The mass spectrometric detector is the most sophisticated hyphenated (refer to the coupling of an independent analytical instrument to provide detection) HPLC detector in use today. In complex samples, mass spectrometry coupled to liquid

chromatography constitutes a powerful technique due to its high sensitivity and selectivity (Chen and others 2007).

Chromatography conditions

The chromatography conditions applied to the analysis of carbohydrates and organic acids are varied, which depends on several factors, such as detector and column used. For example, the RI detector cannot be used with gradient flow rate for separation of analyte, since the baseline becomes unstable. For this reason, when we use the RI detector is used isocratic flow rate. While for ELSD and UV detectors, the use of gradient can be applied without any compromise in baseline. Thus, different types of mobile phase and flow, and the use or non-gradient may be applied.

CG analysis

The gas chromatography (GC) methods gave sample resolution and sensitivity. For carbohydrates analysis the analytes required prior derivatization to make them volatile (Armstrong and Jin 1989), is not widely used for this analysis. However, GC is an attractive alternative to analyze organic acids due to its simplicity, separation efficiency and excellent sensitivity and selectivity (Ballesteros and others 1994; Yang and Choong 2001; Horák and others 2008; Horák and others 2009). Many short-chain organic acids are thermostable and sufficiently volatile, thus fulfilling key requirements for GC measurement (Nollet 2004). Furthermore, the method of choice for volatile acids analysis is by gas chromatography is, instead of the isolation of compounds from the cheese matrix can be carried out by different methods, such as high vacuum

distillation, simultaneous distillation extraction, supercritical fluid extraction or headspace techniques (Fernández-García and others 2002).

Sample preparation

In general, the great complexity of food samples demands an appropriate sample preparation technique before analysis. As a rule, beverages usually implicate in a simple pretreatment such as dilution and/or filtration, but for other food the potential interference of matrix compounds (e.g. fats, vitamins, proteins, polysaccharides) require the employment of more complex pre-treatment and clean-up procedures (Kritsunankul and others 2009; Rovio and others 2010).

Traditional methods such as stream distillation and liquid–liquid extraction are time consuming and environmentally unfriendly (Nollet 2004). The solid-phase extraction (SPE) can be implemented via flow systems, resulting in a dramatically increased the process and reduced analytical cost through decreased reagent consumption (Cherchi and others 2003; Mota and others 2003; Horák and others 2009). Other alternatives such as single-drop microextraction (Saraji and Mousavinia 2006), solid-phase microextraction (Wen and others 2007) and stir-bar sorptive extraction (Horák and others 2008) have also been successfully applied to the analysis of short and medium-chain fatty acids and preservatives in vinegar, beverages and dairy products.

Derivatization

Although, other acids should be derivatized to convert these compounds into less polar and stable derivates suitable for their GC determination (Saraji and Mousavinia

2006; Horák and others 2009). To avoid the derivatization process of organic acids, there are successfully employed capillary GC columns coated with polar stationary phases such polyethylene glycol or nitroterephthalic acid modified polyethylene glycol. When using these columns it is possible to obtain a good chromatographic resolution, avoiding peak tailing (Yang and Choong 2001; Horák and others 2008).

Detection

The flame ionization detector (FID) is the most sensitive gas chromatographic detector for hydrocarbons such as butane or hexane. With a linear range for 6 or 7 orders of magnitude (10^6 to 10^7) and limits of detection in the low picogram or femtogram range, the FID is the most widely and successfully used gas chromatographic detector for volatile hydrocarbons, such as organic acids. However, the presence of oxygen molecules decreases the detector's response. Therefore, highly oxygenated molecules or sulfides might best be detected using another detector instead of the FID. Sulfides determination by the flame photometric detector and aldehydes and ketones analyzed with the photoionization detector are alternatives to the use of the FID for those molecules (Colón, Baird 2004).

In order to measure the characteristics of individual molecules, a mass spectrometry (MS) converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields. A mass spectrometry (MS) is an analytical technique that measures the molecular masses of individual compounds and atoms precisely by converting them into charged ions. Mass Spectrometry has been applied in food chemistry fields for the analysis of toxic compounds and contaminants, for nutraceutics and for the characterization of foodstuff to be applied for production

areas and traceability (Yang and Caprioli 2011). Therefore MS is, today, usually accoplated to HPLC or CG.

Chromatography conditions

The columns and detectors used for determination of carbohydrates and organic acids in foods from animal origin by GC methods are shown in Table 4.

Conclusion

In this review, it could be evidenced that the carbohydrates and organic acids are related to the intrinsic characteristic of the major Food of animal origin, the processing steps that these foods are submitted and the biochemical changes that occur during storage of those products. Although there are various chromatographic techniques that can be applied for the analysis of carbohydrates and organic acids in the food matrix, HPLC appears to be the method of choice due to the chemical structure of these compounds, since they are associated to other nutrients in foods. In addition, these analytical techniques have the advantage of simultaneously analyzing of carbohydrates and organic acids present in the matrix, which speeds up the analysis process. Whilst the CG is mainly used for identification and quantification of fatty acid profile, especially those aimed at those of long chain.

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Author Contributions

Costa M P researched prior studies and interpreted the articles, compiled data and drafted the manuscript.

Conte-Junior C A drafted and corrected the manuscript.

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Table 1: HPLC methods for carbohydrates and organic acids determination in foods of animal origin

Sample	Carbohydrates and Organic Acids	Columns	Detector	Authors/ Year
Whole milk, powdered skim milk, cultured buttermilk, sour cream, yogurt, cottage, sharp, cheddar and blue cheeses	Orotic, citric, pyruvic, lactic, uric, formic, acetic, propionic, butyric and hippuric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	220 and 275 nm	Marsili and others (1981)
Cheddar cheese	Orotic, citric, pyruvic, lactic, uric, formic, acetic, propionic, butyric and hippuric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV 220 and 285 nm	Bouzas and others (1991)
Cheese	Lactic, formic, acetic, pyruvic, citric, orotic and uric acids	Beckman C_8 (250 x 4.6 mm) 5 µm	UV - 214 nm	Bevilacqua and Califano (1992)
Yogurt	Orotic, citric, pyruvic, lactic, uric, formic, acetic. propionic, butyric and hippuric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 210 nm	Fernandez-Garcia and Mcgregor, (1994)
Reggianito cheese	Formic, pyruvic, orotic, uric, lactic, acetic, citric, propionic and butyric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV 214 and 280 nm	Lombardi and others (1994)
Milk and cheese	Citric, succinic, lactic, formic, acetic, propionic, orotic, uric, pyruvic and butyric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 210 nm	Gonzalez de Llano and others (1996)
Milk	Lactose, glucose and galactose	Alphasil SNH_2 and Sugar Pak I	RI	Indyk and others (1996)
Cheddar cheese	Lactic, formic, citric and acetic acids	Dionex IonPac ICE-AS6 (9 x 259 mm)	Conductivity	Mullin and Emmons (1997)
Low-fat cheese	Pyro-glutamic, lactic, pyruvic and uric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV – 210 nm	Skeie and others (1997)

Cheddar cheese	Acetic, citric, butyric, fumaric, formic, hippuric, iso-valeric, lactic, malic, orotic, oxalic, propionic, pyruvic; uric and n-valeric acids	Supelcogel C-610H ion-exchange column (30 cm×7.8 mm)	UV - 210 and 290 nm	Lues and others (1998)
Mozzarella cheese	Formic, pyruvic, orotic, uric, lactic, acetic, citric, propionic and butyric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 214 and 280 nm	Califano and Bevilacqua (1999)
Fermented milk	Benzoic acid	Chromosorb WAW 80/100 as the stationary phase (3 m x 2 mm, i.d.)	UV	Suomalainen, Mâyrâ-Mâkinen (1999)
Gouda cheeses	Formic, orotic, uric, lactic, acetic, citric, pyruvic, propionic and butyric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 214 and 280 nm	Califano and Bevilacqua (2000)
Kefir	Orotic, citric, pyruvic, lactic, uric, acetic, propionic, butyric and hippuric acids	Alltech IOA-1000 organic-acid column (300 mm x 7.8 mm)	UV - 275 nm	Guzel-Seydim and others (2000)
Norvegia cheese	Pyro-glutamic, lactic, pyruvic and uric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV – 210 nm	Skeie and others (2001)
Cheese	Citric, orotic, piruvic, lactic, oxalic, hippuric, formic, acetic, propionic, butyric, isobutyric, valeric and isovaleric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 210 and 290 nm	Zeppa and others (2001)
Yogurt	Acetic, lactic, citric, propionic, butyric, uric and pyruvic acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 220 nm	Adhikari and others (2002)
Pickled White Cheese	Formic, pyruvic, lactic, acetic, orotic, citric, uric, propionic and butyric acids	C18 (120× 5 mm)	UV	Akalin and others (2002)
Cheddar cheese	Acetic, butyric, citric, formic, fumaric, hippuric, isovaleric, lactic, malic, n-valeric,orotic, propionic, pyruvic and uric acids	Supelcogel C-610H ion-exchange column	UV - 210 and 290 nm	Lues and Bekker (2002)
Milk-based formulae	Mono- and disaccharides	Tracer carbohydrates (250 × 4.6 mm i.d.)	RI	Chávez-Servín and others (2004)

Raw milk, yogurt and cheese	Oxalic, citric, formic, succinic, orotic, uric, pyruvic, acetic, propionic, lactic and butyric acids	Atlantis dC18 column (Waters) (250 mm x 4.6 mm) 5 µm	UV - 210 nm	Tormo and others Izco (2004)
Goat milk cheeses	Citric, pyruvic, malic, lactic, formic, acetic, propionic, uric and butyric acids	Supelcogel C-610H ion-exchange column (300×7.8 mm)	UV – 2010 and 290 nm	Buffa and others (2004)
Goat milk cheese	Tartaric, formic, orotic, malic, lactic, acetic, citric, uric, propionic and butyric acids	ODS Hypersil (125 mm × 4 mm) 5 µm	UV - 214 nm	Park and Drake (2005)
Low-fat Feta-type cheese	Lactic, citric and acetic acids	Hamilton column, hydrogen form (305 × 7.8 mm) 10 µm	UV - 210 and 280 nm	Manolaki and others (2006)
Yogurt	Lactic and acetic acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 220 nm	Ong and others (2006)
Goat milk cheese	Acetic, butyric, citric, formic, lactic, malic, isomalic, orotic, propionic, pyruvic, tartaric, isotartaric and uric acids	Hypersil ODS (125 × 4 mm) 5 µm	UV - 214 nm	Park and others (2006)
Monterey Jack goat milk cheeses	Tartaric, formic, orotic, malic, lactic, acetic, citric, uric, propionic and butyric acids	Hypersil ODS (125 mm × 4 mm) 5 µm	UV - 214 nm	Park and Lee (2006).
Yogurt	Lactic, acetic, butyric and propionic acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 220 nm	Donkor and others (2007).
Milk and yogurt	Citric, pyruvic and lactic acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	RI	Kaminaride and others (2007)
Cheddar cheese	Lactic and acetic acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 220 nm	Ong and others (2007).
Cheddar cheese	Lactic, acetic, citric, propionic and butyric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 220 nm	Ong and Shah (2008).
Milk-based formulae	Glucosamine and lactose	Shodex Asahipak NH2P-50 (4.6 x 250 mm)	RI	Xinmin and others (2008)

Halloumi-type cheese	Acetic, pyruvic and lactic acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	RI	Kaminarides and others (2009).
Milk	Lactose, glucose, galactose and oligosaccharides	Waters Sugar Pak I column (6.5 300 mm)		Nguyen and others (2009).
Halloumi cheese	Lactic, citric and acetic acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	220 nm	Ayyash and Shah (2010)
Milk	Lactose and lactulose	Ion exclusion and Hydrogen Bonding	ELSD	Schuster-Wolff-Bühring and others (2010).
Kashar cheese	Citric, lactic, formic, acetic, propionic and butyric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 214 and 280 nm	Andiç and others (2011).
Kefir	Lactic, citric, pyruvic and acetic acids	Diamonsil C18 column (46 x 250 mm) 5 µm	UV - 275 nm	Bensmira and Jiang (2011).
Human and cow's milk	Lactose	ACQUITY UPLC BEH C18 1.7 µm column (2.1 × 100 mm)	Tandem mass spectrometry	Fusch and others (2011)
Sheep milk and Manchego cheese	Citric, pyruvic, lactic, formic, acetic, propionic, butyric, orotic and uric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	210 and 280 nm	Garde and others (2011).
Dairy matrix	Inulin, fructose and glucose		ELSD	Kristo and others (2011).
Cheese	Acetic and lactic acids	Chrompack column (300 x 6.5 mm)	UV	Magalhães and others (2011)
Cheddar cheese	Lactic, formic and oxalic acids	HP1050 equipped with a Prevail (150 x 4.6 mm) 5 µm	UV - 200 nm	Subramanian and others (2011).
Probiotic yogurts	Glucose, lactic and acetic acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 220 nm	Cruz and others (2012).
Skim milk	Lactose, glucose and galactose	Ion-pair	UV - 303 nm and fluorescence	Erich and others (2012).
Cheese	lactose, glucose, galactose, citric, pyruvic,	Aminex HPX-87H ion-exchange column	RI	Garde and others (2012)

	lactic, acetic, propionic and butyric acids	(300 x 7.8 mm)	UV - 210 nm	
Infant formula	Sialic acid	Anion-exchange	Pulsed amperometric and fluorescence	Hurum and Rohrer (2012).
Whey cheese	Succinic, citric, lactic and acetic acids	Aminex HPX ion-exchange column (300 x 7.8 mm)	RI UV - 220 nm	Madureira and others (2012)
Milk	Lactic acid	Aminex HPX ion-exchange column (300 x 7.8 mm)	UV - 210 nm	Milagres and others (2012)
Buffalo cheese	Lactic, acetic, citric, pyruvic, formic, butyric and maleic acids	Shim-Pack C_{18} (LC) column	UV - 214 nm	Murtaza and others (2012).
Liquid milk and powdered milk	Carbohydrates	Hypercarb (100 × 4 mm)	ELSD	Terol and others (2012).
Milk	Benzoic acid	Metrosep A5 250 anion-exchange column (250 mm X 4.0 mm) 5 µm	Conductivity	Wang and others (2013).
Kefir	Citric, succinic, lactic, formic, acetic, propionic and butyric acids	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 210 nm	Leite and others (2013).
Powdered milk	Glucose, galactose, fructose, saccharose and lactose	ZORBAX carbohydrate (4.6 × 250 mm)	RI	Zhou and others (2014).
Dulce de leche	Lactose, sucrose and glucose	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	RI	Gaze and others (2015).
Coarsely ground beef	Lactic	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 210 nm	Nassos and others (1984).
Raw fish meat and dried meat	Lactic, acetic, pyroglutamic, citric, succinic, formic, phosphoric and malic	Shim-Pack SCR-102H (i.d. 0.008 m x 0.30 m x 2) ion-exclusion column	Conductivity	Yoshida and others (1999).
Thai fermented fish	Lactic, acetic, butyric, propionic and gluconic	Alltech Platinum EPS C18 column	UV - 210 nm	Saithong and others (2010).
Thai fermented	Lactic, acetic and formic	Aminex HPX-87H ion-exchange column	UV - 210 nm	Sriphochanart and Skolpap (2011).

sausage		(300 x 7.8 mm)		
Ready-to-eat meat and poultry products	Lactate and acetate	Aminex HPX-87H ion-exchange column (300 x 7.8 mm)	UV - 210 nm	Ahmed and others (2015).
Honey	Monosaccharides	Dionex Carbopac AS-6 pellicular anion-exchange	Pulsed amperometric	Hardy and others (1988).
Honey	Monosaccharides	Dionex 10-rm Carbo Pac anion-exchange (4 X 250 mm)	Pulsed amperometric	Swallow and Low (1990).
Honey	Pyruvic, quinic, malic, isocitric, succinic, fumaric, propionic, galacturonic, gluconic, tartaric, dimethylglyceric, 2-oxopentanoic and glutaric acids	Spherisorb ODS 1S5 (250 x 4.6 mm) 5 µm	UV - 210 nm	Cherchi and others (1994).
Honey	Glucose, fructose, and sucrose	Anion exchange	Pulsed amperometric	Mora and Marioli, (2001).
Honey	Malic, citric, succinic, fumaric and maleic acids	Spherisorb ODS-2 S5	UV - 215 nm	Suárez-Luque and others (2002a).
Honey	Malic, citric, succinic, fumaric and maleic acids	Spherisorb ODS-2 S5	UV - 215 nm	Suárez-Luque and others (2002b).
Honey	Gallic, caffeic, ferulic, benzoic and cinnamic acids	C_{18} column (150 x 4.6 mm, 5 µm)	UV - 280 nm	Aljadi and Yusoff (2003).
Honey	Sugar profile	Anion exchange	Pulsed amperometric	Cordella and others (2003).
Honey	Fructose, glucose, disaccharides, trisaccharides	Carbopac PA1 anion-exchange (4 × 250 mm)	Pulsed amperometric	Ouchemoukh and others (2010).
Honey	Quinic, pyroglutamic, gluconic, lactic, propionic, formic, butyric, pyruvic, galacturonic, glutamic, citramalic, citric, isocitric and cis-aconitic acids	IonPac AS11-HC column (50 x 4 mm) 10 µm		Daniele and others (2012).

Honey	Glucose, fructose, sucrose and maltose	Rezex RCM cation-exchange column (300 x 7.8 mm)	RI	Özbalci and others (2013).
Honey	Fructose, glucose, sucrose, maltose	Prevail carbohydrate ES column	ELSD	Qiangsheng and others (2013).
Honey	Malto-oligosaccharides	Waters ACQUITY BEH amide (2.1 × 50 mm, 1.7 µm)	ELSD	Zhou and others (2014).

Table 2: GC methods for determination of carbohydrates and organic acids in foods from animal origin.

Sample	Carbohydrates and Organic Acids	Columns	Detector	Authors
Coarsely ground beef	N-propyl derivatives of lactic and glutaric acids	Glass column (1.8m x 2.0 mm i.d.) was packed with	Flame ionisation	Nassos and others (1984).
Honey	Disaccharides and trisaccharides			Low, Sporns (1988).
Milano salami	2 organic acids	Capillary coated with a DB-5 stationary phase (30m x 0.32 mm, 1 µm film thickness).	Flame ionisation	Meynier and others (1999).
Fermented milk	Acetic and propionic acids	Chromosorb WAW 80/100 as the stationary phase (3 m x 2 mm, id.)	Flame ionisation	Suomalainen, Mâyrâ-Mâkinen, (1999).
Kefir	Volatile component	Capillary column (DB-5, J&W Scientic, Folsom, CA) (0.32 i.d. x30 x1 µm)	Flame ionisation	Guzel-Seydim and others (2000).
Fresh milk, spoiled milk, fermented milk, yogurt drink and lactic acid beverage,	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, caprylic, capric, lauric, lactic and levulinic	Chrompack CP-Wax column (30 m x 0.53 mm)	Flame ionisation	Yang, Choong (2001).

	acids			
Honey	Gallic, caffeic, ferulic, benzoic and cinnamic acids	CBP1-Shimadzu non-polar column (20 m x 0.2 mm, 0.25 mm film thickness).	Mass spectrometry	Aljadi, Yusoff (2003).
Italian sausages	Acetic, butanoic, 2-methylpropanoic, 3-methylbutanoic and pentanoic acids	Carbowax capillary	Mass spectrometry	Spaziani, Del Torre, Stecchini (2009).
Pecorino di Farindola cheese	Volatile component	Fused silica capillary column coated with a 0.2 μm film of Carbowax 30 m × 0.32 μm i.d.	Mass spectrometry	Suzzi and others (2014).

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3.4 ARTIGO IV: SIMULTANEOUS ANALYSIS OF CARBOHYDRATES AND ORGANIC ACIDS BY HPLC-DAD-RI FOR MONITORING GOAT'S MILK YOGURTS FERMENTATION SUBMETIDO PARA REVISTA TALANTA

Simultaneous analysis of carbohydrates and organic acids by HPLC-DAD-RI for monitoring goat's milk yogurts fermentation

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Abstract

During yogurt manufacture, the lactose fermentation and organic acid production can be used to monitor the fermentation process by starter cultures and probiotic bacteria. In the present work, a simple, sensitive and reproducible high-performance liquid chromatography with dual detectors, diode array detector and refractive index was validated by simultaneous analysis of carbohydrates and organic acids in goat milk yogurts. In addition, pH and bacterial analysis were performed. Separation of all the compounds was performed on an Aminex HPX-87H column (300 x 7.8 mm, 9 µm) utilizing a 3 mmol.L^{-1} sulfuric acid aqueous mobile phase under isocratic conditions. Lactose, glucose, galactose, citric, lactic and formic acids were used to evaluate the following performance parameters: selectivity, linearity, precision, limit of detection (LOD), limit of quantification (LOQ), decision limits (CC_α), detection capabilities (CC_β), recovery and robustness. For the method application a six goat milk yogurts were elaborated: natural, probiotic, prebiotic, symbiotic, cupuassu, and probiotic with cupuassu. The validated method presented an excellent selectivity with no significant matrix effect, and a broad linear study range with coefficients of determination higher than 0.99. The relative standard deviation was lower than 10% under repeatability and within-laboratory reproducibility conditions for the studied analytes. The LOD of the method was defined from 0.001 to 0.003 µg.mL^{-1}, and the LOQ from 0.003 to 0.013 µg.mL^{-1}. The CC_α was ranged from 0.032 to 0.943 µg.mL^{-1}, and the CC_β from 0.053 to 1.604 µg.mL^{-1}. The obtained recovery values were from 78% to 119%. In addition, the method exhibited an appropriate robustness for all parameter evaluated. Base in our data, it was concluded that

the performance parameters demonstrated total method adequacy for the detection and quantification of carbohydrates and organic acids in goat milk yogurts. The application of the method was successfully applied to monitoring different goat milk yogurts during fermentation.

Keywords: Validation, lactose, lactic acid, cupuassu pulp, probiotic, inulin.

1. Introduction

During yogurt fermentation, the lactic acid bacteria hydrolyze lactose into glucose and galactose, and produces organic acids [1]. Furthermore, organic acids in fermented milk are also originated from animal metabolism during milk production, and hydrolysis of milk fat during processing and storage. These compounds are important indicators of bacterial activity in yogurt, and contribute to the development of the characteristics taste and flavor of this type of product. The carbohydrate and organic acid contents could be used, such as pH and acidity, to monitor the fermentation process by starter cultures and probiotic bacteria [2]. Thereunto, high-performance liquid chromatography (HPLC) with dual mode detection systems namely, diode array detector (DAD) and refractive index (RI), is a reliable technique to analyze carbohydrates and organic acids contents in complex food matrices [3,4]. However, the validation of HPLC-methods is an important step to ensure the performance of a bioanalytical assay.

The validation of a methodology reduces possible analytical errors, which improves the reliability and reproducibility of the analysis [5]. For the most international guidelines, there are parameters required for the validation of a method, which including: selectivity, linearity, precision, limit of detection (LOD), limit of quantification (LOQ), recovery and robustness [5–10]. Selectivity estimates the capability of identification of a specific compound without matrix effect interference [6]. Linearity is the ability to obtain analytical values that are directly proportional to the concentration of an analyte in the sample [7]. Precision is the closeness amongst analytical values obtained from a series of measurements of the same homogeneous sample investigated under similar conditions, and obtained from multiple sampling procedures [5–7]. LOD is the minimum concentration value of a certain analyte that is detectable, while LOQ is the minimal amount of an analyte present in the sample that is quantifiable [5–10]. Recovery estimates the yield of an

analytical technique and is obtained by fortifying a biological matrix with a known amount of an analyte, followed by submitting such sample to the analytical procedure [5]. Finally, robustness evaluates the influence of slight variations in the analytical parameters on the final output; robust techniques are unaffected by small variations in such parameters [7].

In terms of the fermentation step during dairy products manufacture, the addition of ingredients such as probiotics, prebiotics and fruit pulp may interfere with the bacterial metabolism resulting on longer of shorter periods of fermentation. Probiotics are live microorganisms that, when ingested in adequate amounts promote health benefits for the host [11]. *Lactobacillus acidophilus* LA-5 is recognized as a probiotic strain, and can also produce metabolites such as lactic acid and acetaldehyde [12] which potentially influence the fermentation of yogurt [13]. Furthermore, prebiotics are non-digestible food ingredients that selectively stimulate the growth of gut health favorable bacteria resulting on the production of desirable metabolites or favoring the competition against pathogenic bacteria [14]. Therefore, prebiotics can also promote the growth of probiotics during yogurt fermentation [15]. In addition, cupuassu (*Theobroma grandiflorum*) pulp contains elevated contents of sucrose as well as glucose and fructose [16], and is a potential source of insoluble dietary fibers [17]. These physicochemical characteristics are favorable for the development and survival of lactic acid bacteria and fermenting microorganism indicating that cupuassu pulp is an interesting novel ingredient for the manufacture of probiotic yogurt [18].

In this context, fast, simple and accurate analytical methods are desirable in dairy industry especially for yogurt fermentation process [3]. Therefore, the aim of the present study was to validate a HPLC method for simultaneous determination of carbohydrates and organic acids contents utilizing DAD and RI detectors to evaluate the influence of the addition of probiotic, prebiotic, and cupuassu pulp on these compounds during goat milk yogurts fermentation.

2. Material and methods

2.1 Standards preparation

Commercial standards of lactose (cat. 61345), galactose (cat. 48259), glucose (cat. G8270), and formic (cat. 09676), citric (cat. C0759) and lactic (cat. 46937) acids were

purchased from Sigma-Aldrich® (St. Louis, MO, USA). Stock solutions of lactose and lactic acid were individually diluted with ultrapure water (Simplicity UV, Millipore, Molsheim, France) to 60 $mg.mL^{-1}$ whereas, for the other standards a final concentration of 40 $mg.mL^{-1}$ was utilized; all stock solutions were stored at 4 °C. For each validation phase (selectivity, linearity, precision, recovery, limit of detection, limit of quantification, robustness and stability), fresh working solutions were prepared by diluting aliquots of each stock solution with ultrapure water to a desired concentration.

2.2 Yogurt Processing

Yogurts were manufactured according to Costa et al. (2015) utilizing UHT-treated goat whole milk (Cappry's, Rio Grande do Sul, Brazil) and thermophilic yogurt cultures (YF-L903; Chr. Hansen, Valinhos, Brazil) at a final concentration of 1% (v/v). A total of six formulations were elaborated: natural (NAT), probiotic (PRO), prebiotic (PRE), symbiotic (SYM), cupuassu (CUP), and probiotic with cupuassu (PWC). All ingredients were added individually to two liters for each formulation prior to fermentation at 43 °C. For the manufacture of probiotic (PRO, SYM, and PWC) formulations, *Lactobacillus acidophilus* strain (LA-5®; Chr. Hansen, Valinhos, Brazil) was inoculated at a concentration of 5% (v/v) of the total milk volume; whereas, treatments with prebiotic (PRE and SYM) 5% (w/v) of inulin (Ingredients & Systems Biotechnology, São Paulo, SP, Brazil) were added. In addition, 10% (w/v) of cupuassu pulp (Polpa de Fruta, Macapá, AP, Brazil) was added to the treatments containing cupuassu (CUP and PWC). Two aliquots of 80 mL were collected every thirty minutes during the fermentation period: one to evaluate the pH and the other was stored at -20 ºC for analysis of carbohydrates and organic acids content. A pH value of 4.6 [6] was considered as the end point of fermentation. The goat milk yogurts were individually packed in flasks of 80 mL and stored at 4 °C for one day. The whole yogurt processing was repeated three times (n = 3).

2.3 Sample extraction

Carbohydrates (lactose, galactose and glucose) and organic acids (formic, citric and lactic) from goat milk and goat milk yogurts were extracted following method described by González et al. [2] with modifications. Briefly, aliquots of 1 g of each sample were

individually homogenized with 5 mL of 45 mmol.L^{-1} H_2SO_4 for 1 min in a vortex (Certomat® MV, B. Braun Biotech International, Melsungen, Germany) at 2500 rpm. Then, the solution was stirred for 30 min in a multi-purpose rotator (TS – 2000 A VDRL shaker, Biomixer®, São Paulo, Brazil) at 240 rpm following another 1 min in vortex. The homogenates were centrifuged at 5,500 × *g* for 20 min at 4 ºC (Sorvall ST16R, Thermo Scientific, São Paulo, Brazil). The supernatant was initially filtered through Whatman no. 1 filter paper, then passed through a 0.45 µm pore size membrane (PVDF, Millipore, Brazil) filter, and finally stored at 4 ºC until analysis.

2.4 Chromatographic conditions

The chromatographic system consisted of a LC-20AT pump integrated with CBM-20A controller, and SPD-M20A diode array detector in-line with RID-10A refractive index serial detector (Shimadzu, Kyoto, Japan). Carbohydrates and organic acids were separated on an Aminex HPX-87H column (300 × 7.8 mm, 9 µm particle size, 8% cross linkage and pH range of 1—3; Bio-Rad, Hercules, CA, USA) utilizing a 3 mmol.L^{-1} sulfuric acid aqueous mobile phase (pH 2.35±0.02) under isocratic conditions. Aliquots of 20 µL (injection volume) were chromatographically separated at 0.5 mL.min^{-1} (flow rate), at a constant 60 °C (column temperature). The total run time was 30 min in which the last analyte eluted at 15.8 min, and the other 14 minutes were used to equilibrate the detectors for the next injection. The wavelength for organic acid detection was set at 210 nm. An aqueous solution of 30 mmol.L^{-1} sulfuric acid (pH 1.66±0.01) was run for 10 min every three samples to flush the HPLC system.

2.5 Validation parameters

The method for the analysis of carbohydrates and organic acids in NAT yogurt was validated in terms of analytical parameters of selectivity, linearity, precision, recovery, limit of detection, limit of quantification, decision limits, detection capabilities, robustness and stability following conventional protocols from international guidelines [6–10]. Carbohydrates and organic acids were identified by comparing the chromatographic retention time of individual peaks with their respective commercial standards. Whereas the quantification was performed based on the external standard method utilizing refractive

index and absorbance at 210 nm to estimate the contents of each individual carbohydrate and organic acid, respectively. Calibration curves were plotted with ten different concentrations of each individual standard.

Selectivity was evaluated by comparing the detectors response between working solutions of standard analytes in water at different concentrations (25, 5.0, 1.0 and 0.125 $mg.mL^{-1}$) and NAT yogurts spiked with the same working solutions at the aforementioned concentrations.

To determine the linearity of the HPLC method, NAT samples were fortified with standard solutions at 10 different concentrations of each standard in six replicates. The concentrations used were: 66, 33, 16.5, 8.2, 4.12, 2.06, 1.03, 0.51, 0.28 and 0.13 $mg.mL^{-1}$ of lactose; 56, 28, 14, 7, 3.5, 1.75, 0.87, 0.44, 0.21 and 0.11 $mg.mL^{-1}$ of lactic acid; 40, 20, 10, 5, 2.5, 1.25, 0.62, 0.31, 0.15 and 0.078 $mg.mL^{-1}$ of galactose and glucose; 30, 15, 7.5, 3.75, 1.87, 0.94, 0.47, 0.23, 0.11 and 0.06 $mg.mL^{-1}$ of citric and formic acids. The linearity of the calibration curve was determined by linear regression analysis. The correlation coefficient was evaluated, and the significance of the slope of the curve was verified.

Two levels of precision were evaluated: (1) repeatability (intra-day analyses, same operator and instrumental calibration) and (2) within-laboratory reproducibility (intermediate precision). (1) The repeatability was established utilizing NAT yogurt spiked with mix standards (lactose, galactose, glucose, and formic, citric and lactic acids) at aqueous solutions at four different concentrations (solutions A, B, C, and D). Solution A contained each analyte at 1.0 $mg.mL^{-1}$; solution B at 0.5 $mg.mL^{-1}$; solution C at 0.25 $mg.mL^{-1}$; and solution D at 0.125 $mg.mL^{-1}$. Each NAT yogurt spiked with mix standards were injected ten times and expressed as the mean, standard deviation (SD), and relative standard deviation (RSD). (2) The within-laboratory reproducibility was estimated utilizing NAT yogurt extracted in triplicate and injected in quintuplicate by three different analysts. This procedure was expressed in the same way as repeatability.

The limits of detection (LOD) and quantitation (LOQ) goat milk samples were spiked with increasingly diluted solutions of each individual analyte to a minimum signal-to-noise (S/N) ratio of 3 and 10, respectively. Five injections of each solution were performed to confirm the S/N values. In addition, decision limits (CC_α) and detection capabilities (CC_β) values were estimated according to European Commission Decision 2002/657/EC [8]. CC_α was calculated as the minimum concentration required performance level for each analyte plus 2.33 fold the within-laboratory reproducibility standard

deviation calculated at this level. CC_β was calculated as CC_α plus 1.64 fold the within-laboratory reproducibility standard deviation [8].

Recovery was estimated by standard addition procedure with four different concentrations (25.0, 5.0, 1.0 and 0.125 $mg.g^{-1}$) of each analyte utilizing the following equation $R = [(C_1 - C_2) / C_3] \times 100$. Where R represents the recovery rate (%), C_1 represents the data obtained from NAT yogurt samples fortified with standard solution of carbohydrates and organic acids, C_2 data from NAT yogurt samples, and C_3 data from standard solution of carbohydrates and organic acids.

The robustness of the method was estimated by evaluating the effect of three parameters: mobile phase (2, 3 and 4 $mmol.L^{-1}$ sulfuric acid); flow rate (0.4, 0.5, and 0.6 $mL.min^{-1}$); and homogenization time in multi-purpose rotator (25, 30 and 35 min). For this purpose, natural goat milk yogurt fortified with mix solution of all the six analytes at 5.0 $mg.mL^{-1}$ each, was used.

The stability of the stock solutions and processed samples was carried out by FDA guidelines [26]. The stock solution and processed sample stability was assessed during nine days at 4 °C. Injections of the samples were performed every three days until the ninth day (0, 3, 6 and 9 days).

2.6 Method application

Goat milk yogurts (NAT, PRO, PRE, SYM, CUP and PWC) were analyzed for carbohydrates and organic acids contents during the fermentation period (every thirty minutes interval totaling nine points). In addition, pH analyses [6] were evaluated in the same period, using a digital pHmeter (pH Model PG1800, Cap Lab®, SP, Brazil).

2.7 Statistical Analysis

The physicochemical parameters were analyzed using XLSTAT software (version 2013.2.03; Addinsoft, Paris, France) by one-way ANOVA, and when difference amongst the means were detected, Tukey's test at 95% of confidence level ($P < 0.05$) was performed. In addition, linear regression analysis was utilized to evaluate linearity, where the significance of the regression curves coefficients (intercept and slope) was investigated at 95% of confidence ($P < 0.05$).

3. Results and discussion

3.1 Method validation

The performance parameters of the proposed method were adequate to detection and quantification of the carbohydrates and organic acids in NAT goat milk yogurt (Fig 1). The selectivity was evaluated by visualizing the calibration curves of carbohydrates and organic acids standards and goat milk yogurt spiked with these standards (Fig. 2), which are parallel, well-adjusted coefficient of determination, suggesting that was not significant matrix interference. The mean retention times in minutes were 9.187 ± 0.052 for lactose; 10.799 ± 0.088 for glucose; 11.477 ± 0.031 for galactose; 9.112 ± 0.068 for citric acid; 14.668 ±0.052 for lactic acid; and 15.838 ± 0.046 for formic acid. By comparing the chromatograms of the samples with the addition of the 25 $mg.mL^{-1}$ standard solution of the six analytes (Fig. 1C) to the samples (Fig. 1B), it was observed that when carbohydrates and organic acids were added to the matrix, detection occurred at the same retention time as when them were added to water (Fig. 1A), exhibiting satisfactory resolution. Hence, because the peaks associated with the six analytes could be distinguished from the other compounds detected in the goat milk yogurt and the retention times were the same in matrix and standard solution, the method was considered selective [6,7,9,10].

The regression equations and the coefficient of determination (R^2) for the analyses of carbohydrates and organic acids added to NAT goat milk yogurt, obtained in the evaluation of linearity in the range from 0.06 to 66.0 $mg.g^{-1}$, are represented in Table 1. The values indicate that the model is adequate, given that the coefficient of determination of the analytical curves was greater than 0.99, which is an evidence of indicating that a fit of the regression equation is well fitted to the experimental data to the regression line. In addition, the curves were considered linear (R^2 at 95% of significance) for all the evaluated analytes. According to the criteria of the European, Brazilian legislations and FDA, values higher than 0.990, 0.990 and 0.995, respectively, are recommended for the linearity tests [5,8,9].

The precision of our methodology was expressed in terms of the repeatability and within-laboratory reproducibility relative standard deviation (RSD) [24]. The repeatability assesses the precision under the same operating conditions within a short period and would

be equivalent to within-run precision. While, intermediate precision (within-laboratory reproducibility) considers the within laboratory variations (different days, analysts or instrumentation) and would be equivalent to between-run precision [8,9]. The present technique exhibited RSD values (Table 2 and 3) in conformity with the criteria established by ANVISA [9] and FDA [5], in which RSD should not exceed 5 and 20%, respectively, to be considered for a bioanalytical analysis. Therefore, the precision of the HPLC-DAD-RI exhibited satisfactory results for all the six analytes studied.

LODs and LOQs values of the present method ranged from 0.001 to 0.003 $\mu g.mL^{-1}$ and from 0.003 to 0.013 $\mu g.mL^{-1}$, respectively (Table 4). These values were lower than those presented by Milagres [19] for lactic acid in bovine milk.

The CCα and CCβ values are reported in Table 4. These parameters allow the assessment of the critical concentration above which the method reliably distinguishes and quantifies an analyte, taking into account the variability of the method and the statistical risk of making a wrong decision [8,20]. CCα and CCβ results ranged from 0.035 to 0.943 $\mu g.mL^{-1}$ and from 0.053 to 1.604 $\mu g.mL^{-1}$, respectively, for each carbohydrate and organic acid, indicating that the HPLC-DAD-RI technique is reliable.

Recovery measures the ability of a method to extract an analyte from a biological matrix. It is important to point out that there is no lower limit value for recovery since a bioanalytical method with a low recovery could be suitable for a certain analyte if the sensitivity of the detection is high enough [5,6,10]. In our study, recovery values are reported at each fortification level ranged from 78 to 109% (Table 4). All recovery values satisfy the performance criteria of acceptable limits of 70–110% as recommended by European Commission [8].

Robustness estimates the ability of a given method to provide reliable results despite variations on the analytical conditions. Although, this parameter is not addressed on several bioanalysis validation guidelines, it is important to consider this parameter in order to guarantee the good performance of routine analysis [21,22]. The changes in mobile phase, flow rate and homogenization time of analysis did not compromise the determination of the levels of carbohydrates and organic acids in the NAT goat milk yogurt (Table 5).

Regarding stock solutions stability, the results confirmed that the solutions were stable during the nine days. Furthermore, the processed sample were stable for five days in

refrigeration at 4 °C, in which no changes ($P > 0.05$) were observed in the content of carbohydrates and organic acids.

3.2 Method application

3.2.1 Carbohydrates

The analytical methodology validated on this study was applied to estimate contents of carbohydrates and organic acids on goat milk yogurts added with cupuassu pulp (CUP and PWC), probiotic (PRO and SYM) and prebiotic (PRE and SYM) during fermentative process (Table 6). In goat whole milk, the values of carbohydrates and organic acids, in $mg.g^{-1}$ were: 58.923 ± 1.042 for lactose, 0.323 ± 0.014 for glucose, 0.113 ± 0.027 for galactose, 3.987 ± 0.016 for citric acid, 0.583 ± 0.026 for lactic acid and 0.250 ± 0.009 for formic acid. These contents are in agreement with other studies [3,23–25]; lactose and citric acid are, the major carbohydrate and organic acid in milk, respectively [18].

Amongst the carbohydrates investigated, all treatments exhibited a similar trend for lactose content: the longer the fermentation period the lower ($P < 0.05$) the lactose content. The inclusion of inulin and cupuassu pulp probably favored the bacterial metabolism, since PRE, SYM, CUP and PWC treatments exhibited a sharp slope on lactose content. In addition, these aforementioned treatments demonstrated a decrease ($P < 0.05$) on this carbohydrate content at 30 minutes of fermentation while PRO and NAT yogurts were, respectively, at 60 and 150 minutes. Furthermore, at the end of fermentation, CUP and PWC yogurts presented the lowest ($P < 0.05$) values for lactose, while PRE and SYM the highest ($P < 0.05$) ones. Although lactose is considered the most common substrate for lactobacilli metabolism, some strains can metabolize oligosaccharides and the long-chain inulin [26]. This fact may explain the higher lactose content at the end of the fermentation period to treatments added with inulin (PRE and SYM), once this could be a substrate for the bacteria.

During fermentation lactose is readily hydrolyzed into galactose and glucose by Group N streptococci enzymatic apparatus. In the homofermentative pathway, also known as Embden-Meyerhoff-Parnas pathway, glucose is metabolized into pyruvate. Posteriorly, pyruvate is used directly as an H-acceptor, and two moles of lactate are formed per glucose

molecule [26]. Therefore, this intricate role of glucose on energy metabolism potentially explains the observed fluctuating behavior ($P < 0.05$) in all treatments. In terms of galactose, the yogurt bacteria lack the essential enzymes involved on this carbohydrate metabolism. In addition, Kaminarides et al [27] also documented an increase ($P < 0.05$) on galactose and a decrease on lactose contents. On PRE, SYM, CUP and PWC treatments at 30 min of fermentation period galactose content exhibited an increase ($P < 0.05$) while lactose content a decrease ($P < 0.05$). Whereas, NAT and PRO demonstrated similar data pattern as the other treatments at 60 min of fermentation.

3.2.2 Organic acids

In yogurts, these compounds are derived mainly from bacterial metabolism during fermentation and storage periods. Lactic acid is the most abundant organic acid found in yogurt [16], and, on average its level on goat milk yogurt is 4.75 $mg.g^{-1}$ (Table 6). In addition, several species of lactic acid bacteria metabolize carbohydrates into trace amounts of acetic acid, formic acid, and ethanol by homofermentative metabolic pathway [28].

Concerning lactic acid content, all treatments (NAT, PRO, PRE, SYM, CUP and PWC) exhibited an increase ($P < 0.05$) throughout the whole fermentation period. While PWC lactic acid content values increased ($P < 0.05$) at the first 60 min, the other treatments documented such increase ($P < 0.05$) only at 120 min of fermentation. In addition, at the end of the fermentation period NAT yogurt presented the greater ($P < 0.05$) lactic acid content than PRO, PRE, SYM, CUP, and PWC.

Furthermore, citric and formic acids contents fluctuated ($P < 0.05$) during fermentation, suggesting production and consumption of such organic acids by bacterial metabolism. Citric acid is the predominant organic acid in milk [18] and its promotion of refreshing taste [29]. According to Güzel-Seydim et al. [30], citric acid is the main substrate for acetoin and diacetyl formation by some lactic acid bacteria. Moreover, the formic acid is potentially associate with the shift of metabolic pathway from homolactic to mix-acid fermentation [31].

3.2.3 pH

All treatments exhibited lower ($P < 0.05$) pH values at the end of the fermentation period than at the beginning (Figure 3). Therefore, the final pH (4.6) in all goat's milk yogurt are in line with the growth of the starter culture and probiotic bacteria [18] and production of lactic acid (Table 6). In addition, CUP and PWC exhibited an initial pH values lower ($P < 0.05$) than the other treatments; resulting on the reduction of fermentation time (210 min) required to reach the end pH value of 4.6. This difference is potentially explained by the inherent acidity of cupuassu pulp indicated by an acidic pH value of 3.4 [16]. The observed decrease on the pH values are in agreement with the growth of the starter culture and probiotic bacteria [18] and production of lactic acid (Table 6).

This pH decline is related to lactose fermentation into mainly lactic acid shifting the pH to a more acidic value [32]. Furthermore, the increase on the organic acids content (Table 6) supports the decrease on the pH values due to carbohydrate bacterial fermentation [3, 34]. Several studies reported that starter bacteria metabolism exert influence on pH values of fermented milk beverages due lactose hydrolysis with subsequent organic acids production [1,25,26].

4. Conclusion

The present HPLC-DAD-RI method demonstrated to be specific, linear, precise, accurate and robust within the validated range for the simultaneous determination of lactose, glucose, galactose, and citric, lactic and formic acids in goat milk yogurts. The method was successfully applied to monitor the fermentation period on different goat milk yogurts, exhibiting that lactic acid was the major organic acid produced in yogurts manufactured. We conclude that the proposed HPLC method can be utilized conveniently in yogurts analysis for monitoring and routine quality control.

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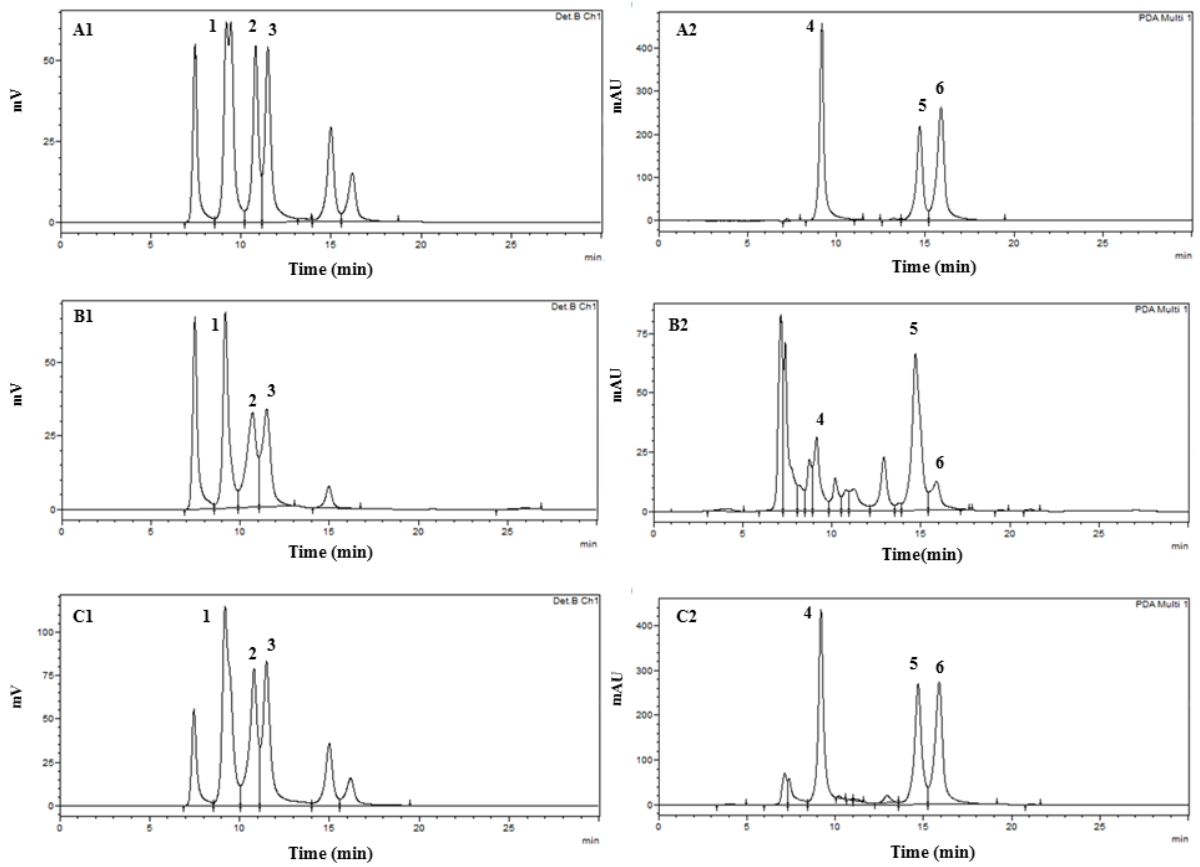

Figure 1. Typical chromatograms of carbohydrates (A1, B1 and C1) and organic acids (A2, B2 and C2): (A) standard mixture of individual compounds at concentrations of 25mg.mL^{-1}; (B) natural goat milk yogurt; (C) natural goat milk yogurt fortified with 25mg.mL^{-1} of each analyte. Numbers over chromatographic peaks indicate lactose (1), glucose (2), galactose (3) citric acid (4), lactic acid (5), and formic acid (6).

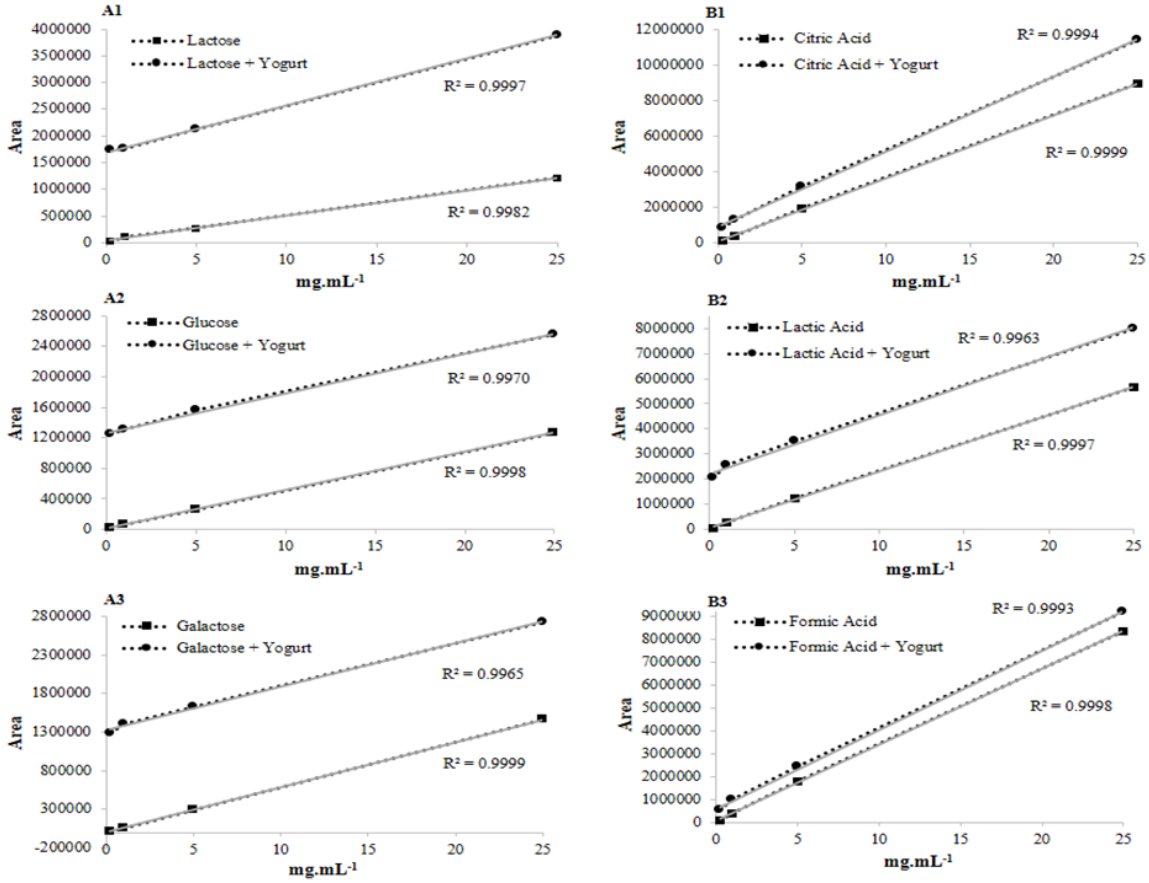

Figure 2. Matrix-fortified and direct standard calibration curves for the evaluation of the matrix effect on carbohydrates (lactose, glucose and galactose) and organic acids (citric, lactic and formic) contents.

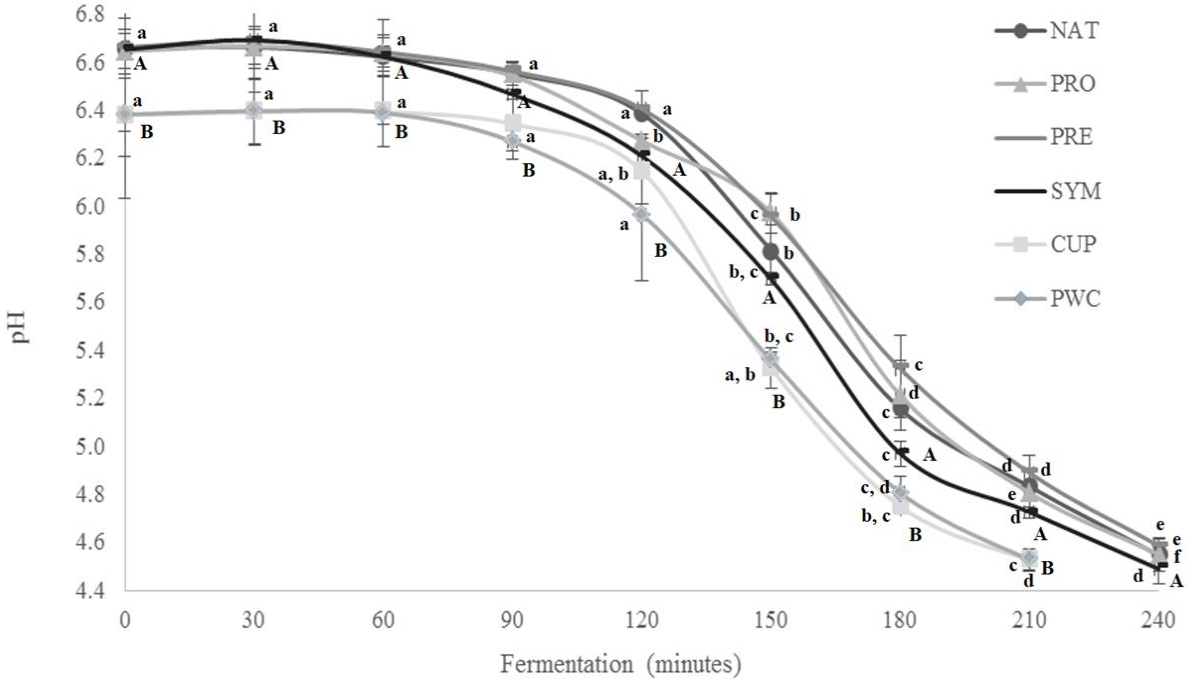

Figure 3. Averages of the pH for different goat's milk yogurts during the fermentation process. Natural (NAT), probiotic (PRO), prebiotic (PRE), symbiotic (SYM), cupuassu (CUP), and probiotic with cupuassu (PWC) goat's milk yogurts. [a-f] Different letters indicate differences among fermentation time ($P < 0.05$). [A-B] Different letters indicate differences among goat's milk yogurts ($P < 0.05$).

Table 1. Linearity of the HPLC-DAD-RI method for simultaneous analysis of carbohydrates and organic acids contents, obtained from ten different analyte concentrations between 0.06 and 60 $mg.mL^{-1}$.

Parameter		Regression equation	Coefficient of Determination (R^2)
Carbohydrates	**Lactose**	y = 45305x -1273.4	0.9952
	Glucose	y = 325728x + 2561.4	0.9979
	Galactose	y = 40611x + 4047.9	0.9981
Organic acids	**Citric**	y = 2000000x + 3379.9	0.9962
	Lactic	y = 566934x + 29264	0.9965
	Formic	y = 535005x +380614	0.9991

Table 2. Data obtained from the repeatability evaluation of the HPLC-DAD-RI method for simultaneous analysis of carbohydrates and organic acids evaluated with four different analyte concentrations (0.125, 0.25, 0.5 and 1.0 $mg.mL^{-1}$) in a standard mix solution.

Parameter		RT	Concentration ($mg.mL^{-1}$)							
			0.125		0.25		0.5		1.0	
			Mean±SD	RSD	Mean±SD	RSD	Mean±SD	RSD	Mean±SD	RSD
Carbohydrates	Lactose	9.187	0.120±0.014	0.021	0.265±0.006	0.023	0.508±0.010	0.021	0.993±0.013	0.013
	Glucose	10.799	0.124±0.004	0.029	0.252±0.004	0.018	0.500±0.008	0.017	1.000±0.020	0.019
	Galactose	11.477	0.124±0.003	0.030	0.250±0.003	0.014	0.499±0.008	0.016	1.001±0.011	0.011
Organic acids	Citric	9.112	0.123±0.001	0.011	0.250±0.003	0.012	0.503±0.007	0.015	0.999±0.005	0.005
	Lactic	14.668	0.123±0.004	0.030	0.251±0.007	0.029	0.502±0.013	0.027	0.999±0.009	0.009
	Formic	15.838	0.124±0.003	0.023	0.253±0.009	0.035	0.498±0.010	0.021	1.001±0.009	0.009

SD: standard deviation

RSD: relative standard deviation

Table 3. Data obtained from the within-laboratory reproducibility evaluation of the HPLC-DAD-RI method for simultaneous analysis of carbohydrates and organic acids performed by three different analysts.

Parameter		Analyst 1		Analyst 2		Analyst 3	
		Mean±SD	**RSD**	**Mean±SD**	**RSD**	**Mean±SD**	**RSD**
Carbohydrate	**Lactose**	44.993±0.102[a]	0.002	44.590±0.261[a]	0.006	45.147±0.847[a]	0.019
	Glucose	0.055±0.021[a]	0.038	0.071±0.001[a]	0.015	0.049±0.019[a]	0.040
	Galactose	8.503±0.361[a]	0.042	8.683±0.037[a]	0.004	8.528±0.157[a]	0.018
Organic acid	**Citric**	0.360±0.010[a]	0.029	0.368±0.019[a]	0.052	0.367±0.010[a]	0.027
	Lactic	7.649±0.011[a]	0.001	7.623±0.038[a]	0.005	7.550±0.271[a]	0.036
	Formic	1.488±0.009[a]	0.006	1.455±0.035[a]	0.024	1.443±0.079[a]	0.055

SD: standard deviation

RSD: relative standard deviation

Table 4. Data obtained from the evaluation of recovery, limit of detection (LOD), limit of quantification (LOQ), decision limit (CC_α), and detection capability (CC_β) of the optimized liquid-chromatography method.

Parameter	**Carbohydrates**			**Organic Acids**		
	Lactose	**Glucose**	**Galactose**	**Citric**	**Lactic**	**Formic**
LOQ (µg.mL^{-1})	0.013	0.011	0.008	0.003	0.006	0.003
LOD (µg.mL^{-1})	0.003	0.003	0.002	0.001	0.003	0.002
CC_α (µg.mL^{-1})	0.943	0.035	0.433	0.032	0.251	0.097
CC_β (µg.mL^{-1})	1.604	0.057	0.736	0.053	0.426	0.164
Recovery (%)						
0.125 (mg.mL^{-1})	97	81	105	95	103	104
1.0 (mg.mL^{-1})	105	85	109	105	106	102
5.0 (mg.mL^{-1})	96	78	107	104	106	97
25.0 (mg.mL^{-1})	98	81	103	106	109	89

LOQ: limit of quantification.

LOD: limit of detection.

CC_α: decision limit.

CC_β: detection capability.

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Table 5. Data obtained from the evaluation of the robustness of the HPLC-DAD-RI method for simultaneous analysis of carbohydrates and organic acids with variations in mobile phase concentration, flow rate, and homogenization time.

Parameters	Carbohydrates			Organic acids		
	Lactose*	**Glucose***	**Galactose***	**Citric***	**Lactic***	**Formic***
Mobile phase						
2 mmol.L^{-1} H_2SO_4	43.315±0.018[a]	0.086±0.103[a]	7.325±0.015[a]	0.566±0.073[a]	5.943±0.046[a]	0.174±0.057[a]
3 mmol.L^{-1} H_2SO_4	43.310±0.010[a]	0.087±0.042[a]	7.327±0.001[a]	0.574±0.066[a]	5.745±0.068[a]	0.202±0.025[a]
4 mmol.L^{-1} H_2SO_4	43.525±0.042[a]	0.089±0.004[a]	7.363±0.117[a]	0.568±0.222[a]	5.858±0.010[a]	0.182±0.005[a]
Flow rate						
0.4 mL.min^{-1}	40.761±0.138[a]	0.058±0.022[a]	7.158±0.462[a]	0.333±0.069[a]	5.259±0.037[a]	0.176±0.014[a]
0.5 mL.min^{-1}	43.012±0.178[a]	0.064±0.133[a]	7.104±0.166[a]	0.340±0.067[a]	5.156±0.067[a]	0.179±0.020[a]
0.6 mL.min^{-1}	40.175±0.191[a]	0.069±0.008[a]	7.297±0.035[a]	0.329±0.002[a]	5.246±0.025[a]	0.181±0.015[a]
Homogenization time						
25 min	40.490±0.099[a]	0.087±0.002[a]	6.505±0.095[a]	0.328±0.010[a]	5.284±0.039[a]	0.167±0.001[a]
30 min	40.322±0.043[a]	0.085±0.001[a]	6.319±0.013[a]	0.349±0.060[a]	5.404±0.006[a]	0.153±0.447[a]
35 min	40.328±0.019[a]	0.084±0.032[a]	6.325±0.011[a]	0.316±0.054[a]	5.166±0.055[a]	0.159±0.023[a]

* Results expressed as mean ± SD (Standard deviation).

[a] Different letters in columns within a parameter represent significantly different averages ($P < 0.05$)

Table 6. Carbohydrate and organic acids values (means ± standard deviation) in $mg.g^{-1}$ of goat milk yogurts during the fermentation period.

Parameter	Treatment	Time points during fermentation period (min)								
		0	30	60	90	120	150	180	210	240
Lactose	NAT	56.729±0.105^{a,B}	55.949±0.665^{a,B}	55.301±0.09^{a,A}	56.296±0.016^{a,A}	54.953±0.031^{a,A}	49.893±0.333^{b,A}	47.288±1.329^{bc,A}	45.660±0.106^{cd,A}	40.164±0.008^{d,C}
	PRO	58.589±0.237^{a,A}	58.669±0.278^{a,A}	54.944±0.475^{b,A}	54.073±1.049^{bc,A}	52.934±0.669^{c,B}	48.403±0.726^{d,AB}	44.644±1.401^{e,B}	42.057±0.066^{f,CD}	40.774±0.536^{g,B}
	PRE	58.263±0.883^{a,A}	53.126±0.630^{b,C}	49.197±0.565^{c,A}	48.270±0.405^{cd,B}	47.961±0.568^{d,CD}	47.277±0.724^{d,BC}	45.391±0.437^{e,AB}	43.895±0.815^{f,B}	41.840±0.076^{g,A}
	SYM	57.881±0.585^{a,A}	55.375±0.817^{b,B}	53.311±0.839^{c,AB}	53.656±2.032^{bc,A}	47.227±0.471^{d,D}	46.273±0.471^{d,C}	42.085±0.359^{e,C}	40.710±1.188^{ef,D}	42.118±0.305^{f,A}
	CUP	54.076±0.414^{a,C}	50.589±0.436^{b,D}	50.356±0.419^{b,B}	50.466±1.046^{b,B}	49.463±1.054^{cb,C}	47.687±1.280^{cd,BC}	45.376±0.028^{d,AB}	39.149±0272^{e,E}	-
	PWC	54.191±0.528^{ab,C}	53.629±0.616^{b,B}	52.889±1.067^{b,AB}	50.710±1.269^{c,B}	49.010±0.487^{cd,CD}	48.578±0.748^{de,AB}	46.752±1.062^{e,AB}	39.219±0.571^{f,E}	-
Glucose	NAT	0.004±0.032^{b,E}	0.228±0.029^{ab,B}	0.287±0.429^{ab,B}	0.565±0.054^{a,D}	0.066±0.003^{b,E}	0.003±0.001^{b,D}	0.016±0.002^{b,C}	0.013±0.001^{b,B}	0.004±0.001^{b,D}
	PRO	0.053±0.017^{c,E}	0.073±0.002^{b,B}	0.027±0.003^{d,B}	0.036±0.005^{d,E}	0.127±0.005^{a,D}	0.002±0.001^{e,D}	0.013±0.001^{e,C}	0.002±0.001^{e,B}	0.084±0.002^{b,C}
	PRE	0.623±0.039^{cd,D}	0.044±0.002^{e,B}	0.853±0.093^{bc,A}	1.132±0.043^{b,A}	1.454±0.043^{a,B}	0.411±0.014^{d,A}	0.747±0.057^{c,A}	0.769±0.004^{c,A}	0.379±0.040^{d,A}
	SYM	0.900±0.0049^{a,B}	0.866±0.019^{b,A}	0.116±0.024^{g,B}	0.971±0.004^{a,B}	0.017±0.004^{h,F}	0.426±0.010^{e,A}	0.782±0.014^{c,A}	0.613±0.053^{d,C}	0.327±0.035^{f,B}
	CUP	1.768±0.014^{a,A}	0.204±0.011^{f,B}	0.938±0.109^{c,A}	0.627±0.027^{d,C}	2.938±0.027^{b,A}	0.352±0.012^{e,B}	0.627±0.026^{d,B}	0.367±0.032^{d,C}	-
	PWC	1.770±0.023^{b,A}	0.997±0.016^{a,A}	1.036±0.105^{a,A}	0.649±0.016^{c,C}	0.328±0.001^{d,C}	0.069±0.006^{e,C}	0.649±0.012^{c,B}	0.620±0.039^{c,C}	-
Galactose	NAT	0.118±0.001^{g,D}	0.021±0.010^{g,E}	0.846±0.077^{f,E}	0.741±0.030^{f,F}	1.572±0.045^{e,F}	3.358±0.074^{d,F}	4.492±0.096^{c,D}	6.490±0.018^{b,E}	7.059±0.073^{a,C}
	PRO	0.355±0.058^{g,D}	0.604±0.003^{g,E}	2.159±0.024^{f,D}	2.620±0.048^{e,E}	2.711±0.047^{e,E}	4.410±0.018^{d,E}	4.832±0.053^{c,D}	7.092±0.110^{b,D}	8.209±0.002^{a,B}
	PRE	3.936±0.571^{g,B}	1.968±0.058^{f,C}	5.311±0.076^{e,A}	7.587±0.321^{d,A}	9.803±0.594^{c,A}	10.750±0.017^{b,A}	10.709±0.113^{b,A}	11.218±0.060^{b,A}	10.532±0.674^{a,A}
	SYM	1.470±0.041^{f,C}	2.473±0.066^{e,B}	2.787±0.088^{e,C}	6.029±0.027^{d,C}	8.543±0.037^{c,B}	9.047±0.027^{c,B}	10.350±0.093^{b,A}	10.769±0.048^{b,A}	11.444±0.035^{a,A}
	CUP	1.100±0.057^{g,A}	1.219±0.051^{f,D}	4.463±0.119^{e,B}	6.775±0.119^{d,B}	6.863±0.015^{d,C}	7.705±0.029^{c,C}	8.635±0.134^{b,B}	9.053±0.146^{a,B}	-
	PWC	1.994±0.033^{e,B}	4.059±0.038^{d,A}	4.202±0.085^{d,B}	4.774±0.053^{c,D}	4.402±0.059^{cd,D}	5.955±0.497^{b,D}	6.306±0.047^{b,C}	7.654±0.020^{a,C}	-
Citric acid	NAT	0.330±0.024^{b,E}	0.594±0.003^{ab,D}	0.472±0.040^{ab,C}	0.498±0.037^{ab,C}	0.439±0.085^{ab,B}	0.772±0.181^{a,C}	0.482±0.006^{ab,A}	0.439±0.037^{ab,A}	0.363±0.025^{b,BC}
	PRO	0.412±0.008^{d,D}	0.608±0.006^{c,C}	0.597±0.016^{c,B}	0.673±0.008^{b,B}	0.765±0.008^{a,AB}	0.750±0.057^{a,C}	0.416±0.045^{d,ABC}	0.419±0.002^{d,A}	0.388±0.020^{d,AB}
	PRE	0.344±0.02^{e,E}	0.577±0.009^{b,E}	0.635±0.042^{b,B}	0.661±0.083^{b,B}	0.983±0.083^{a,A}	0.763±0.007^{b,C}	0.448±0.066^{d,AB}	0.411±0.011^{de,A}	0.332±0.01^{e,C}
	SYM	0.483±0.003^{e,C}	0.577±0.003^{d,E}	0.641±0.009^{c,B}	0.117±0.014^{h,D}	1.071±0.014^{a,B}	0.737±0.012^{b,C}	0.386±0.004^{f,BC}	0.348±0.024^{g,A}	0.420±0.043^{f,A}
	CUP	1.067±0.020^{a,A}	0.891±0.004^{a,B}	0.883±0.033^{a,A}	0.737±0.043^{ab,B}	0.414±0.095^{b,A}	1.101±0.021^{a,B}	0.379±0.060^{b,BC}	0.891±0.054^{a,A}	-
	PWC	0.712±0.007^{ac,B}	0.942±0.007^{a,A}	0.892±0.078^{a,A}	0.856±0.109^{a,A}	0.980±0.009^{ab,A}	1.396±0.051^{a,A}	0.349±0.009^{c,C}	0.670±0.053^{c,A}	-

Lactic acid	**NAT**	0.054±0.012^{e,C}	0.106±0.005^{e,B}	0.150±0.031^{e,B}	0.103±0.010^{e,D}	0.205±0.007^{e,D}	0.894±0.021^{d,B}	2.345±0.021^{c,A}	3.527±0.013^{b,B}	5.186±0.063^{a,A}
	PRO	0.048±0.009^{e,CD}	0.098±0.041^{e,B}	0.154±0.028^{e,B}	0.160±0.009^{e,D}	0.737±0.128^{d,A}	1.298±0.034^{c,A}	2.339±0.046^{b,A}	3.116±0.024^{a,BC}	4.593±0.060^{a,B}
	PRE	0.026±0.009^{f,E}	0.101±0.016^{ef,B}	0.138±0.029^{ef,B}	0.127±0.011^{ef,D}	0.223±0.056^{e,D}	0.463±0.004^{d,D}	1.579±0.104^{c,C}	3.446±0.046^{b,B}	4.891±0.020^{a,AB}
	SYM	0.031±0.012^{d,DE}	0.124±0.012^{d,B}	0.124±0.005^{d,B}	0.428±0.054^{c,B}	0.585±0.062^{c,B}	0.524±0.035^{c,C}	2.196±0.042^{b,B}	2.400±0.093^{b,C}	4.676±0.069^{a,B}
	CUP	0.168±0.008^{de,B}	0.143±0.012^{e,B}	0.188±0.010^{de,B}	0.239±0.026^{de,C}	0.486±0.020^{d,C}	1.386±0.065^{c,A}	2.085±0.052^{b,B}	4.868±0.150^{a,A}	-
	PWC	0.522±0.006^{cd,A}	0.486±0.081^{cd,A}	0.398±0.061^{d,A}	0.496±0.043^{cd,A}	0.654±0.016^{c,AB}	0.689±0.026^{c,BC}	1.170±0.073^{b,D}	5.069±0.031^{a,A}	-
Formic acid	**NAT**	0.039±0.017^{e,B}	0.089±0.029^{d,AB}	0.083±0.009^{d,BC}	0.078±0.015^{de,C}	0.155±0.014^{c,AB}	0.241±0.026^{b,D}	0.267±0.013^{b,A}	0.336±0.029^{a,A}	0.239±0.020^{b,A}
	PRO	0.031±0.019^{d,BC}	0.112±0.014^{bc,A}	0.110±0.038^{bc,AB}	0.080±0.006^{cd,B}	0.148±0.029^{b,AB}	0.632±0.018^{a,A}	0.049±0.017^{d,C}	0.151±0.026^{b,BC}	0.086±0.054^{cd,B}
	PRE	0.020±0.002^{f,BC}	0.022±0.002^{f,C}	0.160±0.075^{c,A}	0.097±0.013^{de,B}	0.419±0.013^{b,A}	0.606±0.010^{a,A}	0.025±0.003^{f,C}	0.155±0.030^{cd,B}	0.094±0.010^{e,B}
	SYM	0.042±0.002^{b,B}	0.091±0.006^{b,A}	0.087±0.020^{b,B}	0.173±0.004^{b,A}	0.327±0.044^{ab,AB}	0.570±0.011^{a,B}	0.108±0.057^{b,B}	0.106±0.003^{b,CD}	0.114±0.015^{b,B}
	CUP	0.204±0.016^{d,A}	0.063±0.009^{g,B}	0.116±0.010^{f,AB}	0.170±0.009^{e,A}	0.481±0.009^{a,A}	0.404±0.024^{b,C}	0.294±0.024^{c,A}	0.053±0.002^{g,D}	-
	PWC	0.007±0.004^{d,C}	0.016±0.003^{d,C}	0.017±0.001^{d,C}	0.015±0.003^{d,D}	0.026±0.008^{cd,B}	0.058±0.008^{ab,E}	0.046±0.007^{bc,C}	0.073±0.028^{a,DE}	-

Natural (CON), probiotic (PRO), prebiotic (PRE), symbiotic (SYM), cupuassu (CUP), and probiotic with cupuassu (PWC) goat's milk yogurts.

$^{a-g}$ Different letters indicate differences among fermentation time ($P < 0.05$).

$^{A-E}$ Different letters indicate differences among goat's milk yogurts ($P < 0.05$).

"-" The CUP and PWC had no the 240 point of fermentation

3.5 ARTIGO V: EFFECT OF DIFFERENT FAT REPLACERS ON THE PHYSICOCHEMICAL, COLOR, APPARENT VISCOSITY AND TEXTURE PROPERTIES OF LOW-FAT CUPUASSU GOAT MILK YOGURTS SUBMETIDO PARA REVISTA LWT - FOOD SCIENCE AND TECHNOLOGY

Effect of different fat replacers on the physicochemical, color, apparent viscosity and texture properties of low-fat cupuassu goat milk yogurts

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ABSTRACT

The effect of inulin (SI), maltodextrin (SM), whey protein (SW) and skim milk powder (SP) on the physicochemical properties, color, texture and apparent viscosity of low-fat cupuassu goat milk yogurts was firstly studied. All fat replacers improved ($P < 0.05$) the physicochemical properties when compared to whole (W) and skimmed milk (S) yogurts. The addition of each carbohydrates (SI and SM) and proteins (SW and SP) influenced ($P < 0.05$) color of the low-fat cupuassu goat milk yogurt. The SP yogurt presented a higher apparent viscosity than W, S, SI, SM and SW yogurts. Furthermore, only SP yogurt increased ($P < 0.05$) the texture analyses. These results obtained in this study suggest that skim milk powder has the potential to be used to improve apparent viscosity and texture parameters on low-fat cupuassu goat milk yogurt.

Keywords: inulin, maltodextrin, whey protein, skim milk powder, instrumental analysis, syneresis.

1. Introduction

Fermented milks, especially yogurt, are commonly associated with healthy foods, once as food vehicles to deliver probiotics and prebiotics to consumers (Costa, Balthazar, Pinto, Cruz, & Conte Junior, 2013). The goat milk yogurt present high digestibility and nutritional value, as well as its therapeutic and dietary characteristics, intrinsic of goat milk (Park, Juárez, Ramos, & Haenlein, 2007). However, this yogurt presents a lower overall acceptance by unusual consumer (Costa et al., 2014), when compared with cow milk yogurt (Costa et al., 2015a). This is due its unpleasant "goaty" taste and consistency, as it is perceived by consumers, even in goat milk yogurt with added strawberry pulp (Senaka Ranadheera, Evans, Adams, & Baines, 2012). Regarding the consistency, the cupuassu pulp is considered an important technological strategy to improve the goat milk yogurts texture (Costa et al., 2015b).

An alternative to improve the taste of goat milk yogurts with fruit pulp is the use of skim milk in the preparation of them. Once, 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). However, the yogurt from skim goat milk can interfere in the physicochemical, apparent viscosity and texture. Some studies used inulin (Crispín-Isidro, Lobato-Calleros, Espinosa-Andrews, Alvarez-Ramirez, & Vernon-Carter, 2015) and maltodextrin (Hadnad et al., 2014) as fat replacers to stabilize the texture. Other possible solution for improving the apparent viscosity and texture of goat milk yogurts comprehend increasing the total solids content of the milk, such as whey protein (Gauche, Tomazi, Barreto, Ogliari, & Bordignon-Luiz, 2009; Wang, Bao, Hendricks, & Guo, 2012) and skim milk powder (Damin, Alcântara, Nunes, & Oliveira, 2009).

In this context, the aim of the research was to investigate the addition of inulin, maltodextrin, whey protein and skim milk powder on physicochemical, color, apparent viscosity and texture parameters of low-fat cupuassu goat milk yogurts.

2. Materials and methods

2.1 Production of goat milk yogurts

Three batches of each cupuassu goat milk yogurt treatment were prepared as described by Costa et al. (2015b). In all treatments, thermophilic yogurt cultures (1% vol/vol; YF-L903®, Chr. Hansen, Valinhos, Brazil) and cupuassu pulp (10% w/vol; Polpa de Fruta®, Macapá, AP, Brazil) were added in UHT whole goat milk and skimmed goat milk (Caprilat®, Paraná, Brazil). The others ingredients were inulin (5% w/vol; Ingredients & Systems Biotechnology®, São Paulo, SP, Brazil), maltodextrin (5% w/vol; Max Titanium®, São Paulo, SP, Brazil), whey protein isolate (5% w/vol; Optimum Nutrition®, Meridian Lake, Aurora, USA) and skim milk powder (5% w/vol; Glória®, São Paulo, SP, Brazil). A total of 6 treatments of cupuassu goat milk yogurt were performed: whole (W); skimmed (S); skimmed with inulin (SI); skimmed with maltodextrin (SM); skimmed with whey protein (SW); skimmed with skim milk powder (SP). The yogurt mixtures were fermented in an oven at 43°C. The fermentation was interrupted when the pH (AOAC, 2012) reached 4.5 ± 0.1. Finally, the product was packaged in 300 mL glass pots and stored at 4 ± 1°C for 24 hours. All analyses were performed on the 1st day of storage (D1).

2.2 Physicochemical analyses

The cupuassu goat milk yogurts were analyzed for pH by digital potentiometer (model PG1800, Cap Lab, SP, Brazil), protein by the Kjeldahl method using a conversion factor of 6.38, fat content by the Gerber method and moisture by oven drying (AOAC, 2012). Syneresis was determined by weight difference of the supernatant and initial yogurt samples (10 g), after centrifugation at 1500 × *g* for 10 min according Ramírez-Sucre & Vélez-Ruiz (2013) with modification.

2.3. Color, Apparent Viscosity and Texture analyses

Color determinations were made at 5°C by means of a Minolta CM-600D spectrophotometer (Minolta Camera Co., Osaka, Japan). The colorimeter was previously calibrated with illuminant D65 and a 2° standard observer (Costa et al., 2015b).

The apparent viscosities of the yogurts samples (300 mL) were measured at 5 °C using a Brookfield concentric cylinder viscometer (DV3T, Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) equipped with rotor n°. 63, mixing at 60 rpm. The apparent viscosity was measured in triplicate.

Firmness and consistency were measured using a texture analyzer (TA-XT.Plus, Stable Micro Systems Ltd., Surrey, UK) equipped with a 5-kg load cell. The back extrusion cell plunger was 3.6 cm in diameter and set at 20 mm above the sample surface. The test cell penetrated with a distance of 2 cm into the sample (300 mL) at 5°C. Firmness was defined as the maximum force (at the topmost point of the textural profile curve) and expressed in g. Consistency was defined as the area of the curve, calculated by the force value multiplied by the corresponding distance and expressed in g/s.

2.4. Statistical analysis

The data obtained for physicochemical, color, texture and rheological data were analyzed by ANOVA and reported as means (± standard deviations). All ANOVA were subjected to Tukey's test at $P < 0.05$ using XLSTAT version 2013.2.03 (Addinsoft, Paris, France). All the experimental replicate (n=3) was done in triplicate.

3. Results and discussions

3.1 Physicochemical parameters

The results of physicochemical proprieties are exhibited in Table 1. SW and SP had higher protein content ($P < 0.05$) than the others treatments (W, S, SI and SM), where milk-fat was substituted by non-protein milk solids. The W had a higher ($P < 0.05$) fat content than the others treatments (S, SI, SM, SW and SP) (Table 1), which is expected, as it was elaborated from whole milk, whereas the reduced milk-fat yogurts were made from skimmed milk. Treatments with fat replacers (SI, SM, SW and SP) presented lower ($P < 0.05$) moisture than W and S yogurts.

Syneresis was affected ($P < 0.05$) by the addition of fat-substitutes, with exception of SM. However, without the addition of whey protein and skimmed milk powder the syneresis in goat milk yogurts (W, S, SI and SM) were higher than 5.65g/100g. Therefore, the higher water holding capacity for the SW and SP yogurts can be explained due to the greater protein content (Table 1). The initial pH in the W

and S goat milk yogurts were 4.46 and 4.45, respectively, and lower ($P < 0.05$) than that of yogurt containing inulin, maltodextrin, whey protein and skimmed milk powder.

3.2. Color, Apparent Viscosity and Texture analyses

The L^* values were affected ($P < 0.05$) by skimmed milk (S, SI, SM, SW and SP). Moreover, the addition of inulin, maltodextrin and whey protein decreased ($P < 0.05$) this parameter; the milk substitution may influence the opacity level of gels (González-Martınez et al., 2002). The W a^* value was ($P < 0.05$) higher than the treatments with skimmed milk (S, SI, SM, SW and SP). The greenness color of cupuassu goat milk yogurts is explained by the presence of natural pigments, such as carotenoids, originating from cupuassu pulp (Rogez et al., 2004; Costa et al., 2015b). The b^* values was different between all treatments, and the S treatment was less yellow than the other treatments (W, SI, SM, SW and SP). The yellowness of treatments can be attributed to the addition of cupuassu pulp, depends on the type and level of fruit or fiber (Costa et al., 2015b).

For apparent viscosity, the SP yogurt was higher than W, S, SI, SM and SW yogurts ($P < 0.05$). However, the addition of inulin, maltodextrin and whey protein also increased ($P < 0.05$) the apparent viscosity (Table 1). These behaviors can be explained due the aggregation of casein micelles and gel formation that is a consequence of biochemical and physicochemical changes during fermentation of milk (Gaygadzhiev, Corredig, & Alexander, 2009).

Regarding texture analyses (firmness and consistency), only cupuassu goat milk yogurt added with skimmed milk powder (SP) differed ($P < 0.05$) from other treatments. The gel structure is the main texture properties, which results for casein

aggregation (Damin et al., 2009). In addition, other parameters, such as milk base composition and total solids, also perform a determinative role in gel structure formation (Akalın, Unal, Dinkci, & Hayaloglu, 2012).

4. Conclusions

Skim milk powder present potential as fat substitute, which improve the apparent viscosity and texture proprieties of low-fat cupuassu goat milk yogurts. Therefore, skim milk powder can be a technological strategy to dairy industry for goat milk yogurt manufacture with fruit pulp.

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Table 1. Physicochemical, apparent viscosity and texture analyses of different cupuassu goat milk yogurts.

Physicochemical analyses		Samples					
		W	S	SI	SM	SW	SP
Protein (% w/w)		2.37±0.70^{c}	2.62±0.21^{c}	2.32±0.18^{c}	2.23±0.47^{c}	6.84±1.69^{a}	3.98±0.69^{b}
Fat (% w/w)		2.75±0.25^{a}	0.36±0.06^{b}	0.23±0.06^{b}	0.27±0.05^{b}	0.38±0.03^{b}	0.39±0.01^{b}
Moisture (% w/w)		87.98±0.24^{b}	90.36±0.06^{a}	85.86±0.26^{cd}	85.33±0.06^{d}	86.17±0.21^{c}	86.13±0.25^{c}
Syneresis (g/100 g)		5.70±0.16^{c}	7.67±0.17^{a}	6.19±0.19^{b}	5.65±0.09^{c}	5.14±0.16^{a}	5.26±0.30^{d}
pH		4.46±0.02^{cd}	4.45±0.02^{d}	4.52±0.02^{b}	4.55±0.05^{b}	4.61±0.02^{a}	4.51±0.06^{b}
Color	***L****	81.89±0.11^{a}	78.79±0.52^{b}	77.36±0.13^{d}	77.61±0.08^{c}	73.52±0.24^{c}	79.18±0.52^{b}
	a*	-0.99±0.02^{a}	-2.01±0.05^{de}	-2.03±0.04^{e}	-1.59±0.02^{c}	-1.36±0.01^{b}	-1.94±0.11^{d}
	b*	9.45±0.07^{c}	7.80±0.09^{e}	8..26±0.17^{d}	9.72±0.02^{bc}	12.23±0.11^{a}	9.98±0.55^{b}
Apparent Viscosity		321.95±8.72^{d}	157.30±3.23^{e}	445.25±10.13^{c}	417.90±8.44^{c}	672.63±1.43^{b}	1091.77±15.59^{a}
Firmness		33.29±0.88^{b}	37.15±0.25^{b}	36.49±0.78^{b}	39.70±0.55^{b}	34.51±0.07^{b}	56.69±0.85^{a}
Consistency		365.98±0.15^{b}	385.72±0.87^{b}	383.62±0.74^{b}	398.81±0.56^{b}	368.74±0.09^{b}	546.63±0.48^{a}

W – whole cupuassu goat milk yogurt; S – skimmed cupuassu goat milk yogurt; SI – skimmed with inulin cupuassu goat milk yogurt; SM – skimmed with maltodextrin cupuassu goat milk yogurt; SW – skimmed with whey protein cupuassu goat milk yogurt; SP – skimmed with milk powder cupuassu goat milk yogurt.

Values were expressed as mean ± standard deviation.

$^{a-d}$ Different lower case letters in the same line represent significant differences ($p < 0.05$); n = 3.

*L**- lightness; *a** - redness; and *b** - yellowness

4 CONSIDERAÇÕES FINAIS

Em relação aos resultados obtidos nesta tese pode-se concluir que a polpa de cupuaçu demonstrou grande potencial como ingrediente na elaboração de iogurtes a partir de leite de cabra, sendo uma estratégia tecnológica importante para a indústria de laticínios de cabra. Além disso, a adição da polpa de cupuaçu melhorou os atributos sensoriais do iogurte a partir do leite de cabra, melhorando a aceitação do mesmo frente a consumidores não habituais de derivados caprinos. Ademais, o efeito da informação de saúde dos compostos antioxidante pode ser utilizado como estratégia sensorial para favorecer alguns atributos sensoriais, como aroma ácido e alcóolico. Outrossim, este estudo demonstrou, por meio de validação cromatográfica, um método de CLAE específico, linear, exato, preciso e robusto para a determinação simultânea de carboidratos e ácidos orgânicos em iogurtes de leite de cabra, podendo ser utilizado para o monitoramento e o controle de qualidade do período fermentativo de iogurtes de leite de cabra.

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6 ANEXO

6.1 COMPROVANTE DE SUBMISSÃO ARTIGO I

Elsevier Editorial System(tm) for International Dairy Journal

Manuscript Draft

Manuscript Number:

Title: Consumer perceptions, health information and instrumental parameters of cupuassu (Theobroma grandiflorum) in goat milk yogurt

Article Type: Research Article

Corresponding Author: Mrs. Marion Pereira da Costa,

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Abstract: The objective of this study was to investigate consumers' perceptions of new goat milk yogurt manufactured with cupuassu pulp, including the effect of antioxidant health information on consumer acceptance and purchase intention. A positive expectation regarding linking and familiarity to goat's milk products and products with cupuassu pulp were obtained. Based on PCA, PLSR, JAR and penalty analysis, the addition of cupuassu pulp improved some sensory attributes of the goat milk yogurt such as cupuassu aroma, cupuassu flavor, yellow color, consistency and viscosity, which positively influenced product acceptance. In addition, antioxidant health information increased the acceptance and purchase intention of cupuassu goat milk yogurts. Taking into account the parameters investigated in this study, the optimal formulation was goat milk yogurt containing 10% cupuassu pulp. Our results suggest that cupuassu pulp can be considered a potential ingredient in goat milk yogurt.

6.2 ARTIGO II

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Cupuassu (*Theobroma grandiflorum*) pulp, probiotic, and prebiotic: Influence on color, apparent viscosity, and texture of goat milk yogurts

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ABSTRACT

Cupuassu is an acidic fruit that has a characteristic aroma, flavor, and texture; its fiber-rich pulp can provide a different consistency than other fruit pulps. Goat milk is an excellent source of amino acids, fatty acids, and minerals, and is widely used for processing fermented milks, such as yogurt. However, compared with cow milk yogurts, it is difficult to make goat milk yogurts with a good consistency. Therefore, it is necessary to use certain technological strategies. This study was carried out to investigate the possibility of adding cupuassu pulp, probiotic (*Lactobacillus acidophilus* LA-5), and prebiotic (inulin) to improve the texture of goat milk yogurt. A total of 6 treatments were performed: natural (N), probiotic (Pro), prebiotic (Pre), synbiotic (S), cupuassu (C), and probiotic with cupuassu (PC). The viability of probiotic in yogurts (Pro, S, and PC) was evaluated. In addition, instrumental analyses (pH, color, apparent viscosity, and texture) were performed to evaluate the influence of these different ingredients on goat milk yogurts. The probiotic bacteria remained viable ($\geq$7 log cfu·mL^{-1}) throughout the 28 d of refrigerated storage, which exceeded the minimum count required to confer probiotic physiological benefits. The pH levels of the yogurts inoculated with *L. acidophilus* (Pro, S, and PC) were lower than others yogurts (N, Pre, and C). However, all yogurt samples underwent gradual decreases in pH until 7 to 14 d of storage. The lightness (L^*) was affected initially by addition of all ingredients (cupuassu pulp, probiotic, and prebiotic). The addition of cupuassu pulp (C and PC) increased the L^* during the period of storage. Apparent viscosity and firmness decreased in the PC yogurt. The consistency was highest in the yogurts with added prebiotic (Pre and S) than the other yogurts (N, Pro, C, and PC) at the end of the storage period (d 28). The cohesiveness remained constant in all yogurts (N, Pro, Pre, S, C, and PC). Based on the results obtained from the current study, it was concluded that cupuassu pulp addition improves the texture of goat milk yogurts. Therefore, this pulp could be an important technological strategy for the dairy goat industry.

Key words: instrumental analysis, *Lactobacillus acidophilus* LA5, consistency, caprine milk

INTRODUCTION

Cupuassu (*Theobroma grandiflorum*) is a tropical fruit native to the Brazilian Amazon. Cupuassu has a high economic potential because of its excellent characteristics such as aroma, flavor, and texture (Faber and Yuyama, 2015). However, because of its distinctive flavor, cupuassu pulp is used as an ingredient in the manufacture of ice cream, juice, liquors, wines, jellies, and other products, such as yogurts, rather than being consumed *in natura* (Vriesmann and Petkowicz, 2009; Salgado et al., 2013). Cupuassu is a potential source of dietary fiber, mainly soluble fiber (Salgado et al., 2011). The cupuassu pulp has a particular chemical composition, rich in fibers, and contains a considerable amount of starch as well as pectin polysaccharides (Vriesmann et al., 2009), which can provide a different texture than other fruit pulps.

Goat milk is an excellent source of FA, protein, and minerals. When compared with cow milk, goat milk has the following characteristics: (1) less soluble and more insoluble contents of volatile FA, (2) a higher percentage of medium- and short-chain FA, (3) casein micelle with a lower percentage of α_{S1}-casein fraction, (4) smaller size of casein micelle, and (5) more calcium and inorganic phosphorus (Park et al., 2007). Furthermore, the importance of goat milk as a functional food is due to its high digestibility and nutritional value, as well as its therapeutic and dietary characteristics (Park et al., 2007; Fonseca et al., 2013). For these reasons, it is an excellent substitute for cow milk in the nutrition of children and elderly persons (Park et al., 2007; Kapila et al., 2013). Goat milk is widely used for processing fermented milks and other dairy products. Yogurt is the most widely produced and consumed fermented milk and is used as a vehicle for probiotic cultures and

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prebiotics (Costa et al., 2013; Costa and Conte-Junior, 2013). However, compared with cow milk yogurt, it is difficult to make goat milk yogurt with an appropriate flavor (Costa et al., 2014) and consistency, which is mainly due to the difference in casein composition and content (Li and Guo, 2006). Micelle structures of goat milk differ from cow milk in average diameter, hydration, and mineralization (Park et al., 2007). Therefore, it is necessary to use certain technological strategies. One alternative is the addition of inulin or another type of fiber, such as that present in fruit pulp (Buriti et al., 2014).

Inulin is one of the most studied and widely used prebiotics, with advantageous technological and nutritional properties (Paseephol et al., 2008). Prebiotics are selectively fermented ingredients that allow specific changes in the composition, activity, or both, of gastrointestinal microbiota, which confers a health benefit on the host (Gibson, 2007). Depending on the concentration, inulin may increase its effect on the structure and texture of dairy products, such as yogurt. Addition of inulin can change the texture and rheological properties of dairy foods (Paseephol et al., 2008).

Probiotics are live microorganisms, which when administered in adequate amounts, may benefit the health of the host (Sanders, 2009). *Lactobacillus acidophilus* LA-5 strain exhibits viability in milk matrix, such as fermented milks (Costa et al., 2015). However, no reports are present in the literature that this probiotic can improve the texture of goat milk yogurt. Certain strains of *Lactobacillus*, such as *Lactobacillus delbrueckii* ssp. *bulgaricus*, have this ability (Shihata and Shah, 2002).

In this context, the aim of the present study was to improve the texture of goat milk yogurt by adding cupuassu pulp, probiotic, prebiotic, or a combination of these.

MATERIALS AND METHODS

Goat Milk Yogurts

Ten liters of goat milk yogurts were produced as described by Costa et al. (2014) with modifications. In all treatments, thermophilic yogurt cultures (1% vol/vol; YF-L903, Chr. Hansen, Valinhos, Brazil) were added in UHT goat whole milk (Cappry's, Rio Grande do Sul, Brazil). A total of 6 treatments were performed: natural (**N**) containing milk and yogurt cultures; probiotic (**Pro**) containing milk, yogurt cultures, and probiotic; prebiotic (**Pre**) containing milk, yogurt cultures, and inulin; synbiotic (**S**) containing milk, yogurt cultures, probiotic, and inulin; cupuassu (**C**) containing milk, yogurt cultures, and cupuassu pulp; and probiotic with cupuassu (**PC**) containing milk, yogurt cultures, probiotic, and cupuassu pulp. For treatments with a probiotic (Pro, S, and PC), *L. acidophilus* culture (LA-5; Chr. Hansen) was inoculated at a concentration of 5% (vol/vol) in relation to the total milk volume used to produce the probiotic. For treatments with a prebiotic (Pre and S), 5% (wt/vol) of inulin (Ingredients & Systems Biotechnology, São Paulo, SP, Brazil) was added. The inulin polymer has a degree of polymerization from 2 to 50 with an average degree of polymerization of 9. For the treatments with cupuassu (C and PC), 10% (wt/vol) pasteurized cupuassu pulp (Polpa de Fruta, Macapá, AP, Brazil) was added.

The yogurt mixtures were fermented in an oven at 43 ± 2°C. The fermentation was interrupted when the pH (AOAC International, 2012) reached 4.5. Finally, the product was packaged in 500-mL plastic pots and stored at 4 ± 2°C for 28 d. The physicochemical analysis and probiotic viability assay were performed during the storage period (0, 7, 14, 21, and 28 d). This experiment was repeated 3 times (n = 3), and all analyses were performed in triplicate.

Bacteriological Analysis and Survivability of Probiotic

Streptococcus thermophilus and *Lactobacillus delbrueckii* ssp. *bulgaricus* were analyzed after the yogurt was prepared (d 1) to characterize the fermented product as yogurt. Enumeration of *S. thermophilus* was performed on M17 agar with lactose, which was incubated under aerobiosis at 37°C for 2 d. The count of *L. delbrueckii* ssp. *bulgaricus* on de Man, Rogosa and Sharpe (**MRS**) agar with pH 5.4 was performed after incubation under anaerobiosis at 37°C for 3 d (Codex Alimentarius, 2010). The probiotic (*L. acidophilus* LA-5) was counted according to the procedures of Costa et al. (2014), during the storage period (0, 7, 14, 21, and 28 d). *Lactobacillus acidophilus* was grown on MRS agar supplemented with 0.15% (wt/vol) bile salts, and aerobically incubated at 37°C for 2 d.

Physicochemical Analysis

pH Determination. Samples of goat milk yogurts were also analyzed for pH, using a digital pH meter (model PG1800, Cap Lab, SP, Brazil; AOAC International, 2012).

Instrumental Color. Color determinations were made at 5°C by means of a Minolta CM-600D spectrophotometer (Minolta Camera Co., Osaka, Japan). The colorimeter was previously calibrated with illuminant D65 and a 2° standard observer. Yogurt samples (50 mL) at 5°C were stirred and placed in an aluminum cyl-

inder (outside diameter 55 mm), with the surface optically flat before measuring, and the sensor was mounted directly on top of the cylinder to prevent ambient light noise. The color space of the yogurts was studied, and the following color coordinates were determined: lightness (L^*, 100 = white, 0 = black), redness (a^*, +red, −green), and yellowness (b^*, +yellow, −blue). These analyses were performed in triplicate.

Apparent Viscosity and Instrumental Texture Analysis

The apparent viscosities of the yogurts samples (100 mL) were measured at 5°C using a Quimis viscometer (Viscosímetro Rotativo Microprocessado, Q860M21, SP, Brazil) equipped with rotor no. 3, mixing at 60 rpm. The apparent viscosity was measured in triplicate.

Texture was assessed using a texture analyzer (TA-XT.Plus, Stable Micro Systems Ltd., Surrey, UK) equipped with a 50-kgf load cell, according to Iličić et al. (2014). Texture profile analysis (**TPA**) was used, analyzing firmness, consistency, and cohesiveness. The samples (100 mL) were compressed at 10% of original height with a back extrusion cell (A/BE) disc (diameter 36 mm; distance 30 mm; speed 0.001/ms), at a temperature of 4°C, with 3 measurements per sample averaged for data analysis. The tests were carried out in a standard size back extrusion container (50 mm in diameter). The extrusion disc was positioned centrally over the sample container.

Statistical Analysis

The results for color, pH, apparent viscosity, texture, and *L. acidophilus* LA-5 were subjected to one-way ANOVA, considering treatments and days 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 mean bacteria counts were calculated and expressed as $\log_{10}$ cfu·g^{-1}.

RESULTS AND DISCUSSION

Bacteriological Analysis

The counts of *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were evaluated to characterize the products made with yogurts, which was analyzed only on d 1. The yogurts contained, respectively, for *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*: 11.37 and 7.30 (N), 11.34 and 7.62 (Pro), 11.44 and 10.73 (Pre), 9.10 and 7.97 (S), 9.02 and 7.9 (C), and 11.16 and 11.13 (PC) log cfu·g^{-1}. Thus, the fermented milks produced in all treatments (N, Pro, Pre, S, C, and PC) were considered to be yogurt, the starter cultures in all the products were higher than 7 log cfu·g^{-1} (Codex Alimentarius, 2010).

For the probiotic yogurts, *L. acidophilus* LA-5 initial values were 11.01, 9.11, and 11.29 log cfu·g^{-1} for Pro, S, and PC yogurts, respectively. In general, the addition of inulin did not influence the probiotic viability (Bedani et al., 2013). However, in our study the treatment with inulin (S) had the lowest initial value of probiotic, which suggests an interference of this ingredient in the development of this microorganism. Figure 1 demonstrates the behavior of the probiotic in all probiotic goat milk treatments. The viability of the probiotic bacteria decreased ($P < 0.05$) in all treatments (Pro, S, and PC) during the first week of storage. The decrease of *L. acidophilus* LA-5 can be explained by 3 mechanisms: the depletion of some nutrients needed by probiotic bacteria; probiotic may have upset the desirable relationship between the yogurt starter culture; and probiotic in the yogurt may have initially produced higher concentrations of antimicrobials such as bacteriocins, H_2O_2, or organic acids that may have eventually inhibited more *L. acidophilus* (Olson and Aryana, 2008).

Thereafter, they were stable, and all probiotic yogurts maintained counts ≥7 log cfu·g^{-1} during 4 wk (28 d) of storage. *Lactobacillus acidophilus* LA-5 demonstrated variable viability in the yogurts, with final counts of 9.40, 8.02, and 8.43 log cfu·g^{-1} for Pro, S, and PC yogurts, respectively. These counts exceeded the minimum count required to confer probiotic physiological benefits (Bedani et al., 2013; Costa et al., 2013). Regarding the lower viability of the PC yogurts than Pro and S, Kailasapathy et al. (2008) suggested that probiotic strains can be influenced by the pH of the fruit preparation.

pH Analysis

The pH of the goat milk used to produce the yogurts was 6.62 ± 0.03. The pH values of the N, Pro, Pre, S, C, and PC yogurts are presented in Table 1. The reduction ($P < 0.05$) of milk pH after yogurt production (d 0), in all treatments, was in line with the growth of the starter culture and the probiotic bacteria. The pH of all yogurt samples decreased ($P < 0.05$) gradually until 7 to 14 d of storage, and then increased ($P < 0.05$) in Pre and C treatments. The high bacterial metabolic activity ferments lactose and produces lactic acid, which decreases the pH of yogurts (Gaspar et al., 2013). However, when the sugar sources are exhausted, microorganisms begin to consume proteins and start to produce other metabolites, such as biogenic amines (Costa et al., 2015), which increase the pH (Vahedi et al., 2008). This explains the pH increase of Pre and C

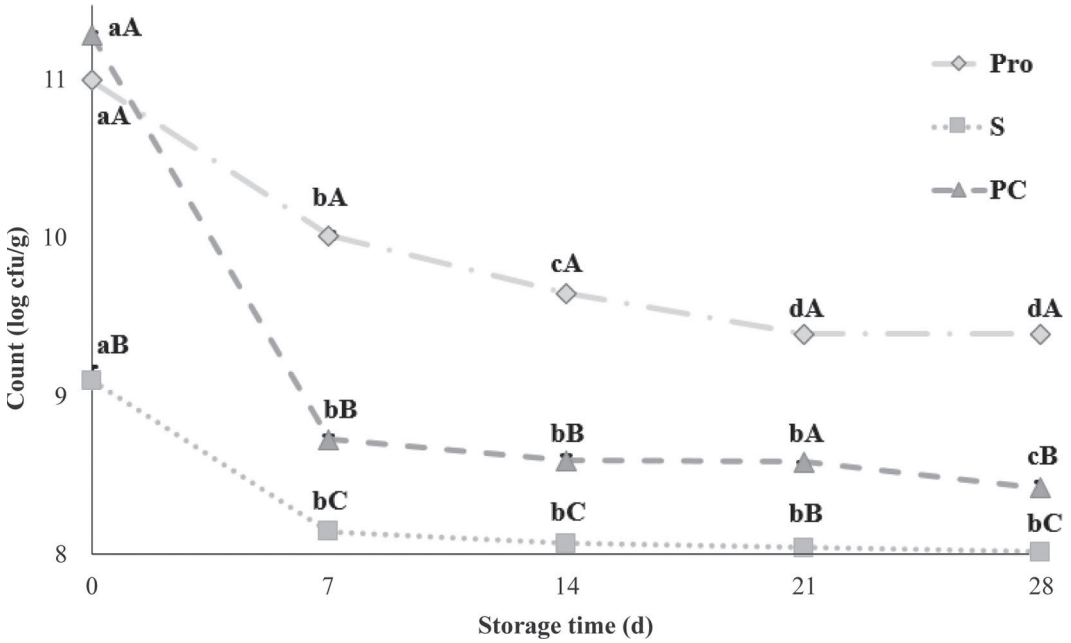

Figure 1. Counts of *Lactobacillus acidophilus* LA-5 (log cfu·g^{-1}) in goat milk yogurts with added probiotic (Pro), synbiotic (S) and probiotic with cupuassu (PC) goat milk yogurts during 28 d of storage. Different uppercase letters (A–C) indicate significant differences among goat milk yogurts, $P < 0.05$; different lowercase letters (a–d) indicate significant differences among storage times, $P < 0.05$.

yogurts ($P < 0.05$) at the end of the storage period (21 and 28 d).

Although all yogurts were cooled at pH 4.5, the pH levels of the yogurts inoculated with *L. acidophilus* (Pro, S, and PC) were lower ($P < 0.05$) than the pH levels of the remaining yogurts (N, Pre, and C) at the end of storage. Espírito Santo et al. (2011) observed similar behavior, and suggested that the occurrence of fatty acid consumption as a carbon source after sugar depletion and fiber pectin degradation to uronic acids could explain the pH reduction. Moreover, the probiotic bacteria may have produced organic acids (Olson and Aryana, 2008), which contributes to decreasing pH.

Instrumental Color Analysis

The color parameters L^*, a^*, and b^* exhibited some differences ($P < 0.05$), and these changes in color in the 6 goat milk yogurts (N, Pro, Pre, S, C, and PC) stored at 4°C for 28 d are presented in Table 2.

The L^* is lightness, in which 100 represents white, whereas zero represents the black. The L^* values were significantly affected by the addition of the cupuassu pulp, probiotic, and prebiotic (Pro, Pre, S, C and PC) on the initial day ($P < 0.05$); however, at the end of the storage period no difference were found between treatments. The L^* values in all yogurt (N, Pro, Pre, S, C, and PC) samples increased ($P < 0.05$) during the 28 d of storage. The white color of milk results from the presence of colloidal particles, such as milk fat globules and casein micelles, capable of scattering light in the visible spectrum (García-Pérez et al., 2005). In addition, the goat milk has the absence of β-carotene because of a physiological process of the goats. This substance is converted into vitamin A (Park et al., 2007), which explains the high L^* values, mainly in N yogurt. The goat milk yogurt sample containing cupuassu pulp (C and PC) had a lower L^* value than others (N, Pro, Pre, and S). These results suggest that the cupuassu pulp decreased the lightness values of the yogurts, which can be related to this fruit pulp color. Silva and Silva (1999) observed that cupuassu pulp exhibits a light yellow color (L^* 70.04, a^* 26.36, and b^* 19.73), which consequently can change the yogurt color (C and PC). This difference probably could be well accepted by consumers, as it would reflect the presence of cupuassu. Changes in yogurt color are in agreement with milk substitution, which may be attributed to the different opacity level of gels. This fact increases with the casein proportion and their aggregation level (González-Martınez et al., 2002).

As for the storage period, the L^* value increased ($P < 0.05$) in all treatments (N, Pro, Pre, S, C, PC). Although, the greatest change occurred in Pro, where the L^* value increased from 89.24 to 92.39. As the result of Pre and S, some studies achieved the same effect, which demonstrated that inulin increase L^* value (Noziere et al., 2006; Villegas et al., 2010). However, this result dif-

Table 1. pH values (means ± standard deviation) of goat milk yogurts measured during the storage period at 4°C

	Storage period (d)				
Treatment[1]	0	7	14	21	28
N	$4.57^{a,A} \pm 0.04$	$4.42^{c,A} \pm 0.01$	$4.48^{bc,B} \pm 0.01$	$4.51^{b,A} \pm 0.03$	$4.57^{a,A} \pm 0.01$
Pro	$4.45^{ab,B} \pm 0.08$	$4.38^{bc,B} \pm 0.05$	$4.47^{a,B} \pm 0.02$	$4.38^{c,B} \pm 0.01$	$4.37^{c,C} \pm 0.03$
Pre	$4.55^{a,A} \pm 0.01$	$4.41^{b,AB} \pm 0.05$	$4.54^{b,A} \pm 0.08$	$4.51^{a,A} \pm 0.06$	$4.47^{ab,B} \pm 0.01$
S	$4.42^{a,B} \pm 0.04$	$4.27^{c,D} \pm 0.05$	$4.35^{ab,C} \pm 0.05$	$4.26^{c,C} \pm 0.01$	$4.34^{b,C} \pm 0.01$
C	$4.43^{c,B} \pm 0.02$	$4.35^{d,C} \pm 0.05$	$4.60^{a,A} \pm 0.08$	$4.51^{b,A} \pm 0.02$	$4.53^{b,AB} \pm 0.03$
PC	$4.50^{a,AB} \pm 0.01$	$4.28^{bc,D} \pm 0.01$	$4.24^{c,D} \pm 0.01$	$4.28^{bc,C} \pm 0.02$	$4.30^{b,C} \pm 0.01$

[a–d]Different lowercase superscripts indicate significant differences among storage times, $P < 0.05$.

[A–D]Different uppercase superscripts indicate significant differences among goat milk yogurts, $P < 0.05$.

[1]N = natural; Pro = probiotic; Pre = prebiotic; S = synbiotic; C = cupuassu; PC = probiotic with cupuassu.

fers from those found by Mani-López et al. (2014), who observed no changes in color parameters during storage. This difference may related to distinct factors such as the probiotic strain (*L. acidophilus*, *Lactobacillus casei*, and *Lactobacillus reuteri*), the absence of inulin, and the type of milk (cow milk), which depending on combination of the ingredients could generate a unique color profile (Mani-López et al., 2014).

Regarding a^* (greenness-redness) initial values, treatments added with prebiotic (Pre and S) and cupuassu pulp (C and PC) differed from control ($P < 0.05$). However, Pre and S had lower values, whereas C and PC had higher values. Kim et al. (2011) reported the same behavior of Pre and S treatments, which can be explained by the addition of inulin that increases the water-holding capacity. In all treatments (N, Pro, Pre, S, C, and PC), during storage, an increase of a^* values was observed ($P < 0.05$), indicating an increase in the redness of the yogurts. Estrada et al. (2011) explained this increase through the gel stirring and acidity changes in yogurt during refrigerated storage, because they may cause changes in tissue structure that result in leakage of natural pigments, such as carotenoids, to the yogurt matrix.

The b^* (blueness-yellowness) values was different between all treatments, and the N treatment was less yellow than the other treatments (Pro, Pre, S, C, and PC). The greater yellowness ($P < 0.05$) of Pro, Pre, S, C, and PC can be attributed to the addition of cupuassu pulp, and probiotic and prebiotic ingredients, which all differed from N. Yellowness of the yogurt depends on the type and level of fruit or fiber. Similar results were described for yogurts fortified with commercial apple fiber (Staffolo et al., 2004), orange fiber (García-Pérez

Table 2. The color values (means ± standard deviation) of goat milk yogurt measured at 4°C during the storage period

		Storage period (d)				
Property[1]	Treatment[2]	0	7	14	21	28
L^*	N	$90.05^{e,A} \pm 0.01$	$90.22^{d,A} \pm 0.05$	$90.40^{c,A} \pm 0.02$	$90.71^{b,A} \pm 0.02$	$92.78^{a,A} \pm 0.02$
	Pro	$89.24^{d,C} \pm 0.01$	$89.90^{c,B} \pm 0.04$	$90.06^{c,B} \pm 0.02$	$90.88^{b,A} \pm 0.08$	$92.39^{a,A} \pm 0.10$
	Pre	$89.41^{e,B} \pm 0.02$	$89.83^{d,B} \pm 0.01$	$90.13^{c,B} \pm 0.03$	$90.89^{b,A} \pm 0.18$	$92.43^{a,A} \pm 0.03$
	S	$89.06^{d,D} \pm 0.01$	$89.45^{c,C} \pm 0.00$	$89.68^{c,C} \pm 0.01$	$90.58^{b,A} \pm 0.17$	$92.05^{a,A} \pm 0.02$
	C	$87.76^{c,F} \pm 0.01$	$88.44^{b,D} \pm 0.01$	$87.90^{c,E} \pm 0.04$	$88.33^{b,B} \pm 0.08$	$90.17^{a,B} \pm 0.13$
	PC	$88.09^{c,E} \pm 0.01$	$88.33^{c,E} \pm 0.01$	$88.07^{c,D} \pm 0.02$	$88.78^{b,B} \pm 0.23$	$89.70^{a,B} \pm 0.01$
a^*	N	$-1.74^{d,B} \pm 0.02$	$-1.69^{d,A} \pm 0.03$	$1.99^{c,A} \pm 0.01$	$2.09^{b,C} \pm 0.02$	$2.37^{a,B} \pm 0.01$
	Pro	$-1.74^{d,B} \pm 0.01$	$-1.86^{e,B} \pm 0.04$	$1.85^{c,A} \pm 0.01$	$2.32^{a,A} \pm 0.03$	$2.20^{b,D} \pm 0.02$
	Pre	$-1.78^{d,C} \pm 0.01$	$-2.01^{e,C} \pm 0.02$	$1.62^{c,A} \pm 0.02$	$2.19^{b,BC} \pm 0.05$	$2.21^{a,DC} \pm 0.01$
	S	$-1.78^{de,C} \pm 0.01$	$-2.04^{e,C} \pm 0.02$	$1.81^{c,A} \pm 0.04$	$2.35^{a,A} \pm 0.01$	$2.24^{b,DC} \pm 0.01$
	C	$-1.32^{d,A} \pm 0.01$	$-1.68^{e,A} \pm 0.01$	$1.78^{c,A} \pm 0.07$	$2.27^{b,AB} \pm 0.01$	$2.90^{a,A} \pm 0.01$
	PC	$-1.35^{d,A} \pm 0.01$	$-1.72^{e,A} \pm 0.01$	$2.09^{c,A} \pm 0.01$	$2.37^{a,A} \pm 0.02$	$2.26^{b,C} \pm 0.01$
b^*	N	$8.23^{a,D} \pm 0.02$	$8.08^{b,E} \pm 0.05$	$6.89^{c,E} \pm 0.01$	$4.86^{d,D} \pm 0.05$	$4.55^{e,D} \pm 0.01$
	Pro	$8.41^{b,C} \pm 0.01$	$8.57^{a,C} \pm 0.07$	$6.94^{c,D} \pm 0.01$	$4.51^{e,C} \pm 0.03$	$4.85^{d,C} \pm 0.01$
	Pre	$8.30^{b,D} \pm 0.03$	$8.40^{a,D} \pm 0.03$	$7.56^{c,B} \pm 0.03$	$4.92^{d,DB} \pm 0.01$	$4.96^{d,C} \pm 0.03$
	S	$8.50^{b,B} \pm 0.03$	$8.80^{a,B} \pm 0.04$	$7.09^{c,C} \pm 0.02$	$4.97^{e,B} \pm 0.02$	$5.22^{d,B} \pm 0.04$
	C	$10.32^{a,A} \pm 0.01$	$10.15^{a,A} \pm 0.03$	$8.76^{b,A} \pm 0.02$	$7.38^{d,A} \pm 0.02$	$7.40^{c,A} \pm 0.14$
	PC	$10.32^{a,A} \pm 0.01$	$10.17^{b,A} \pm 0.01$	$8.55^{c,A} \pm 0.02$	$7.09^{d,A} \pm 0.01$	$7.11^{d,A} \pm 0.01$

[a–f]Different lowercase superscripts indicate significant differences among storage times, $P < 0.05$.

[A–F]Different uppercase superscripts indicate significant differences among goat milk yogurts, $P < 0.05$.

[1]Measured L^*, a^*, and b^* values were used as indicators of lightness (L^*), redness (a^*), and yellowness (b^*).

[2]N = natural; Pro = probiotic; Pre = prebiotic; S = synbiotic; C = cupuassu; PC = probiotic with cupuassu.

et al., 2005), and wheat bran (Hashim et al., 2009). The b^* values decreased significantly in all yogurts (N, Pro, Pre, S, C, and PC) during the 28 d of refrigerated storage ($P < 0.05$).

These results (increased a^* and decreased b^*) indicate that the reddish color was reinforced, which should be attributed to the goat milk, due to carotenoids (Noziere et al., 2006) and lipid oxidation (Xia et al., 2012), because all the yogurts exhibited the same behavior. Statistical analyses demonstrated that, although the pattern was the same, the treatments with and without cupuassu pulp differed ($P < 0.05$). Other studies have presented the same performance (increased a^* and decreased b^*) when fruit (pomegranate) and vegetal (yam) ingredients were added to yogurt (Kim et al., 2011; Trigueros et al., 2014).

Apparent Viscosity Analysis

The effects of addition of a probiotic, a prebiotic, and cupuassu pulp on the apparent viscosity of the goat milk yogurts (N, Pro, Pre, S, C, and PC) during storage are presented in Figure 2. On the initial day, the viscosities of the Pre, S, C, and PC yogurts were higher than N goat milk ($P < 0.05$; i.e., the addition of cupuassu pulp and inulin increased the apparent viscosity). The addition of inulin may increase the structure of dairy products, which can change the viscosity and rheological properties of dairy foods. Also, it can be technologically used as a fat substitute (Paseephol et al., 2008). The cupuassu pulp has a particular chemical composition, being rich in fiber (mainly soluble fiber) and containing a considerable amount of starch as well as pectin polysaccharides (Vriesmann et al., 2009), which could improve the apparent viscosity of C and PC yogurts.

Regarding the period of storage, the apparent viscosity remained constant until d 7 of storage, in all goat milk yogurts, and then decreased ($P < 0.05$). The decrease in apparent viscosity might have been caused by the whey separation with increasing storage time (Al Mijan et al., 2014). This behavior is in agreement with the results of Wang et al. (2012), who compared the apparent viscosity of goat and cow milk yogurts.

The development of apparent viscosity in yogurts is associated with the aggregation of casein micelles and gel formation, which is a consequence of biochemical and physicochemical changes during fermentation of milk (Gaygadzhiev et al., 2009; Singh and Kim, 2009). The apparent viscosity also increases as the pH of

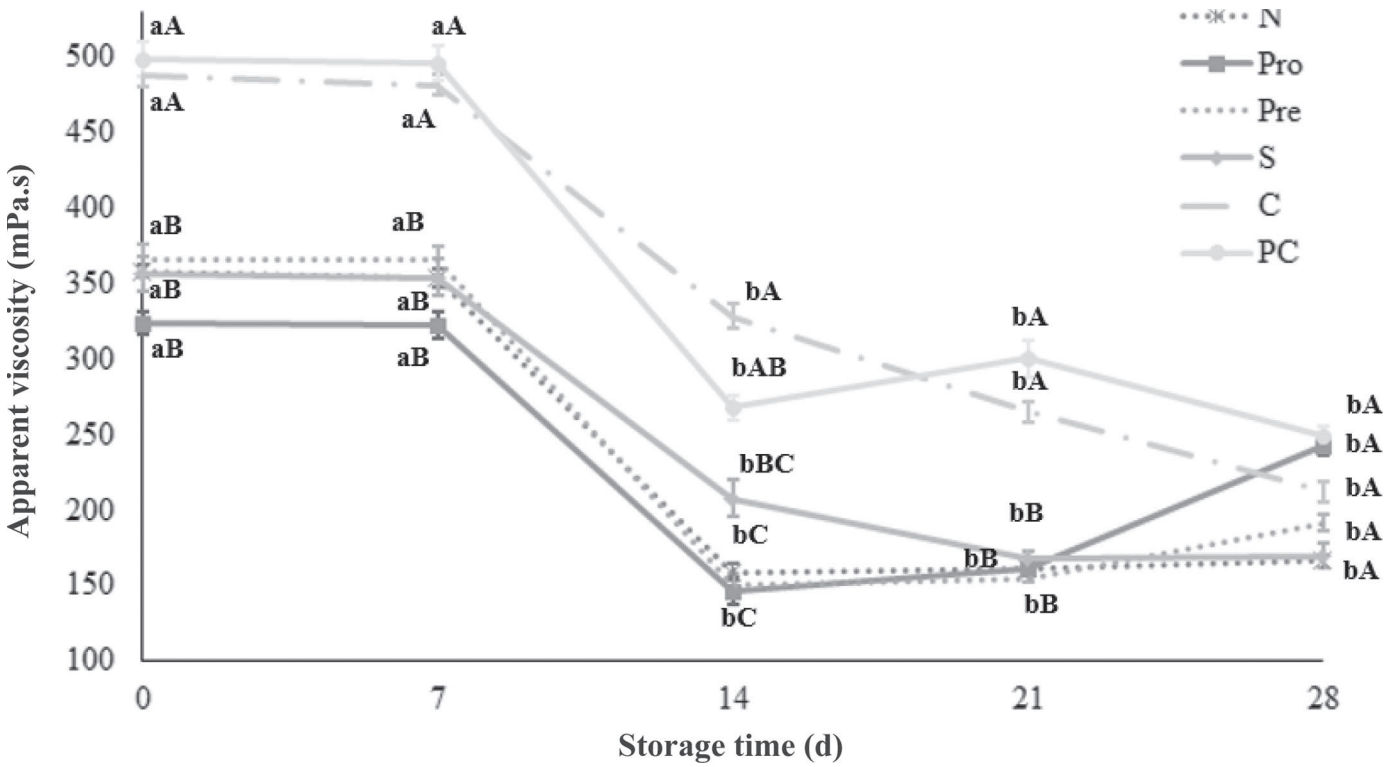

Figure 2. Apparent viscosity of the natural (N), probiotic (Pro), prebiotic (Pre), synbiotic (S), cupuassu (C), and probiotic with cupuassu (PC) goat milk yogurts during 28 d of refrigerated storage. Different uppercase letters (A–C) indicate significant differences among goat milk yogurts, $P < 0.05$; different lowercase letters (a, b) indicate significant differences among storage times, $P < 0.05$.

Table 3. Firmness, consistency, and cohesiveness values (means ± standard deviation) of goat milk yogurts measured at 4°C during the storage period

TPA[1] parameter	Treatment[2]	Storage period (d)				
		0	7	14	21	28
Firmness (g)	N	$22.28^{a,B} \pm 0.12$	$21.42^{a,A} \pm 0.20$	$20.81^{a,A} \pm 0.13$	$21.27^{a,A} \pm 0.18$	$20.70^{a,AB} \pm 0.11$
	Pro	$22.32^{a,B} \pm 0.11$	$21.85^{a,A} \pm 0.06$	$20.52^{ab,A} \pm 0.28$	$19.30^{ab,A} \pm 0.62$	$17.57^{b,B} \pm 0.12$
	Pre	$21.92^{a,B} \pm 0.21$	$22.81^{a,A} \pm 0.47$	$21.41^{a,A} \pm 0.17$	$21.17^{a,A} \pm 0.14$	$21.45^{a,A} \pm 0.32$
	S	$22.14^{b,A} \pm 0.16$	$21.59^{a,A} \pm 0.11$	$20.52^{a,A} \pm 0.28$	$21.59^{a,A} \pm 0.31$	$20.16^{a,AB} \pm 0.02$
	C	$21.85^{ab} \pm 0.23$	$21.88^{a,A} \pm 0.02$	$21.78^{a,A} \pm 0.35$	$20.88^{a,A} \pm 0.02$	$20.73^{a,AB} \pm 0.12$
	PC	$26.16^{a,A} \pm 0.52$	$21.45^{b,A} \pm 0.18$	$21.42^{b,A} \pm 0.17$	$20.73^{b,A} \pm 0.03$	$20.88^{b,AB} \pm 0.16$
Consistency (g/s)	N	$122.86^{a,AB} \pm 0.15$	$121.27^{a,A} \pm 0.01$	$122.50^{a,B} \pm 0.08$	$123.23^{a,A} \pm 0.07$	$118.14^{a,B} \pm 0.19$
	Pro	$126.92^{a,B} \pm 0.13$	$121.77^{a,A} \pm 0.20$	$118.69^{ab,B} \pm 0.17$	$103.22^{b,A} \pm 0.19$	$98.07^{b,B} \pm 0.16$
	Pre	$121.31^{a,AB} \pm 0.44$	$126.43^{a,A} \pm 0.30$	$125.98^{a,AB} \pm 0.32$	$127.23^{a,A} \pm 0.05$	$127.30^{a,A} \pm 0.31$
	S	$120.12^{a,AB} \pm 0.29$	$117.63^{a,A} \pm 0.16$	$115.34^{a,B} \pm 0.43$	$113.96^{a,A} \pm 0.06$	$123.13^{a,A} \pm 0.06$
	C	$122.09^{a,AB} \pm 0.12$	$122.18^{a,A} \pm 0.05$	$124.25^{a,A} \pm 0.21$	$123.55^{a,A} \pm 0.10$	$122.46^{a,B} \pm 0.20$
	PC	$127.29^{a,A} \pm 0.67$	$122.46^{a,A} \pm 0.31$	$120.56^{a,B} \pm 0.04$	$120.25^{a,A} \pm 0.11$	$122.6^{a,B} \pm 0.14$
Cohesiveness (g)	N	$-30.29^{a,AB} \pm 0.16$	$-31.48^{a,A} \pm 0.32$	$-30.54^{a,A} \pm 0.25$	$-30.72^{a,A} \pm 0.55$	$-30.97^{a,A} \pm 0.05$
	Pro	$-32.45^{a,B} \pm 0.65$	$-30.29^{a,A} \pm 0.11$	$-31.55^{a,A} \pm 0.10$	$-29.75^{a,A} \pm 0.48$	$-29.83^{a,A} \pm 0.04$
	Pre	$-29.75^{a,AB} \pm 0.53$	$-30.58^{a,A} \pm 0.57$	$-30.79^{a,A} \pm 0.30$	$-31.48^{a,A} \pm 0.50$	$-30.97^{a,A} \pm 0.10$
	S	$-31.84^{a,AB} \pm 0.50$	$-29.36^{a,A} \pm 0.32$	$-31.30^{a,A} \pm 0.50$	$-30.87^{a,A} \pm 0.55$	$-32.05^{a,A} \pm 0.32$
	C	$-29.75^{a,AB} \pm 0.17$	$-30.87^{a,A} \pm 0.50$	$-31.04^{a,A} \pm 0.10$	$-31.08^{a,A} \pm 0.15$	$-32.23^{a,A} \pm 0.30$
	PC	$-29.39^{a,A} \pm 0.54$	$-29.76^{a,A} \pm 0.10$	$-29.86^{a,A} \pm 0.16$	$-31.73^{a,A} \pm 0.10$	$-28.89^{a,A} \pm 0.16$

[a,b]Different lowercase superscripts indicate significant differences among storage times, $P < 0.05$.

[A,B]Different uppercase superscripts indicate significant differences among goat milk yogurts, $P < 0.05$.

[1]TPA = texture profile analysis.

[2]N = natural; Pro = probiotic; Pre = prebiotic; S = synbiotic; C = cupuassu; PC = probiotic with cupuassu.

milk decreases, which is attributable to the additional swelling of casein micelles. At pH 5.4 to 5.3, the initial increase of apparent viscosity can be observed, at this stage indicating the initiation of aggregation. In the pH range of 5.1 to 4.6, the apparent viscosity of goat products increases (Park, 2007), which typically occurs in yogurts. In our study, this increase on apparent viscosity effect by pH happened in all treatments (N, Pro, Pre, S, C, and PC).

However, the casein micelles of goat milk contain more calcium, inorganic phosphorus, and noncentrifugal casein, and are less solvated, less heat stable, and lose β-casein more readily than bovine casein micelles (Park et al., 2007). This fact is related to the large difference in the apparent viscosity of yogurt made with goat milk compared with cow milk.

Instrumental Texture Analysis

The TPA parameters well represented the yogurt textural characteristics. Firmness, consistency, and cohesiveness are commonly evaluated in determining yogurt texture (Espírito Santo et al., 2012; Buriti et al., 2014; Iličić et al., 2014). Different goat milk yogurts were measured, as presented in Table 3.

Gel formation is one of the main texture properties of yogurt. This structure is result of casein aggregation by pH decreasing and disulfide bonding between κ-casein and denatured whey proteins (Damin et al., 2009). In addition, other parameters, such as milk base composition, heat treatment applied, fermentation process, storage conditions, and starter culture, also perform a determinative role in gel structure formation (Akalın et al., 2012).

Regarding firmness, no statistical difference ($P > 0.05$) was found between the treatments. The firmness decreased in all yogurts (N, Pro, Pre, S, C, and PC) during 28 d of storage (Table 3). However, despite similar behavior in the different treatments, this decline was statistically significant ($P < 0.05$) only in the PC yogurt. Therefore, the addition of each ingredient (cupuassu pulp and probiotic) separately did not affect the firmness, although together, they changed this parameter. Oliveira et al. (2001) reported that the firmness of fermented milks is highly dependent on the culture composition, TS, and protein content of the product. Moreover, the type of protein and the interaction between the ingredients used and the composition of the culture can affect the firmness of the product (Oliveira et al., 2001). This fact may explain the significant decline in the yogurt PC, which has lower lactic protein content when compared with other treatments. The firmness of yogurts is also related to the bacteria *L. delbrueckii* ssp. *bulgaricus*. The incorporation of this microorganism into the yogurt starter culture improved the firmness, which in general is due to the attachment of mucogenic strains to the protein matrix via the exopolysaccharides (Shihata and Shah, 2002).

The consistency of the samples was significantly high ($P < 0.05$) in the yogurts with added prebiotic (Pre and

S) compared with the others (N, Pro, C, and PC) at the end of storage (d 28). Furthermore, the consistency of the Pre and S goat milk yogurt remained constant ($P > 0.05$) during the storage period (Table 3). A similar result was obtained for the yogurt consistency with the addition of the inulin (Pimentel et al., 2012, 2013). This prebiotic helped to increase this physical property, but up to a certain concentration. The interactions between whey proteins and κ-casein make the micelles less sensitive to the pH decline, increasing their solubility. Inulin is a soluble fiber and a water-structuring agent. In addition, this prebiotic can form complexes with the protein aggregates, and it must be part of the structural network that is formed during fermentation and structuring of the stirred yogurt (Srisuvor et al., 2013).

The cohesiveness values indicated that the predominance of protein in the composition of the yogurt caused the large number of casein–casein linkages broken during stress application to reform after the stress was released (Peng et al., 2009). The cohesiveness values are provided in Table 3. In this study, the cohesiveness in all treatments remained constant ($P > 0.05$) during refrigeration storage. Therefore, the addition of the cupuassu pulp, probiotic, and prebiotic did not affect the cohesiveness. Hence, cohesiveness should not be considered a good parameter because all treatments showed the same results. The cohesiveness value together with the springiness may indicate a predominance of protein in the composition of the yogurt, which led to a large promoted number of broken casein–casein linkages during stress application, which reformed after the stress was released (Sandoval-Castilla et al., 2004). A possible explanation for the similar behavior of this parameter in all yogurts is that they have a proximate milk protein content.

CONCLUSIONS

We conclude that cupuassu pulp is potentially useful in the manufacture of goat milk yogurts to improve their texture. In this way, cupuassu is an important technological strategy for the dairy goat industry.

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6.3 ARTIGO III

Comprehensive REVIEWS in Food Science and Food Safety

Chromatographic Methods for the Determination of Carbohydrates and Organic Acids in Foods of Animal Origin

Marion Pereira da Costa and Carlos Adam Conte-Junior

Abstract: Carbohydrates are ubiquitous and range from simple monosaccharides to large complex polysaccharides. Organic acids are compounds with acidic properties. Both occur naturally in many foods and in fermented products. Organic acids are usually derived from the hydrolysis of carbohydrates by microorganisms such as lactic acid bacteria. These bacteria convert carbohydrates into energy required for growth, since they are not equipped with the enzymes necessary for respiration and are unable to perform oxidative phosphorylation. Determination of carbohydrates and organic acids in foods of animal origin is important, since they contribute to flavor and texture. Their presence and proportions can affect the chemical and sensory characteristics of a food matrix and they can provide information on nutritional properties of food and the means to optimize selected technological processes. Furthermore, the levels of carbohydrate and organic acid are important to monitor bacterial growth and activity. Actually, these compounds can be quantified by several methods including high-performance liquid chromatography (HPLC) and gas chromatography (GC). High-performance liquid chromatography has been widely used to analyze carbohydrates and nonvolatile organic acids, while gas chromatography has been used to determine the volatile organic acids in complex matrices. This contribution provides an overview of chromatographic methods (HPLC and GC) used to analyze carbohydrates and organic acids in foods of animal origin.

Keywords: carbohydrates, honey, HPLC, meat, milk

Introduction

Carbohydrates are structurally classified as monosaccharides, oligosaccharides, and polysaccharides. Monosaccharides and some oligosaccharides have a sweet taste. Polysaccharides, in combination with proteins, lipids, and nucleic acids, play an important role in animal metabolic systems. In food systems, carbohydrates provide flavor, structure, and texture (Manthey and Xu 2009).

The term "organic acid" refers to organic compounds with acidic properties which contain carbon. These are generally not considered nutrients, but they give a characteristic taste to food. Therefore, they are among the major contributors to flavor, besides sugars and volatile compounds (Urbach 1997). Organic acids occur naturally in a number of foods, mainly in fermented products as a result of hydrolysis, biochemical metabolism, and microbial activity (Leroy and De Vuyst 2004). Organic acids have been widely used as food additives and preservatives to prevent deterioration and extend the food shelf life (Chen and others 2006; Jurado-Sánchez and others 2011). Organic acids primarily act as acidulants and reduce bacterial growth by lowering the pH of food products to levels that will inhibit bacterial growth (Hinton 2006; Conte-Junior and others 2010). The acid in its undissociated state is able to penetrate the microbial cell, which is not able to tolerate a major change in its internal pH (Adams and Hall 1988; Goosen and others 2011).

Determination of carbohydrate and organic acid contents in food products is important, since they contribute to the flavor, texture, and aromatic properties (Tormo and Izco 2004; Farajzadeh and Assadi 2009; Kritsunankul and others 2009). The presence and relative proportions of carbohydrates and organic acids can affect the chemical and sensory characteristics of the food matrix (including pH, total acidity, and microbial stability) and can provide information on nutritional properties of food and the means of optimizing selected technological processes (Chinnici and others 2005). The quantitative determination of carbohydrates and organic acids is also important to monitor bacterial growth and activity (Izco and others 2002). High-performance liquid chromatography (HPLC) has been widely used to analyze carbohydrates and nonvolatile organic acids (Murtaza and others 2012; Terol and others 2012; Leite and others 2013; Wang and others 2013; Zhou and others 2014; Gaze and others 2015), while gas chromatography (GC) has been used to determine the volatile organic acids in complex matrixes (Yang and Choong 2001; Aljadi and Yusoff 2003; Spaziani and others 2009; Suzzi and others 2014).

This review discusses the main chromatographic methods used in the analysis of carbohydrates and organic acids in food of animal

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origin, providing an overview of the types of carbohydrates and organic acids in different products of animal origin, and the different methods used (HPLC and GC) to analyze these compounds.

Carbohydrates and Organic Acids in Foods of Animal Origin

The type and concentration of carbohydrate will vary depending on the animal product. The monosaccharides glucose and fructose occur naturally in honey. Free glucose is also found in animal fluids (blood, lymph, and cerebrospinal fluid). The pentose monosaccharides arabinose, xylose, and ribose and the hexoses mannose and galactose rarely occur free in nature, except as breakdown products during fermentation. Of the disaccharides, lactose is the most abundant in milk and milk products, and occurs solely in mammary tissue products (Ball 1990).

Organic acids in foods of animal origin result from the metabolism of large-molecular-mass compounds, such as carbohydrates, lipids, and proteins. These acids are also found in many products as compounds added to food to carry out some hygienic or technological function (Brul and Coote 1999). Organic acids such as lactic and acetic acids are used as direct antimicrobial activity products and are incorporated into human foods (Cruz-Romero and others 2013), because of their ability to lower the pH, resulting in instability of bacterial cell membranes (Mani-Lopez and others 2012). These acids can accumulate over time as they are produced by fermentation activity of indigenous or added starter cultures of microorganisms (Ricke 2003; Costa and Conte-Junior 2013).

Milk and derivatives

Lactose is the major carbohydrate in milk from all mammalian species, such as goat, sheep, and bovine. The lactose content in milk is relatively constant, although it varies among different dairy products. Lactose is a disaccharide composed of glucose and galactose molecules, and it is synthesized in the mammary gland. Small amounts of free glucose and galactose may also be present (Park 1994; Haenlein 2004). Other minor carbohydrates found in milk are oligosaccharides, glycopeptides, glycoproteins, and nucleotide sugars, although in very small amounts (Park and others 2007).

The organic acid content of milk varies in the range of 0.12% to 0.21%, or around 1.2% dry matter. Citric acid, the predominant organic acid in milk, is present in the form of citrate (Walstra and others 2005). During storage, citric acid disappears rapidly as a result of bacterial growth. Lactic and acetic acids are degradation products of lactose. Other acids are produced from the hydrolysis of lactose, citric acid, and fat. Milk also contains nitrogenous acidic compounds such as orotic acid and hippuric acid. The orotic acid concentration is mainly influenced by diet and stage of lactation (Tormo and Izco 2004).

During milk fermentation, the lactic acid bacteria (LAB) utilize lactose and synthesize organic acid byproducts (Costa and others 2013). The first step is hydrolysis of lactose to its component monosaccharides by β-galactosidase, for most species of bacteria, or by phospho-β-galactosidase. In fermented milk, generally, the production of some organic acids, such as lactic, formic, acetic, and succinic, is the result of the metabolic activity of the starter cultures (Ammor and others 2006). These acids contribute to the flavor of fermented milk, especially lactic acid that is important in the formation of various typical flavor products. Lactic acid gives a sharp, acidic, and refreshing taste to yogurt and other fermented milks. During fermentation, there is an appreciable increase in the level of some organic acids such as lactic and citric acids. The level of organic acids in any type of milk product depends on several variables such as the starter cultures, type of milk, and incubation temperature and time (Akalin and others 1997).

Cheese ripening is a complex process that involves several concurrent and interlinked reaction pathways. The primary biochemical events of ripening include metabolism of lactose, lactate, and citrate, and lipolysis and proteolysis. The products of primary events such as free fatty acids, organic acids, and amino acids are further catabolized to smaller volatile and nonvolatile flavor compounds (Subramanian and others 2011). For cheese ripening, the decrease of the sugars and the evolution of organic acids, directly or indirectly, determine the chemical composition, as well as the sensory characteristics, and hence the quality (Zeppa and others 2001). The organic acids present in the various types of cheese can vary according to the manufacturing process and cheese starter culture.

Meat and derivatives

Meat is a major source of proteins, particularly those containing amino acids essential to human health, and it is also a good source of iron, zinc, and vitamin B_{12} (Bax and others 2013), although it is not a good source of carbohydrates. Carbohydrates are used for energy production, by 2 main alternative routes, the oxidative and glycolytic pathways. Glycolysis is an important metabolic pathway in the postmortem period, and this pathway changes glycogen, a polymer of glucose and the major energy reserve in muscle, into lactate (Choe and others 2008). The lactate formed is also converted back to pyruvate to be used oxidatively via the tricarboxylic acid cycle (Pösö and Puolanne 2005). Meat processing, such as in the production of sausages and frankfurters, can increase the carbohydrate content by adding sugars, starch products, and others (Costa-Lima and others 2014).

The predominant acid in muscle tissue is the lactic acid formed by glycolysis, followed by glycolic and succinic acids. Pyruvate, generated as the end product of glycolysis, is converted to lactic acid by lactic dehydrogenase, and since the metabolic waste products cannot be removed without blood flow, the lactic acid accumulates in the muscle. Other acids of the Krebs cycle are present in negligible amounts (Greaser 2001; Kauffman 2001). The aerobic mechanism in muscle produces energy from glycogen, which normally comprises about 1% of the muscle weight. When the muscle is contracting rapidly, its oxygen supply becomes inadequate to support ATP resynthesis via aerobic metabolism. Under these conditions, the aerobic metabolism supplies energy for a short time, converting glycogen to lactic acid, especially after slaughtering. In beef muscle, 48 h post mortem, the glycogen level drops rapidly from the initial value and the lactic acid level increases (Savenije and others 2002).

Various microorganisms produce organic acids and alcohols by anaerobic fermentation of food substrates, which then inhibit other organisms that are present and may spoil the food or make it toxic. Lactic acid, for example, is an effective inhibitory agent that is frequently used to preserve fresh meat (Theron and Lues 2007). Other organic acids may cause discoloration and production of pungent odors (Zhou and others 2010). For example, Samelis and others (2005) evaluated combinations of nisin with or without lactic and acetic acids as inhibitors of *Listeria monocytogenes* in sliced pork bologna. Lactic and acetic acids may be present in meat, because they are used in the beef industry to decontaminate carcasses or meat products. The effectiveness of these acids depends on the concentration and temperature of the acid solution, exposure time and application pressure, application stage in the slaughtering

process, tissue type, group of microorganisms, and initial concentration (Li and others 2015). Therefore, a higher concentration of lactic and/or acetic acid might be expected in meats treated with these acids (Carpenter and others 2011).

In fermented meat products, the production of organic acids by bacteria is undoubtedly the determining factor for the shelf life and safety of the final product. This is due to the immediate and rapid formation of acids at the beginning of the fermentation process, and the production of sufficient amounts of organic acids to lower the pH below 5.1 (Maijala and others 1993). Several factors can affect the type of organic acid present, including the microorganism involved in the fermentation process. The homofermentation routes produce more than 85% lactic acid as a major end product of glucose catabolism, while the hetero- or mixed-acid fermentation routes yield not only lactic acid (50%), but also formic and acetic acids as byproducts (Stiles and Holzafel 1997). However, few studies have assessed the production of organic acids in meat products.

Fish and derivatives

As with animal meats, fish meat is also a poor source of carbohydrates. Processing of fish can increase the carbohydrate content by the same means as described above. Lactic acid is also the main organic acid in fish meat. During the storage of fish, some organic acids formed include formic, acetic, propionic, n-butyric, isobutyric, n-valeric, and isovaleric acids (Osako and others 2005). As they are for animal meats, organic acids are also used as additives for the conservation of fish and derivatives (Mejlholm and Dalgaard 2007; Calo-Mata and others 2008; Tomé and others 2008; García-Soto and others 2014).

The fermentation process of fish products is similar to that at fermented meat, with lactic acid as the major product. In their study of Thai fermented fish under 4 different treatments, Saithong and others (2010) evaluated the production of 5 organic acids (lactic, acetic, butyric, propionic, and gluconic). They observed that lactic and gluconic acids were present in all treatments, but their behavior differed depending on the treatment. Butyric, succinic, acetic, and propionic acids were not detected in any treatment during fermentation. There is a lack of information about organic acids in the meat of different fish species and their derived products.

Honey

Honey is a natural product produced by honeybees which collect nectar from flowers, convert it with regard to composition, and store it in honeycomb cells to mature (Codex Alimentarius 2001). Sugars and water are the main chemical constituents of honey (>95%), and proteins, flavor- and aroma-producing compounds, pigments, vitamins, free amino acids, and numerous volatile compounds constitute the minor components. The honey carbohydrate content mainly includes a complex mixture of 70% monosaccharides (glucose and fructose), 10% disaccharides, and small amounts of trisaccharides and tetrasaccharides (White and Winters 1988). Due to its composition, honey can be adulterated in various ways. One method of honey adulteration is the addition of syrups made from different sugars (Tosun 2013) such as glucose. Chromatographic analysis can be used to detect changes caused by the addition of other carbohydrates such as cornstarch.

Honey acidity is mainly due to its content of less than 0.5% organic acids. The acidity contributes to the flavor, stability in the presence of microorganisms, enhancement of chemical reactions, and antibacterial and antioxidant activities. Gluconic acid, resulting from the action of honey's glucose oxidase on glucose, contributes most to the acidity and is in equilibrium with gluconolactone. Other organic acids, together with inorganic anions, also contribute to the acidity of honey (Cavia and others 2007). The acid level is mostly dependent on the time elapsed between the nectar collection by bees and the final honey density in the honeycomb cells. Other acids, such as acetic, butyric, lactic, citric, succinic, formic, malic, maleic, and oxalic acids, are also present in small amounts. There are also differences in composition of organic acids in the monofloral honey varieties. Therefore, the acids can be used as internal standards in order to detect honey adulteration (Daniele and others 2012).

The organic acids comprise a small proportion of honey (0.5%) and together with the total acidity can be used as an indicator of deterioration due to storage or aging, or to measure the purity and authenticity (Cavia and others 2007). They are also components of the honey flavor. Some organic acids identified in honey may be useful for characterizing different honey types. For example, the citric acid concentration is used as a reliable parameter for the differentiation of 2 main types of honey, floral and honeydew (Daniele and others 2012).

Carbohydrate Metabolism and Organic Acid Production by Lactic Acid Bacteria

Lactic acid bacteria (LAB) are Gram-positive, microaerophilic, acid-tolerant, nonspore-forming, mainly nonmotile rods or cocci. They are characterized by the majority production of L (+) and/or D (−) lactic acid from the fermentation of sugars, including lactose. The main characteristic of LAB, which renders this group of organisms ideal as a starter culture in the fermentation of food, is their ability to produce organic acids and thereby also to decrease the pH in food (Røssland and others 2005). Lactic acid bacteria occur naturally in various foodstuffs; either their growth is enhanced, or they are added deliberately to produce a range of fermented foods. These include fish, meat, various dairy products, cereals, fruits, and vegetables including legumes. This important group of starter cultures is used in the production of a wide range of fermented foods; they contribute to the enhancement of the characteristics of food; and they have been recognized as contributing to the microbial safety of fermented food (O'Sullivan and others 2002). The LAB have an important antimicrobial function, due to their production of certain metabolites such as organic acids (Messens and Vuyst 2002).

Lactic acid bacteria lack the enzymes necessary for respiration, and they are therefore unable to perform oxidative phosphorylation. Consequently, their energy requirements are met solely through substrate-level production of adenosine triphosphate (ATP) or its equivalent from carbohydrates. In addition, lactic acid bacteria can use homolactic or heterolactic fermentation metabolic pathways (Kandler 1983). Bacterial homolactic fermenter strains are able to convert the fermented carbohydrate into products other than lactate, and the end products are represented with the enzymes catalyzing the reactions. Heterolactic fermentation can simultaneously produce various other metabolites in addition to lactic acid, such as acetic acid, fumaric acid, ethanol, malic acid, and soon. These LAB metabolize citrate or induce oxidase enzyme activity; oxidase acts on NADH producing acetic acid, ethanol and other carbonylic compounds (Laleye and others 1990).The amount of these metabolites can significantly influence the downstream process and the quality of the L(+)-lactic acid produced (Wang and others 2005). Not all LAB produce the same lactic acid isomer (Gravesen and others 2004). The levels and also the type of organic acids that are produced during a fermentation

process are, therefore, dependent on the LAB species or strains, growth conditions, and food composition (Ammor and others 2006).

HPLC Analysis

The analysis of carbohydrates and organic acids in different food items such as dairy products, meat products, and honey is of great interest for the food industry. These compounds are responsible for sensory properties, deterioration, and authenticity, identification, and they may also influence the stability of these matrixes (Rodrigues and others 2007). For this reason, different HPLC techniques have been used for the separation and identification of these compounds in different foods (Van Hees and others 1999), such as those of animal origin. HPLC methods have gained importance in these analyses because of the speed, selectivity, sensitivity, and reliability of this technology (Chen and others 2006). Table 1 shows the different HPLC methods for the determination of carbohydrates and organic acids in foods of animal origin.

Sample preparation

The extraction is usually performed using an acid, which may be the only mobile phase, but with a higher concentration, such as sulfuric and phosphoric acids. However, for meat samples, perchloric acid (PCA) is the most often used and the most efficient. The centrifugation may be used or not, depending mainly on the type of food to be analyzed. Most investigators who apply centrifugation use a force range from 6000 to 17000 × *g*; however, in dairy products, the use of 5000 × *g* of rotation is sufficient (Gaze and others 2015). The supernatant generally is filtered through a 0.22- or 0.45-μm cellulose acetate filter, and the preparation obtained is then ready to inject into the apparatus (González de Llano and others 1996; Suárez-Luque and others 2002a,b; Kaminarides and others 2007; Leite and others 2013; Gaze and others 2015). The use of centrifugation in the analysis of carbohydrates and organic acids in complex matrices facilitates the extraction, yielding a purer final extract.

Separation columns

Liquid chromatography has simplified the analysis of various food constituents, including carbohydrates and organic acids. In chromatography, the selection of the stationary phase is essential in order to achieve a suitable separation. A number of different separation mechanisms have been widely employed in different matrixes, including ion-exchange, ion-exclusion, ion-pair, hydrophilic interaction, and reverse-phase. The choice of method is dictated essentially by the type and extent of analyte to be determined, as well as by the nature of the food matrix (Quirós and others 2009; Churms 1996). For the determination of carbohydrates and organic acids in foods of animal origin the most usual method is ion-exchange chromatography (Leite and others 2013; Wang and others 2013; Gaze and others 2015) followed by reverse-phase chromatography (Murtaza and others 2012; Terol and others 2012; Zhou and others 2014).

For carbohydrates, hydrophilic interaction chromatography (HILIC) and ion-exchange chromatography (Dvořáčková and others 2014) are widely used. Although both hydrophilic interaction and ion exchange are effective in the separation, the former is more commonly used in the separation of mono- and oligosaccharides, and the latter for mono- and disaccharides.

The ready ionization of organic acids has long been exploited for their isolation by ion-exchange chromatography, which involves the use of an ion-exchange resin as the stationary phase (Sriphochanart and Skolpap 2011; Leite and others 2013; Wang and others 2013; Ahmed and others 2015; Gaze and others 2015). This separation technique is widely used nowadays, and the column most frequently used for this purpose is the Aminex HPX-87H (300 × 7.8 mm) from Bio-Rad Laboratories (Hercules, CA, USA) (Fernandez-Garcia and Mcgregor 1994; Gonzalez de Llano and others 1996; Zeppa and others 2001; Adhikari and others 2002; Ong and others 2006; Donkor and others 2007; Kaminarides and others 2007; Ong and others 2007; Kaminarides and others 2009; Sriphochanart and Skolpap, 2011; Madureira and others 2013; Leite and others 2013). One of the main reasons for the use of this particular column is its length (300 mm), which provides a good separation of peaks, facilitating the simultaneous analysis of carbohydrates and organic acids.

The stationary phases that are most often used in bonded-phase chromatography in its reversed-phase mode are based on octyl (C8 columns) and octadecyl (C18 columns) functionality. The difference between the 2 columns lies in the length of the carbon chain attached to the silica surface; for organic-acid analysis, the C18 column is most often used (Bevilacqua and Califano 1992; Tormo and Izco 2004; Saithong and others 2010; Bensmira and Jiang 2011; Murtaza and others 2012).

Detection

The detectors most frequently used in HPLC for analysis of carbohydrates and organic acids are the conductivity (CD), the pulsed amperometric (PAD), the refractive index (RI), the evaporative light scattering detector (ELSD), and the ultraviolet (UV), as well as the mass spectrometric (MS) detectors. In general, most frequently, detectors used for carbohydrate analysis are the CD, PAD, RI, and ELSD, and for the organic acids are the RI, ELSD, and UV (Yoshida and others 1999; Leite and others 2013; Qiangsheng and others 2013; Wang and others 2013; Zhou and others 2014; Gaze and others 2015). Nowadays, HPLC is widely used, with a dual-wavelength detection mode UV-VIS detector and RI detector for analyzing carbohydrates and nonvolatile organic acids in complex matrixes, in the same chromatographic run (Bouzas and others 1991; Eyéghé-Bickong and others 2012).

CDs were originally employed in ion chromatography for determination of inorganic ions, and later for organic acids. However, the inherent difficulties with these detectors have deterred potential users from applying them to food analyses. The reasons are that this type of detector has low selectivity; and the solute-conductivity measurements require prior elimination of eluent background conductivity, using a conventional suppressing column or a more modern alternative such as a cation-exchange membrane. Currently, due to its limitations, this type of detector is not widely used (Blanco 2000). However, it can be used for the analysis of carbohydrates in different food matrices, including foods of animal origin (Mullin and Emmons 1997; Yoshida and others 1999; Wang and others 2013).

The PAD operates using a triple-step potential waveform to combine amperometric detection with alternating anodic and cathodic polarization to clean and reactivate the electrode surface. This waveform exploits the surface-catalyzed oxidation of the amine group, activated by the transient formation of surface oxides on noble metals (Welch and others 1990). In alkaline solutions, which are useful for anion-exchange separation of carbohydrates, the PAD is significantly more sensitive than the CD. However, the CD provides a linear response for higher concentrations than those observed for PAD (Welch and others 1988). The combination

Table 1–HPLC methods for carbohydrates and organic acids determination in foods of animal origin

Sample	Carbohydrates and organic acids	Columns	Detector	Chromatographic conditions	Reference
Whole milk, powdered skim milk, cultured buttermilk, sour cream, yogurt, cottage, sharp, cheddar and blue cheeses	Orotic, citric, pyruvic, lactic, uric, formic, acetic, propionic, butyric, and hippuric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 and 275 nm	[a]0.009 N H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C; [d]10 μL	Marsili and others (1981)
Food	Lactic acid	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 nm	[a]0.009 N H_2SO_4; [b]0.7 mL/min isocratically; [d]10 μL	Ashoor and Knox (1984)
Coarsely ground beef	Lactic	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 nm	[a]0.048 N H_2SO_4; [b]0.8 mL/min isocratically; [c]60 °C; [d]20 μL	Nassos and others (1984)
Honey	Monosaccharides	Dionex Carbopac AS-6 pellicular anion-exchange (4.6 × 250 mm)	PAD	[a]22 mM NaOH; [b]1.0 mL/min isocratically	Hardy and others (1988)
Raw milk, yogurt, Blue, Provolone, Port Salut and Quartirolo cheeses	Formic, acetic, pyruvic, propionic, uric, erotic, citric, lactic, and butyric acids	Beckman C_8 (250 × 4.6 mm, 5 μm)	UV 214 nm	[a]Aqueous 0% to 5% (w/v) (NH4)2PO4–0.2% (v/v) acetonitrile; [b]1.2 mL/min isocratically; [c]room temperature; [d]20 μL	Bevilacqua and Califano (1989)
Honey	Monosaccharides	Dionex 10-rm Carbo Pac anion-exchange (4 × 250 mm)	PAD	[a]NaOH; [b]0.7 mL/min gradient; [d]50 μL	Swallow and Low (1990)
Cheddar cheese	Orotic, citric, pyruvic, lactic, uric, formic, acetic, propionic, butyric, and hippuric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 and 285 nm	[a]0.009 N H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C	Bouzas and others (1991)
Cheese	Lactic, formic, acetic, pyruvic, citric, orotic, and uric acids	Beckman C_8 (250 × 4.6 mm, 5 μm)	UV 214 nm	[a]Aqueous 0% to 5% (w/v) (NH4)2PO4–0.2% (v/v) acetonitrile; [b]1.2 mL/min isocratically; [c]room temperature; [d]20 μL	Bevilacqua and Califano (1992)
Honey	Pyruvic, quinic, malic, isocitric, succinic, fumaric, propionic, galacturonic, gluconic, tartaric, dimethylglyceric, 2-oxopentanoic, and glutaric acids	Spherisorb ODS 1S5 (250 × 4.6 mm, 5 μm)	UV 210 nm	[a]H_2SO_4 (pH 2.45); [b]0.7 mL/min isocratically	Cherchi and others (1994)
Yogurt	Orotic, citric, pyruvic, lactic, uric, formic, acetic, propionic, butyric and hippuric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 nm	[a]0075N H_2SO_4; [b]0.7 mL/min isocratically; [c]room temperature; [d]10 μL	Fernandez-Garcia and Mcgregor (1994)
Reggianito cheese	Formic, pyruvic, orotic, uric, lactic, acetic, citric, propionic, and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 214 and 280 nm	[a]0.009 N H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C; [d]10 μL	Lombardi and others (1994)
Milk and cheese	Citric, succinic, lactic, formic, acetic, propionic, orotic, uric, pyruvic, and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 nm	[a]3 mmol/L H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C; [d]20 μL	Gonzalez de Llano and others (1996)
Milk	Lactose, glucose, and galactose	Alphasil SNH_2 and Sugar Pak I (25 cm × 4.6 mm)	RI	[a]Acetonitrile:water (75:25, v/v); [b]1.0 mL/min isocratically; [c]room temperature. [a]Water held at 60 °C; [b]0.5 mL/min isocratically; [c]90 °C	Indyk and others (1996)
Cheeses	Formic, pyruvic, lactic, acetic, orotic, citric, uric, propionic, and butyric acids	Machery Nagel C18 (120 × 5 mm)	UV 214 nm	[a]Aqueous 0.5% (wt/vol) $(NH_4)_2HPO_4$ (0.038 M)–0.2% (vol/vol) acetonitrile (0.049 M); [b]0.3 mL/min isocratically; [c]room temperature	Akalin and others (1997)

(*Continued*)

Table 1–Continued

Sample	Carbohydrates and organic acids	Columns	Detector	Chromatographic conditions	Reference
Cheddar cheese	Lactic, formic, citric, and acetic acids	Dionex IonPac ICE-AS6 (9 × 259 mm)	PAD	[a]0.4 mM heptafluorobutyric acid; [b]1.0 mL/min isocratically	Mullin and Emmons (1997)
Low-fat cheese	Pyroglutamic, lactic, pyruvic, and uric acids	Aminex HPX 87 H ion-exchange column (300 × 7.8 mm)	UV 210 nm	[a]3 mmol/L H_2SO_4; [b]0.5 mL/min isocratically; [c]40 °C; [d]25 μL	Skeie and others (1997)
Cheddar cheese	Acetic, citric, butyric, fumaric, formic, hippuric, isovaleric, lactic, malic, orotic, oxalic, propionic, pyruvic, uric, and n-valeric acids	Supelcogel C-610H ion-exchange column (30 cm × 7.8 mm)	UV 210 and 290 nm	[a]0.1% H_3PO_4; [b]1.0 mL/min isocratically; [c]room temperature; [d]40 μL	Lues and others (1998)
Mozzarella cheese	Formic, pyruvic, orotic, uric, lactic, acetic, citric, propionic, and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 214 and 280 nm	[a]0.009 N H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C	Califano and Bevilacqua (1999)
Fermented milk	Benzoic acid	C18 column Spherisorb ODS (3.2 × 250 mm, packed with 5 mm)	UV	Isocratic reversed phase liquid chromatography	Suomalainen and Mäyrä-Mäkinen (1999)
Raw fish meat and dried meat	Lactic, acetic, pyroglutamic, citric, succinic, formic, phosphoric, and malic	Shim-Pack SCR-102H (i.d. 0.008 m × 0.30 m × 2) ion-exclusion column	CD	–	Yoshida and others (1999)
Gouda cheeses	Formic, orotic, uric, lactic, acetic, citric, pyruvic, propionic, and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 214 and 280 nm	[a]0.009 N H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C	Califano and Bevilacqua (2000)
Kefir	Orotic, citric, pyruvic, lactic, uric, acetic, propionic, butyric, and hippuric acids	Alltech IOA-1000 organic-acid column (300 mm × 7.8 mm)	UV 275 nm	[a]0.009 N H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C; [d]10 μL	Guzel-Seydim and others (2000)
Norvegia cheese	Pyroglutamic, lactic, pyruvic, and uric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 nm and RI	[a]0.013 mmol/L H_2SO_4; [b]0.8 mL/min isocratically; [c]65 °C; [d]25 μL	Skeie and others (2001)
Cheese	Citric, orotic, piruvic, lactic, oxalic, hippuric, formic, acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 and 290 nm	[a]30 mmol/L H_2SO_4; [b]0.4 mL/min isocratically; [c]30 °C; [d]25 μL	Zeppa and others (2001)
Yogurt	Acetic, lactic, citric, propionic, butyric, uric, and pyruvic acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 nm	[a]0.01 N H_2SO_4; [b]0.6 mL/min isocratically; [c]60 °C	Adhikari and others (2002)
Pickled White Cheese	Formic, pyruvic, lactic, acetic, orotic, citric, uric, propionic, and butyric acids	Machery Nagel C18 (120 × 5 mm)	UV 214 nm	[a]Aqueous 0.5% (wt/vol) $(NH_4)_2HPO_4$ (0.038 M)–0.2% (vol/vol) acetonitrile (0.049 M); [b]0.3 mL/min isocratically; [c]room temperature	Akalin and others (2002)
Cheddar cheese	Acetic, butyric, citric, formic, fumaric, hippuric, isovaleric, lactic, malic, n-valeric, orotic, propionic, pyruvic, and uric acids	Supelcogel C-610H ion-exchange column (30 cm × 7.8 mm)	UV 210 and 290 nm	[a]0.1% phosphoric acid mobile phase; [b]1.0 mL/min isocratically; [c]room temperature	Lues and Bekker (2002)
Honey	Malic, citric, succinic, fumaric, and maleic acids	Spherisorb ODS-2 S5 (4.6 mm × 250 mm)	UV 215 nm	[a]4.5% metaphosphoric acid; [b]0.7 mL/min isocratically; [c]25 °C; [d]20 μL	Suárez-Luque and others (2002a)
Honey	Malic, citric, succinic, fumaric, and maleic acids	Spherisorb ODS-2 S5 (4.6 mm × 250 mm)	UV 215 nm	[a]4.5% metaphosphoric acid; [b]0.7 mL/min isocratically; [c]25 °C; [d]20 μL	Suárez-Luque and others (2002b)
Honey	Gallic, caffeic, ferulic, benzoic, and cinnamic acids	C_{18} column (150 × 4.6 mm, 5 μm)	UV 280 nm	–	Aljadi and Yusoff (2003)
Honey	Sugar profile	CarboPac column–anion exchange (4 × 250 mm)	PAD	[a]Water and NaOH (48:52, v/v); [b]0.6 mL/min isocratically	Cordella and others (2003)

(*Continued*)

Table 1–Continued

Sample	Carbohydrates and organic acids	Columns	Detector	Chromatographic conditions	Reference
Milk-based formulae	Mono- and disaccharides	Tracer carbohydrates (250 × 4.6 mm i.d.)	RI	[a]Acetonitrile–water (75:25, v/v); [b]1.8 mL/min isocratically; [c]25 °C; [d]20 μL	Chávez-Servín and others (2004)
Raw milk, yogurt and cheese	Oxalic, citric, formic, succinic, orotic, uric, pyruvic, acetic, propionic, lactic, and butyric acids	Atlantis dC18 column (Waters) (250 mm × 4.6 mm, 5 μm)	UV 210 nm	[a]1% of acetonitrile in 20 mM phosphate buffer adjusted at pH 2.20 with phosphoric acid (Solvent A) and acetonitrile (Solvent B); [b]1.5 mL/min gradient; [c]room temperature; [d]10 μL	Tormo and Izco (2004)
Goat milk cheeses	Citric, pyruvic, malic, lactic, formic, acetic, propionic, uric, and butyric acids	Supelcogel C-610H ion-exchange column (300 × 7.8 mm)	UV 2010 and 290 nm	[a]0.1 N H_3PO_4; [b]1.0 mL/min isocratically; [d]40 μL	Buffa and others (2004)
Goat milk cheese	Tartaric, formic, orotic, malic, lactic, acetic, citric, uric, propionic, and butyric acids	ODS Hypersil (125 mm × 4 mm, 5 μm)	UV 214 nm	[a]0.5% (w/v) $(NH_4)_2HPO_4$; [b]0.3 mL/min isocratically; [d]50 μL	Park and Drake (2005)
Low-fat Feta-type cheese	Lactic, citric, and acetic acids	Hamilton column, hydrogen form (305 × 7.8 mm, 10 μm)	UV 210 and 280 nm	[a]0.014 N H_2SO_4; [b]0.6 mL/min isocratically; [c]62 °C	Manolaki and others (2006)
Yogurt	Lactic and acetic acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 nm	[a]0.009 N H_2SO_4; [b]0.6 mL/min isocratically; [c]65 °C	Ong and others (2006)
Goat milk cheese	Acetic, butyric, citric, formic, lactic, malic, isomalic, orotic, propionic, pyruvic, tartaric, isotartaric, and uric acids	Hypersil ODS (125 × 4 mm, 5 μm)	UV 214 nm	[a]0.5% (w/v) $(NH_4)_2HPO_4$; [b]0.3 mL/min isocratically; [d]50 μL	Park and others (2006)
Monterey Jack goat milk cheeses	Tartaric, formic, orotic, malic, lactic, acetic, citric, uric, propionic, and butyric acids	Hypersil ODS (125 mm × 4 mm, 5 μm)	UV 214 nm	[a]0.5% (w/v) $(NH_4)_2HPO_4$; [b]0.3 mL/min isocratically; [d]50 μL	Park and Lee (2006)
Yogurt	Lactic, acetic, butyric, and propionic acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 nm	[a]0.01 M H_2SO_4; [b]0.6 mL/min isocratically; [c]65 °C	Donkor and others (2007)
Milk and yogurt	Citric, pyruvic, and lactic acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	RI	[a]5 mM H_2SO_4; [b]0.5 mL/min isocratically; [c]35 °C; [d]20 μL	Kaminaride and others (2007)
Cheddar cheese	Lactic and acetic acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 nm	[a]0.009 N H_2SO_4; [b]0.6 mL/min isocratically; [c]65 °C	Ong and others (2007)
Cheddar cheese	Lactic, acetic, citric, propionic, and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 nm	[a]0.009 N H_2SO_4; [b]0.6 mL/min isocratically; [c]65 °C	Ong and Shah (2008)
Milk-based formulae	Glucosamine and lactose	Shodex Asahipak NH2P-50 (4.6 × 250 mm)	RI	[a]Water–acetonitrile (30/70, v/v); [b]1.0 mL/min isocratically; [d]20 μL	Xinmin and others (2008)
Halloumi-type cheese	Acetic, pyruvic, and lactic acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	RI	[a]5 mM H_2SO_4; [b]0.5 mL/min isocratically; [c]35 °C; [d]20 μL	Kaminarides and others (2009)
Cheddar cheese	Lactic, acetic, citric, propionic, and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 nm	[a]0.009 N H_2SO_4; [b]0.6 mL/min isocratically; [c]65 °C	Ong and Shah (2009)
Milk	Lactose, glucose, galactose, and oligosaccharides	Waters Sugar Pak I column (6.5 × 300 mm)		[a]Water; [b]0.4 mL/min isocratically; [c]80 °C; [d]20 μL	Nguyen and others (2009)
Halloumi cheese	Lactic, citri,c and acetic acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 220 nm	[a]0.009 N H_2SO_4	Ayyash and Shah (2010)
Honey	Fructose, glucose, disaccharides, trisaccharides	Carbopac PA1 anion-exchange (4 × 250 mm)	PAD	[a]Water and 0.2 M NaOH; [b]0.5 mL/min gradient; [d]25 μL	Ouchemoukh and others (2010)
Thai fermented fish	Lactic, acetic, butyric, propionic, and gluconic	Alltech Platinum EPS C18 column (4.6 × 150 mm)	UV 210 nm	[a]0.05 M KH_2PO_4; [b]1.0 mL/min isocratically; [d]20 μL	Saithong and others (2010)

(*Continued*)

Table 1–Continued

Sample	Carbohydrates and organic acids	Columns	Detector	Chromatographic conditions	Reference
Milk	Lactose and lactulose	Rezex RCM-Monosaccharide Ca^{+} (300 × 7.8), Prevail Carbohydrate ES (250 × 4.6), Sphere Clone NH2 (250 × 4.6), Zorbax Carbohydrate Analysis (250 × 4.6)	ELSD	[a]Acetonitrile and water (70:30, v/v); [b]0.9 mL/min isocratically; [c]25 °C	Schuster-Wolff-Bühring and others (2010)
Kashar cheese	Citric, lactic, formic, acetic, propionic, and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 214 and 280 nm	[a]0.009 N H_2SO_4; [b]0.6 mL/min isocratically; [c]65 °C	Andiç and others (2011)
Kefir	Lactic, citric, pyruvic, and acetic acids	Diamonsil C18 column (46 × 250 mm, 5 μm)	UV 275 nm	[a]0.05% CH_3OH; [c]30 °C	Bensmira and Jiang (2011)
Human and cow's milk	Lactose	ACQUITY UPLC BEH C18 1.7 μm column (2.1 × 100 mm)	MS	[a]Water with 0.1% formic acid and acetonitrile with 0.1% formic acid; [b]0.3 mL/min gradient; [c]35 °C	Fusch and others (2011)
Sheep milk and Manchego cheese	Citric, pyruvic, lactic, formic, acetic, propionic, butyric, orotic, and uric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 and 280 nm	[a]3 mM H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C; [d]20 μL	Garde and others (2011a)
Ovine milk cheese	Lactic acid	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 and 280 nm	[a]3 mM H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C; [d]20 μL	Garde and others (2011b)
Dairy matrix	Inulin, fructose, and glucose	Rezex RCMMonosaccharide column (300 × 7.8 mm)	ELSD	[a]Water; [b]0.5 mL/min isocratically; [c]80 °C; [d]20 μL	Kristo and others (2011)
Cheese	Lactose, acetic, and lactic acids	Chrompack column (300 × 6.5 mm)	UV	[a]5 mM H_2SO_4; [b]0.5 mL/min isocratically; [c]60 °C; [d]20 μL	Magalhães and others (2011)
Thai fermented sausage	Lactic, acetic, and formic	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 nm	[a]0.02 M H_2SO_4; [b]0.6 mL/min isocratically; [c]60 °C	Sriphochanart and Skolpap (2011)
Cheddar cheese	Lactic, formic, and oxalic acids	HP1050 equipped with a Prevail (150 × 4.6 mm, 5 μm)	UV 200 nm	[a]25 mM KH_2PO; [b]1.5 mL/min isocratically; [d]10 μL	Subramanian and others (2011)
Skim milk	Lactose, glucose and galactose	RP-C_{18} column HyPurity (150 × 4 mm)	UV 303	[a]20 mM $TBAHSO_4$ in sodium phosphate buffer (0.1 M, pH 6.5) with methanol (50:50, v/v) adjusted to each pH value by adding orthophosphoric acid and 20 mM $TBAHSO_4$ in sodium phosphate buffer (0.05 M, pH 6.5) adjusted to each pH value by adding orthophosphoric acid; [b]0.5 mL/min gradient; [d]10 μL	Erich and others (2012)
Cheese	Lactose, glucose, galactose, citric, pyruvic, lactic, acetic, propionic and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	RI UV 210 nm	[a]3 mM H_2SO_4; [b]0.7 mL/min isocratically; [c]65 °C; [d]20 μL	Garde and others (2012)
Infant formula	Sialic acid	Scientific Dionex CarboPac PA20 column (2.1 × 100 mm)	PAD	[a]Sodium acetate; [b]0.5 mL/min gradient; [c]30 °C; [d]10 μL	Hurum and Rohrer (2012)
Milk	Lactic acid	Aminex HPX ion-exchange column (300 × 7.8 mm)	UV 210 nm	[a]1.056 N H_3PO_4; [b]0.6 mL/min isocratically; [c]50 °C; [d]50 μL	Milagres and others (2012)
Buffalo cheese	Lactic, acetic, citric, pyruvic, formic, butyric, and maleic acids	Shim-Pack C_{18} (LC) column (3.9 × 150 mm)	UV 214 nm	[a]Aqueous 0.5% (w/v) $(NH_4)_2HPO_4$ (0.038 M)–0.2% (v/v) acetonitrile (0.049 M); [b]0.5 mL/min isocratically; [c]room temperature	Murtaza and others (2012)
Liquid milk and powdered milk	Carbohydrates	Hypercarb (100 × 4 mm)	ELSD	[b]1.0 mL.min^{-1}; [d]20 μL	Terol and others (2012)
Whey cheese	Succinic, citric, lactic, and acetic acids	Aminex HPX ion-exchange column (300 × 7.8 mm)	RI UV 220 nm	[a]13 mmol/L H_2SO_4; [b]0.8 mL/min isocratically; [c]65 °C; [d]20 μL	Madureira and others (2013)

(*Continued*)

Table 1–Continued

Sample	Carbohydrates and organic acids	Columns	Detector	Chromatographic conditions	Reference
Kefir	Citric, succinic, lactic, formic, acetic, propionic, and butyric acids	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV - 210 nm	[a]3 mM H_2SO_4; [b]0.5 mL/min isocratically; [c]65 °C; [d]50 μL	Leite and others (2013)
Milk	Benzoic acid	Metrosep A5 250 anion-exchange column (250 mm × 4.0 mm, 5 μm)	CD	[a]3.2 mmol/L aqueous Na_2CO_3 and 1.0 mmol/L aqueous $NaHCO_3$; [b]0.7 mL/min isocratically; [c]30 °C; [d]20 μL	Wang and others (2013)
Honey	Glucose, fructose, sucrose, and maltose	Rezex RCM cation-exchange column (300 × 7.8 mm)	RI	[a]Water; [b]0.6 mL/min isocratically; [c]80 °C; [d]20 μL	Özbalci and others (2013)
Honey	Fructose, glucose, sucrose, maltose	Prevail carbohydrate ES column (250 × 4.6 mm)	ELSD	–	Qiangsheng and others (2013)
Honey	Malto-oligosaccharides	Waters ACQUITY BEH amide (2.1 × 50 mm, 1.7 μm)	ELSD	[a]0.2% triethylamine in pure water and 0.2% triethylamine in acetonitrile.; [b]0.5 mL/min gradient; [c]60 °C; [d]20 μL	Zhou and others (2014)
Ready-to-eat meat and poultry products	Lactate and acetate	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	UV 210 nm	[a]0.005 M H_2SO_4; [b]0.6 mL/min isocratically; [c]60 ° C; [d]20 μL	Ahmed and others (2015)
Dulce de leche	Lactose, sucrose, and glucose	Aminex HPX-87H ion-exchange column (300 × 7.8 mm)	RI	[a]Water; [b]0.5 mL/min isocratically; [c]60 °C; [d]20 μL	Gaze and others (2015)

CD, conductivity detector; PAD, pulsed amperometric detector; RI, refractive index detector; ELSD, evaporative light scattering detector; UV, ultraviolet detector; MS, mass spectrometric detector.
[a]Mobile phase; [b]flow; [c]temperature; [d]injection volume.

Table 2–GC methods for determination of carbohydrates and organic acids in foods of animal origin

Sample	Organic acids	Columns	Detector	Chromatographic conditions	Authors
Coarsely ground beef	N-propyl derivatives of lactic and glutaric acids	Glass column (1.8 m × 2.0 mm i.d.) was packed with 80/100 mesh Chromosorb W-HP coated with 10% AT-1000	FI	[a]Helium; [b]linear velocity of 30 cm/s; [c]100 to 180 °C at a rate of 8 °C/min held at 240 °C for 6 min	Nassos and others (1984)
Milano salami	2 organic acids	Capillary coated with a DB-5 stationary phase (30 m × 0.32 mm, 1-μm film thickness)	FI	[a]Hydrogen; [b]linear velocity of 3 mL/min; [c]40 °C for 5 min and then increased to 200 °C at 3 °C/min	Meynier and others (1999)
Fermented milk	Acetic and propionic acids	Chromosorb WAW 80/100 as the stationary phase (3 m × 2 mm, i.d.)	FI	–	Suomalainen and Mäyrä-Mäkinen (1999)
Kefir	Volatile component	Capillary column (DB-5, J&W Scientific, Folsom, Calif., U.S.A.) (0.32 i.d. × 30 × 1 μm)	FI	[a]Helium; [b]linear velocity of 30 mL/min.; [c]20 to 30 °C at 5 °C/min and 30 to 220 °C at 10 °C/min	Guzel-Seydim and others (2000)
Fresh milk, spoiled milk, fermented milk, yogurt drink, and lactic acid beverage	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, caprylic, capric, lauric, lactic and levulinic acids	Chrompack CP-Wax column (30 m × 0.53 mm)	FI	[a]Helium; [b]linear velocity of 3 mL/min; [c]75 °C for 1 min, raised to 180 °C at 6 °C/min, then increased to 230 °C at 10 °C/min, and held at 230 °C for 5 min	Yang and Choong (2001)
Italian sausages	Acetic, butanoic, 2-methylpropanoic, 3-methylbutanoic and pentanoic acids	Carbowax capillary (30 m × 0.25 mm i.d., film thickness 0.25)	MS	[a]Helium; [b]linear velocity of 35 cm/s; [c]40 °C for 5 min, ramped to 240 °C at 4 °C/min and held at 240 °C for 15 min	Spaziani and others (2009)
Pecorino di Farindola cheese	Volatile component	Fused silica capillary column coated with a 0.2 μm film of Carbowax (30 m × 0.32 μm i.d.)	MS	[a]Helium; [c]50 °C for 2 min, increased at 1 ∘C/min to 65 °C and increased at 5 °C/min to 220 °C and held for 22 min	Suzzi and others (2014)

FI, flame ionization detector; MS, mass spectrometric detector.
[a]Gas; [b]pressure; [c]ramp.

of these 2 detectors may be a useful strategy to improve the resolution in the chromatograms. Some studies have used this detector for the analysis of carbohydrates in foods of animal origin (Mora and Marioli 2001; Cordella and others 2003; Hurum and Rohrer 2012).

The RI detector responds to a difference in the refractive index of the column effluent as it passes through the detector flow cell. RI detection has been used successfully for the analysis of sugars, triglycerides, and organic acids (Swartz 2010). The RI detector is a bulk-property detector that responds to all solutes, if the refractive index of the solute is sufficiently different from that of the mobile phase. These detectors are somewhat sensitive to changes in pressure, temperature, and composition of the mobile phase, which requires strict control of the chromatographic conditions and the use of isocratic elution. Despite its limitations, the RI detector has the advantage of being usable for determining other components of interest, such as carbohydrates, simultaneously in a single chromatographic analysis (Morgan and Smith 2011).

Evaporative light scattering detection works by nebulizing the column effluent, forming an aerosol that is further converted into a droplet cloud for detection by light scattering. Therefore, ELSD requires the vaporization of the compounds analyzed. Consequently, the chromatography eluent is dependent of the detection system. Currently, ELSD is gaining popularity due to its ability to detect analytes on a nonselective basis. This type of detector has been applied to studies of carbohydrates (Wei and Ding 2000; Liu and others 2012; Dvořáčková and others 2014), and lipids (Rodríguez-Alcalá and Fontecha 2010; Imbert and others 2012; Kobayashi and others 2013).

The most widely used detectors in modern HPLC are photometers based on ultraviolet (UV) and visible light (VIS) absorption (Saithong and others 2010; Sriphochanart and Skolpap 2011; Leite and others 2013; Ahmed and others 2015). They have a high sensitivity for many solutes, including organic acids, but samples must absorb in the UV region (Swartz 2010). These detectors are no doubt the most frequently used at present for determining organic acids in food. They can be used for analysis of underivatized organic acids, with detection at 206 to 220 nm, which usually poses no serious problem for the determination of major organic acids (Blanco 2000; Saithong and others 2010; Sriphochanart and Skolpap 2011; Murtaza and others 2012; Madureira and others 2013; Leite and others 2013). Nevertheless, this detector is not used for carbohydrate analysis. These compounds absorb light at wavelengths within the 190 to 200 nm range, which corresponds to the spectrum region of many organic compounds present in foods and organic solvents (Paredes and others 2006).

The mass spectrometric detector is the most sophisticated hyphenated HPLC detector in use today ("hyphenated" refers to the coupling of an independent analytical instrument to provide detection). For complex samples, mass spectrometry (MS) coupled with liquid chromatography is a powerful technique, due to its high sensitivity and selectivity (Chen and others 2007).

Chromatography conditions

Selection of the chromatography conditions used for the analysis of carbohydrates and organic acids depends on several factors, such as the detector and column used. For example, the RI detector

cannot be used with a gradient flow rate to separate the analyte, since the baseline becomes unstable, and an isocratic flow rate is necessary. For ELSD and UV detectors, a gradient can be used with no effect on the baseline. The chromatographic conditions are extremely variable. Therefore, different types of mobile phase and flow, and a gradient, may or may not be applied.

GC Analysis

GC methods provide good sample resolution and sensitivity. For carbohydrates, the analytes require prior derivatization to make them volatile (Armstrong and Jin 1989), and GC is not widely used for this analysis. However, GC is an attractive alternative to analyze organic acids, because of its simplicity, separation efficiency, and excellent sensitivity and selectivity (Ballesteros and others 1994; Yang and Choong 2001; Horák and others 2008 2009). Many short-chain organic acids are thermostable and sufficiently volatile, thus fulfilling key requirements for GC measurement (Grosch 2004). Furthermore, the method of choice for analysis of volatile acids is GC, instead of the isolation of compounds from the cheese matrix, which can be carried out by different methods, such as high-vacuum distillation, simultaneous-distillation extraction, supercritical fluid extraction, or headspace techniques (Fernández-García and others 2002).

Sample preparation

In general, the great complexity of food samples demands an appropriate sample preparation technique before analysis. As a rule, beverages usually require only a simple pretreatment such as dilution and/or filtration, but for other foods the potential interference of matrix compounds (fats, vitamins, proteins, polysaccharides) requires the employment of more complex pretreatment and clean-up procedures (Kritsunankul and others 2009; Rovio and others 2010).

Traditional methods, such as liquid–liquid extraction, are time-consuming and environment unfriendly (Grosch 2004). Solid-phase extraction (SPE) can be implemented via flow systems, resulting in dramatically increased efficiency and reduced analytical cost through decreased reagent consumption (Cherchi and others 1994; Mota and others 2003; Horák and others 2009). Other alternatives such as single-drop microextraction (Saraji and Mousavinia 2006), solid-phase microextraction (Wen and others 2007) and stir-bar sorptive extraction (Horák and others 2008) have also been successfully applied for the analysis of short- and medium-chain fatty acids and preservatives in vinegar, beverages, and dairy products.

Derivatization

Other acids must be derivatized in order to convert these compounds into less polar and stable derivatives that are suitable for GC determination (Saraji and Mousavinia 2006; Horák and others 2009). To avoid the need for derivatization of organic acids, some investigators have successfully employed capillary GC columns coated with polar stationary phases such as polyethylene glycol or nitroterephthalic acid-modified polyethylene glycol. With these columns it is possible to obtain good chromatographic resolution, avoiding peak tailing (Yang and Choong 2001; Horák and others 2008).

Detection

The flame ionization detector (FID) is the most widely and successfully used gas chromatographic detector for volatile hydrocarbons such as organic acids. However, the presence of oxygen molecules decreases the detector's response. Therefore, highly oxygenated molecules or sulfides might best be detected by using another detector instead of the FID. Determination of sulfides by the flame-photometric detector and analysis of aldehydes and ketones with the photoionization detector are alternatives to the use of the FID for these molecules (Colón and Baird 2004).

In order to measure the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields. MS is an analytical technique that precisely measures the molecular masses of individual compounds and atoms by converting them into charged ions. MS has been applied in food chemistry for the analysis of toxic compounds and contaminants, for nutraceuticals, and for the characterization of foodstuffs to be applied for production areas and traceability (Yang and Caprioli 2011). However, there are few studies using MS for analysis of organic acids in honey, sausages, and cheese (Aljadi and Yusoff 2003; Spaziani and others 2009; Suzzi and others 2014). Thus, this methodology is not widely used in the analysis of carbohydrates and organic acids in food of animal origins. Therefore, more studies are needed on the application of MS to analyze these compounds in these matrixes.

Chromatography conditions

The chromatography conditions used for the analysis of carbohydrates and organic acids by GC depend on several factors, such as the column used and compound analyzed. The chromatographic conditions are extremely variable. Therefore, the columns used and compound analyzed for the determination of carbohydrates and organic acids in foods of animal origin by GC methods are shown in Table 2.

Conclusion

The chromatographic techniques are more relevant in some foods of animal origin, such as honey and milk products. Also, GC and HPLC provide different advantage for carbohydrates and organic acids considering the matrix analyzed.

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Author Contributions

Costa MP researched prior studies and interpreted the articles, compiled data, and drafted the manuscript. Conte-Junior CA edited and corrected the manuscript.

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6.4 COMPROVANTE DE SUBMISSÃO ARTIGO IV

Elsevier Editorial System(tm) for Talanta
Manuscript Draft

Manuscript Number:

Title: Simultaneous analysis of carbohydrates and organic acids by HPLC-DAD-RI for monitoring goat's milk yogurts fermentation

Article Type: Research Paper

Keywords: Validation; lactose; lactic acid; cupuassu pulp; probiotic; inulin

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Abstract: During yogurt manufacture, the lactose fermentation and organic acid production can be used to monitor the fermentation process by starter cultures and probiotic bacteria. In the present work, a simple, sensitive and reproducible high-performance liquid chromatography with dual detectors, diode array detector and refractive index was validated by simultaneous analysis of carbohydrates and organic acids in goat milk yogurts. In addition, pH and bacterial analysis were performed. Separation of all the compounds was performed on an Aminex HPX-87H column (300 x 7.8 mm, 9 µm) utilizing a 3 mmol.L-1 sulfuric acid aqueous mobile phase under isocratic conditions. Lactose, glucose, galactose, citric, lactic and formic acids were used to evaluate the following performance parameters: selectivity, linearity, precision, limit of detection (LOD), limit of quantification (LOQ), decision limits (CCα), detection capabilities (CCβ), recovery and robustness. For the method application a six goat milk yogurts were elaborated: natural, probiotic, prebiotic, symbiotic, cupuassu, and probiotic with cupuassu. The validated method presented an excellent selectivity with no significant matrix effect, and a broad linear study range with coefficients of determination higher than 0.99. The relative standard deviation was lower than 10% under repeatability and within-laboratory reproducibility conditions for the studied analytes. The LOD of the method was defined from 0.001 to 0.003 µg.mL-1, and the LOQ from 0.003 to 0.013 µg.mL-1. The CCα was ranged from 0.032 to 0.943 µg.mL-1, and the CCβ from 0.053 to 1.604 µg.mL-1. The obtained recovery values were from 78% to 119%. In addition, the method exhibited an appropriate robustness for all parameter evaluated. Base in our data, it was concluded that the performance parameters demonstrated total method adequacy for the detection and quantification of carbohydrates and organic acids in goat milk yogurts. The application of the method was successfully applied to monitoring different goat milk yogurts during fermentation.

6.5 COMPROVANTE DE SUBMISSÃO ARTIGO V

Elsevier Editorial System(tm) for LWT - Food Science and Technology

Manuscript Draft

Manuscript Number:

Title: Effect of different fat replacers on the physicochemical, color, apparent viscosity and texture properties of low-fat cupuassu goat milk yogurts

Article Type: Short communication

Keywords: inulin; maltodextrin; whey protein; skim milk powder; instrumental analysis

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Abstract: This study examined changes to the physicochemical properties, color, apparent viscosity, and texture resulting from the addition of inulin, maltodextrin, whey protein and skim milk powder to a low-fat goat milk yogurts containing cupuassu pulp. In comparison to yogurts from whole or skim milk, all of the fat replacers improved the physicochemical properties ($P < 0.05$). The addition of the carbohydrates (inulin and maltodextrin) and proteins (whey protein and skim milk powder) also influenced the color of the low-fat cupuassu goat milk yogurt ($P < 0.05$). The skim milk powder yogurt presented a higher apparent viscosity than yogurts made with whole or skim milk, inulin, maltodextrin, or whey protein. Furthermore, only the addition of skim milk powder increased the texture parameters ($P < 0.05$). These results suggest that skim milk powder could potentially be used to improve the apparent viscosity and texture properties of low-fat goat milk yogurts containing cupuassu.