GLAUCIA AGUIAR ROCHA SELMI

DESENVOLVIMENTO DE MICROCÁPSULAS DE EDULCORANTES PRODUZIDAS POR DUPLA EMULSÃO SEGUIDA DE COACERVAÇÃO COMPLEXA E ESTUDO DE SUA FUNCIONALIDADE EM GOMAS DE MASCAR

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UNIVERSIDADE ESTADUAL DE CAMPINAS

Faculdade de Engenharia de Alimentos

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Tese apresentada à Faculdade de Engenharia de Alimentos, da Universidade Estadual de Campinas, como parte dos requisitos exigidos para a obtenção do título de Doutora em Alimentos e Nutrição, na área de concentração Consumo e Qualidade de Alimentos.

Orientadora: Profª. Drª. Helena Maria Andre Bolini

Co-orientadora: Profª. Drª. Carmen Silvia Favaro Trindade

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RESUMO GERAL

Em todo o mundo, tem-se observado o aumento do interesse da população em ingerir produtos isentos de açúcar e com menor teor calórico. Esse interesse tem impulsionado o mercado dos produtos chamados *diet/light* e as gomas de mascar desempenham importante papel neste setor. Os edulcorantes utilizados nesse segmento podem ser microencapsulados, com o objetivo de reduzir a higroscopicidade desses compostos e aumentar a duração do gosto doce. A microencapsulação é uma tecnologia que vem se mostrando viável para diversas aplicações industriais. O prolongamento do gosto doce é bastante desejado em alguns produtos, especialmente as gomas de mascar, que permanecem um período maior na boca antes da ingestão. Nesse contexto, os objetivos deste trabalho foram desenvolver e caracterizar microcápsulas com diferentes tipos de edulcorantes, aplicá-las em gomas de mascar e determinar o perfil tempo-intensidade de doçura, além da aceitação dos produtos em relação à aparência, aroma, sabor, textura e impressão global. Mesmo sendo compostos hidrofílicos, foi possível encapsular os edulcorantes aspartame, sucralose e estévia por meio da adição da técnica de dupla emulsão ao processo de encapsulação por coacervação complexa. As microcápsulas formadas eram esféricas, multinucleadas e apresentaram baixos valores de solubilidade e higroscopicidade. As gomas de mascar produzidas com edulcorante microencapsulado apresentaram um perfil de liberação do gosto doce mais duradouro em relação às amostras produzidas com edulcorante na forma livre, sem perda da aceitação pelos consumidores, o que indica a potencialidade do uso desse tipo de microcápsulas em gomas de mascar visando aumentar o tempo de duração do gosto doce.

ABSTRACT

Recently, the interest of the population in consuming lower-calorie sugar-free products has grown. This interest has driven the market of light and diet products, and chewing gums play an important role in this sector. Microencapsulation is a technology that has proven viable for several industrial applications. With regard to sweeteners, the process may reduce the hygroscopicity of such compounds and extend the sensation of sweetness. Extending the sweet taste is quite interesting for some products, especially chewing gums, which remain a longer period in the mouth during consumption. In this context, this study aimed at developing and characterizing microcapsules containing different types of sweeteners, applying the microcapsules in chewing gum and determining the time-intensity sweetness profile and acceptance of products, concerning the parameters appearance, aroma, flavor, texture and overall impression. Although sweeteners are hydrophilic compounds, it was possible to encapsulate aspartame, sucralose and stevia using double emulsion stage prior to complex coacervation process. The microcapsules formed were spherical, multinucleated and exhibited low values of solubility and hygroscopicity. The chewing gum produced with microencapsulated sweetener showed a more prolonged sweetness release profile than the samples containing sweetener in free form, without affecting consumer acceptance, indicating the potential use of such microcapsules in chewing gum aimed at extending sweetness duration.

SUMÁRIO

Dedico esse trabalho

aos meus pais, Leila e Umberto

e ao meu marido, Rodrigo.

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INTRODUÇÃO GERAL

Nos últimos anos, os consumidores além de desejar um produto com características sensoriais, como gosto, sabor, cor e textura agradáveis, muitas vezes também desejam que este seja reduzido em gorduras, açúcar e calorias e possibilite a manutenção ou melhoria de sua saúde e bem estar. Com a obesidade tendo se tornado epidêmica e com o aumento cada vez maior do interesse da população por alimentos com benefícios à saúde, os edulcorantes têm recebido especial atenção.

Edulcorantes artificiais são frequentemente utilizados no desenvolvimento de alimentos e bebidas *diet/light* para a manutenção do dulçor desejável, sem comprometimento do sabor e, ao mesmo tempo, sem o aporte de calorias inerente à sacarose.

A microencapsulação é uma tecnologia que tem sido empregada com êxito na indústria de cosméticos, farmacêutica e alimentícia. Essa técnica tem solucionado limitações no emprego de ingredientes e aditivos alimentícios, uma vez que pode mascarar *flavors* indesejáveis, reduzir a volatilidade, a higroscopicidade e a reatividade, além de possibilitar um aumento na estabilidade destes em condições ambientais adversas.

Este trabalho será apresentado conforme descrito a seguir:

Revisão Bibliográfica: Apresenta uma revisão de literatura sobre a definição e situação de mercado das gomas de mascar; descrição e características dos edulcorantes aspartame, sucralose e estévia; abordagem sobre as técnicas sensoriais de tempo-intensidade e análise de aceitação e, por último, as definições sobre microencapsulação, com enfoque na técnica de coacervação complexa.

Artigo de Pesquisa 1: Nesse artigo são apresentados os resultados obtidos para a microencapsulação de aspartame. As microcápsulas produzidas foram avaliadas quanto à morfologia, características físico-químicas, rendimento de encapsulação,

espectroscopia, isotermas de sorção e perfil de liberação em diferentes temperaturas.

Artigo de Pesquisa 2: Nesse segundo artigo apresentado estão os resultados obtidos para avaliação das microcápsulas de sucralose em relação à morfologia, análises físico-químicas, espectroscopia, rendimento de encapsulação e calorimetria.

Artigo de Pesquisa 3: Com algumas das microcápsulas de sucralose produzidas no trabalho anterior e com sucralose não encapsulada, foram elaboradas amostras de gomas de mascar. Nesse estudo são apresentadas as avaliações sensoriais de tempo-intensidade e aceitação assim como as avaliações de cor e textura realizadas nas amostras de gomas de mascar.

Artigo de Pesquisa 4: Nesse estudo são apresentadas a caracterização das microcápsulas de estévia por meio de morfologia, avaliações físico-químicas, espectroscopia, rendimento de encapsulação e calorimetria. Além disso, foram elaboradas amostras de goma de mascar com as microcápsulas produzidas e com estévia livre e são apresentados os resultados da análise de tempo-intensidade realizada nas gomas, com o objetivo de avaliar o perfil temporal do gosto doce nas amostras.

Artigo de Pesquisa 5: O último artigo apresentado se refere à avaliação sensorial de amostras comerciais de goma de mascar. Foram realizados os testes de tempo-intensidade e aceitação. O objetivo inicial desse estudo foi permitir um primeiro contato e treinamento da equipe sensorial com avaliação de gomas de mascar, antes que iniciassem as avaliações das amostras elaboradas nessa pesquisa.

1. REVISÃO BIBLIOGRÁFICA

1.1. Goma de mascar

Goma de mascar é o produto constituído por uma base gomosa, elástica, mastigável e não deglutível, podendo conter outros ingredientes, desde que não descaracterizem o produto. Pode apresentar recheio, cobertura e formato variados (BRASIL, 2004). As gomas de mascar são populares e apreciadas em praticamente todos os países e recentemente têm sido relacionadas à higiene oral e uma alternativa ao hábito de fumar (McGOWAN et al., 2005). Muitas gomas de mascar são produzidas sem sacarose. Nos Estados Unidos, cerca de 60% das gomas de mascar consumidas são *diet* em sacarose, o que é uma tendência mundial acompanhada também pelo Brasil (MOLDERO, 2004).

Objetivando o desenvolvimento de produtos altamente aceitáveis e agradáveis, deve-se aproveitar a característica de diversos ingredientes que oferecem tecnologias para substituir açúcares e gorduras e para reduzir calorias. Estes ingredientes incluem agentes de corpo com reduzido teor calórico, edulcorantes de alta intensidade e substitutos de gordura (KOPCHIK, 1995).

Os produtos resultantes devem ter sabor e aparência tão bons ou melhores do que seus similares adoçados com açúcar. Para isso, os ingredientes alternativos devem apresentar características que sejam tão similares quanto possível das apresentadas pelos ingredientes tradicionais. Além disso, devido aos ingredientes alternativos serem mais caros do que os tradicionais, eles devem fornecer, sempre que possível, produtos com benefícios à saúde do consumidor, tais como menor valor calórico ou menor requerimento de insulina (OLINGER, 1995).

1.2. Edulcorantes

Os edulcorantes são substâncias utilizadas na substituição da sacarose que interagem com os receptores gustativos produzindo a sensação do gosto doce

(MONTIJANO et. al., 1998). Eles são considerados não calóricos pelo fato de não serem metabolizados pelo organismo ou por serem utilizados em quantidades tão pequenas, que o aporte calórico torna-se insignificante (VERMUNT et al., 2003).

Existem diferentes razões para substituir a sacarose nas formulações dos alimentos, tais como proporcionar mais opções de alimentos e bebidas para pessoas que devem ou desejam controlar a ingestão calórica e de carboidratos, ajudar no controle ou redução de peso, facilitar o controle de cáries dentais e fornecer doçura quando não há açúcar disponível (CARDELLO et al., 1999).

Há vários edulcorantes permitidos para utilização em alimentos e bebidas dietéticas, porém cada um possui características específicas em relação à intensidade e persistência do gosto doce e presença ou não de gosto residual (ZHAO & TEPPER, 2007; BOLINI-CARDELLO & DAMASIO, 1997; HIGGINBOTHAM, 1983). As propriedades sensoriais de aparência, aroma, textura, gosto e gosto residual são de principal importância (LARSON-POWERS & PANGBORN, 1978). Esses fatores são determinantes na aceitação, preferência e escolha por parte dos consumidores.

A legislação brasileira divide os edulcorantes em naturais e artificiais e estabelece limites máximos de uso para diferentes alimentos. Os edulcorantes de uso permitido no Brasil são: acessulfame de potássio; ácido ciclâmico e seus sais de cálcio, potássio e sódio; apartame; eritritol; esteviosídeo; isomalte; lactitol; maltitol; manitol; neotame; sacarina e seus sais de cálcio, potássio e sódio; sorbitol; sucralose; taumatina e xilitol (BRASIL, 2008).

Para ser considerado ideal, um edulcorante deve apresentar as seguintes características: (i) poder adoçante igual ou superior ao da sacarose; (ii) ausência de cor e de odor; (iii) perfil de sabor agradável, tão próximo possível quanto ao da sacarose, sem sabor residual; (iv) facilidade de dissolução; (v) compatibilidade química com outros aditivos e demais componentes dos alimentos; (vi) estabilidade química e térmica, tanto em meio ácido, quanto básico; (vii) baixo teor calórico (< 2 kcal/g); (viii) não cariogenicidade; (ix) segurança em termos de saúde pública; (x) metabolismo normal ou resistência à digestão; (xi) facilidade de

produção, transporte e estocagem; (xii) disponibilidade comercial e (xii) custo competitivo em comparação à sacarose e outros edulcorantes (SALMINEN & HALLIKAINEN, 1990; STAMP, 1990; VERDI & HOOD, 1993; MONTIJANO et al., 1998; NABORS, 2002; LINDLEY, 2002). De modo geral, nenhum edulcorante preenche todos os requisitos enumerados anteriormente e, em vista disto, a utilização de combinações de edulcorantes é uma forma de compensar as limitações individuais de cada um deles, proporcionando o desenvolvimento de produtos com melhor sabor, maior vida útil e, muitas vezes, com custos de formulação reduzidos (MONTIJANO et al,1998).

As propriedades temporais de um determinado edulcorante descrevem a evolução da sensação de dulçor ao longo do tempo, o que é fundamental para prever seu desempenho em diferentes tipos de alimentos e bebidas. Entre tais propriedades, encontra-se a intensidade máxima de dulçor, o tempo de duração até a queda do dulçor máximo e o tempo de persistência do sabor doce (HOOD & CAMPBELL, 1990). Embora o perfil de dulçor seja um aspecto crucial na utilização de edulcorantes, as propriedades sensoriais e físico-químicas de cada alimento como um todo influenciam diretamente o tipo e a quantidade de edulcorantes a serem utilizados (REDLINGER & SETSER, 1987; SALMINEN & HALLIKAINEN, 1990).

1.2.1. Aspartame

O aspartame é um dipeptídeo composto pelo ácido L-aspártico e pelo éster metílico da fenilalanina, ambos aminoácidos naturalmente encontrados em alimentos (NEWSOME, 1986; BUTCHKO et al, 2001). A molécula desse edulcorante ($C_{14}H_{18}N_2O_5$ – 294,3 g/mol) é composta por 39,5% ácido aspártico, 50% fenilalanina e 10,5% éster metílico. O aspartame é solúvel em água e álcool e insolúvel em óleos e gorduras, sendo sua solubilidade dependente de temperatura e pH. Apresenta maior estabilidade na faixa de pH 3,0 a 5,0 e seu ponto isoelétrico

é 5,2, pH em torno do qual se encontra a maioria dos alimentos (DZIEZAK, 1986; WELLS, 1989; FERNANDEZ, 1990; BUTCHKO et al, 2001).

Como o aspartame é cerca de 160-220 vezes mais doce que a sacarose, a quantidade deste edulcorante normalmente adicionada aos alimentos é significativamente menor que a do açúcar. Em vista disto, apesar de ambos os compostos apresentarem a mesma quantidade de calorias por grama, o aporte calórico conferido pelo aspartame é muito menor que o da sacarose. O seu perfil de sabor é descrito como limpo e doce, como o da sacarose, sem residual amargo ou metálico normalmente associados a certos edulcorantes como acessulfame-K, ciclamato e sacarina. A curva de tempo-intensidade do aspartame, comparada à da sacarose, é caracterizada por um desenvolvimento mais lento e persistente da sensação de dulçor (HOMLER, 1984; DZIEZAK, 1986; VALLEJO et al., 1990; BUTCHKO et al, 2001).

Na forma sólida, o aspartame apresenta-se estável por longos períodos, porém em meios líquidos, sob determinadas condições de temperatura e pH, pode ser degradado, resultando em perda do gosto doce (GIESE, 1992; NABORS, 2002). Apesar de sofrer hidrólise sob condições excessivas de calor, o aspartame suporta processamento térmico a alta temperatura, por curto período de tempo, como é o caso de certos produtos lácteos e sucos. Por outro lado, sua utilização não é adequada para aplicações em que seja empregado aquecimento muito drástico por tempo prolongado, como por exemplo, o forneamento, a esterilização e a fritura (STAMP, 1990; NABORS, 2002). A estabilidade do edulcorante está bastante relacionada à temperatura de estocagem do alimento. Em baixa temperatura, a velocidade de degradação é significativamente reduzida, enquanto que, em condições de calor excessivo, o potencial de hidrólise ou ciclização do aspartame pode limitar suas aplicações (HOMLER, 1984; BUTCHKO et al, 2001).

Entre as aplicações mais comuns do aspartame encontram-se bebidas carbonatadas, refrescos em pó, iogurtes, bebidas lácteas, adoçantes de mesa, confeitos, sobremesas em pó, gomas de mascar, chás instantâneos, produtos a

base de cereais e sorvetes (DZIEZAK, 1986; BUTCHKO et al, 2001). Seu uso no Brasil é permitido desde 1988 (Brasil, 1988a).

1.2.2. Sucralose

A sucralose ($C_{12}H_{19}Cl_3O_8$ – 397,64 g/mol) também conhecida como triclorogalactosacarose (TGS), é o primeiro adoçante de baixa caloria derivado da sacarose, por meio de substituição seletiva de 3 grupos hidroxilas, por átomos de cloro, o que acarreta em aumento substancial da doçura (WALLIS, 1993; CAMPOS 2002).

A sucralose é altamente solúvel em água, etanol e metanol. Em solução, apresenta viscosidade similar à da sacarose e seu efeito a tensão superficial é praticamente desprezível, o que significa que o uso deste edulcorante em produtos líquidos normalmente não implica em problemas de dissolução ou formação de espuma (GOLDSMITH & MERKEL, 2001). A cloração da molécula da sacarose acarreta alterações conformacionais, que resultam em aumento da estabilidade a ácidos e enzimas em relação à molécula original (HOOD & CAMPBELL, 1990; WALLIS, 1993).

Este edulcorante não é tóxico, é quimicamente inerte, não sofre reação de Maillard, é cerca de 60 vezes mais estável à hidrólise ácida do que a sacarose e pode ser utilizado em produtos esterilizados, pasteurizados e forneados. É um ingrediente versátil e não apresenta problemas de interação com outros compostos. É muito estável em formulações secas, como refrescos em pó, sobremesas e adoçantes de mesa, mantendo sua estabilidade por cerca de 4 anos, a 20°C (HOUGH & KHAN, 1989; CÂNDIDO & CAMPOS, 1996).

A sucralose apresenta intensidade de dulçor de 400 a 1000 vezes maior que a da sacarose (em média, 600 vezes, fator aplicável à maioria dos alimentos e bebidas em que o edulcorante é utilizado) e perfil de tempo e intensidade de dulçor bastante similar ao deste açúcar, destacando-se o gosto doce agradável, sem notas amargas ou metálicas residuais (GRENBY, 1991; HOOD & CAMPELL,

1990; GOLDSMITH & MERKEL, 2001). Seu uso é permitido no Brasil desde 1995 (Brasil, 1995).

1.2.3. Esteviosídeo

O esteviosídeo, composto extraído das folhas de estévia, é um edulcorante que se destaca entre os edulcorantes naturais. Este extrato é um pó branco, composto pelo esteviosídeo propriamente dito e por seus anômeros, os rebaudiosídeos, que são os responsáveis pela doçura do composto (HIGGINBOTHAM, 1983; PARPINELLO et al., 2001). O extrato é bastante estável e fornece um gosto doce que perdura por bastante tempo na boca, mas apresenta gosto residual e amargor bastante acentuados, que limitam seu uso em grandes concentrações (PARPINELLO et al., 2001; NEWSOME, 1993). Por esta razão, muitos esforços foram feitos no intuito de mascarar esta propriedade indesejável da estévia. Estudos permitiram o desenvolvimento de um novo extrato, com uma maior proporção de rebaudiosídio A, que fornece um produto com menor residual amargo (HOUGH, 1996).

O esteviosídeo é pouco solúvel em água e altamente solúvel em etanol, é bastante estável em uma ampla faixa de pH e ao calor. Possui, em média, um potencial edulcorante 250-300 vezes superior ao da sacarose. Apresenta efeito sinérgico com outros edulcorantes, como aspartame, ciclamato e acessulfame-K (STAMP, 1990; GRENBY, 1991; GIESE, 1992; KINGHORN et al., 2001).

Entre suas aplicações destacam-se: refrigerantes, bebidas acidificadas em geral, balas e confeitos, gomas de mascar, sorvetes, iogurtes, gelatinas, sobremesas em geral e adoçantes de mesa (ALMEIDA-MURADIAN & PENTEADO, 1990; GIESE, 1993; CARDELLO & DAMÁSIO, 1997). Assim como o aspartame, seu uso é permitido no Brasil desde 1988 (Brasil, 1988b).

1.3. Análise Sensorial

A análise sensorial é uma ciência usada para medir, analisar e interpretar as reações que as características dos alimentos e materiais provocam no ser humano (AMERINE, PANGBORN & ROESSLER, 1965). É a única forma de se avaliar a aceitação de alimentos e bebidas, sendo, portanto, uma ferramenta insubstituível. Ela pode ser uma ferramenta extremamente útil no entendimento de quais características sensoriais de um produto são importantes para o consumidor e na tradução destas características em requerimentos de ingredientes ou formulações do produto (BURGER, 1992).

Substâncias doces raramente apresentam as mesmas características de doçura ou todos os aspectos associados com o gosto e textura ideais produzidos pela sacarose. Elas frequentemente apresentam diferenças no tempo de detecção, na intensidade e na qualidade de doçura, algumas vezes com presença de sabores residuais, metálicos, alcoólicos e amargor (HOUGH, 1996). Para que edulcorantes sejam aplicados na substituição da sacarose, é necessário que, além de sua segurança absoluta, eles apresentem características sensoriais agradáveis, com doçura semelhante à da sacarose. A única forma de se avaliar a aceitação de um edulcorante é a aplicação da análise sensorial (BURGER, 1992).

1.3.1. Tempo – Intensidade

A metodologia Tempo-Intensidade (T-I) é um tipo de análise descritiva que avalia as mudanças na percepção de um determinado atributo ao longo do tempo. Este teste foi definido por Amerine et al. (1965) como a medida da velocidade, duração e intensidade percebidas por um estímulo único.

A avaliação sensorial de T-I é uma tentativa de munir os provadores com uma oportunidade de mensurar suas sensações percebidas em função do tempo. O método, portanto, fornece ao analista sensorial e aos usuários dos dados informações temporais potencialmente importantes acerca das sensações

percebidas. Como os provadores estão continuamente monitorando suas sensações percebidas, do começo ao fim, o analista sensorial está apto para quantificar as contínuas mudanças na percepção que ocorrem no respectivo atributo ao longo do tempo. Quando muitos atributos são analisados, o perfil de sabor ou textura de um alimento complexo pode demonstrar diferenças entre produtos que mudam através do tempo (LAWLESS & HEYMANN, 1999).

A percepção da doçura é um processo dinâmico que requer avaliação temporal para a completa caracterização. Na avaliação de alimentos cuja substituição de um ingrediente altera as propriedades temporais, os estudos de T-I são essenciais na análise dos fatores que afetam o processamento (NOBLE et al.,1991).

Avaliar o comportamento temporal de substâncias edulcorantes é importante para prever sua aceitação pelo mercado, na medida em que cada uma possui características específicas de intensidade, persistência do gosto doce e presença ou não de gosto residual. Estas características, por sua vez, podem variar em função do alimento ao qual são adicionadas (CAVALLINI & BOLINI, 2005; BOLINI-CARDELLO, 1996; KETELSEN et al., 1993). A maioria dos métodos utilizados para quantificar sensações percebidas em um alimento pede que o provador estime a intensidade percebida da sensação, fornecendo uma medida única. O provador deve, então, fazer uma "média temporal", a fim de fornecer um valor único de intensidade, ou então relatar somente o pico de intensidade percebido, causando perda de informações importantes. É possível que diferentes produtos com perfis de tempo médio iguais ou similares difiram na ordem em que diferentes sabores ocorrem ou em quando estas sensações atingem suas intensidades máximas (LAWLESS & HEYMANN, 1999). No caso de edulcorantes, conforme exposto por DUBOIS et al. (1977), determinados compostos considerados idênticos em termos de seu dulçor global podem apresentar diferenças marcantes quando avaliados quanto às respectivas curvas T-I.

Até década de 70, poucos trabalhos utilizavam a técnica tempo-intensidade. Porém, com o desenvolvimento da informática houve o surgimento de diversos

programas específicos para serem aplicados a este tipo de análise e a metodologia pôde ser incorporada à avaliação sensorial de alimentos e bebidas (CAVALLINI & BOLINI, 2005). Através destes programas, provadores selecionados e treinados registram, com o auxílio do "mouse", estímulos percebidos em uma escala mostrada na tela do computador. Esta metodologia permite a obtenção de informações sobre a substância de interesse, através da associação do insubstituível instrumento de percepção que é o ser humano, aliado à precisão da informática (UMBELINO, 2005). De maneira geral, os parâmetros frequentemente analisados das curvas obtidas por esta metodologia são: a área total sob a curva, o tempo total de duração e a intensidade máxima do estímulo, bem com o tempo em que a intensidade máxima foi atingida (CAVALLINI & BOLINI, 2005; UJIKAWA & BOLINI, 2004; BOLINI-CARDELLO, 1996; KETELSEN et al., 1993; OTT et al., 1991).

1.3.2. Teste de Aceitação – Consumidor

Nos testes de aceitação, os provadores avaliam o quanto gostam ou desgostam de um determinado produto, com o uso de escalas hedônicas. De todas as escalas testadas, a escala hedônica de nove pontos é a considerada mais eficiente em testes de aceitação, devido à simplicidade para descrição e à facilidade no uso e compreensão por parte dos consumidores. Seus extremos são geralmente ancorados em "desgostei extremamente" e "gostei extremamente". Podem ser avaliados com essa escala atributos como aparência, aroma, sabor, textura e impressão global. (STONE & SIDEL, 2004).

Os dados obtidos destes testes são submetidos a Análise de Variância (ANOVA), para verificar se existe diferença significativa entre as amostras, e teste de médias de Tukey, que avaliam a diferença significativa entre as médias das notas obtidas por cada amostra. As análises geralmente são feitas ao nível de significância de 5% (STONE & SIDEL, 2004; MEILGAARD et al., 1999).

É comum expressar os resultados obtidos por testes de consumidor em Mapa de Preferência, que considera a variabilidade individual dos dados (MORAES, 2004). O mapa de preferência interno é uma técnica onde as maiores fontes de variação da preferência são identificadas e extraídas como dimensões de preferência. Os mapas podem ser então construídos onde as amostras aparecem como pontos e os critérios principais de preferência dos consumidores como vetores. Teoricamente, a inspeção visual do mapa fornece informação relevante para a identificação de grupos de consumidores com diferentes padrões de preferência (COSTELL et al., 2000). Aliado à análise de variância e teste de médias, o Mapa de Preferência Interno pode complementar a análise de aceitação de um produto, explicando as preferências dos consumidores e tornando as informações obtidas mais valiosas (CARDELLO & FARIA, 2000).

1.4. Microencapsulação

Microencapsulação é um processo no qual um agente encapsulante envolve um núcleo, com o objetivo de protegê-lo das condições adversas do meio, tais como luz, umidade, oxigênio e interações com outros compostos, estabilizando o produto, aumentando a vida útil e promovendo a liberação controlada da microcápsula em condições pré-estabelecidas. Nos últimos anos, as definições e empregos da microencapsulação têm sido ampliados e esta técnica tem se mostrado promissora para solucionar a dificuldade de incorporação de alguns ingredientes e aditivos em alimentos (SHAHIDI & HAN, 1993; GOUIN, 2004; FÁVARO-TRINDADE et al., 2008).

Embora a técnica de microencapsulação venha sendo estudada desde a década de 30, sua primeira aplicação comercial ocorreu por volta de 1950, por Green e Scheicher, para a produção de papel cópia sem carbono, utilizando tinta microencapsulada por coacervação complexa. Ao serem pressionadas, as microcápsulas, localizadas no verso da folha, rompiam-se e liberavam a tinta contida em seu interior, tingindo a folha abaixo (ARSHADY, 1990; DUBEY et al.,

2009). Depois foram microencapsulados produtos como óleo de laranja para aplicações em indústrias de aroma, combustíveis para foguetes e produção de pílulas e comprimidos na indústria farmacêutica (DZIEZAK, 1988). Na área de alimentos, os primeiros estudos foram nos anos 60, com a microencapsulação de óleos essenciais para a prevenção da oxidação e perda de compostos voláteis e para o controle da liberação do aroma (RÉ, 2000).

Os estudos da técnica de microencapsulação permitiram a ampliação de seu uso para diversos tipos de indústrias, tais como alimentícia, farmacêutica, de aromas, química e agrícola (FANGER, 1974; POTHAKAMURY & BARBOSA-CÁNOVAS, 1995; RÉ, 1998; WIELAND-BERGHAUSEN et al., 2002). No campo da pesquisa, segundo Gouin (2004), o crescente interesse de pesquisadores no potencial oferecido pela microencapsulação é comprovado pelo grande crescimento no número de publicações ao longo dos anos, desde a metade dos anos 50 até os dias atuais.

Microcápsulas podem ser descritas como embalagens extremamente pequenas, compostas por um polímero como agente encapsulante e um material ativo chamado de núcleo (ARSHADY, 1993). De acordo com Ré (1998), para ser considerada uma microcápsula, a partícula deve apresentar um tamanho entre 0,2 e 500 µm. Abaixo de 0,2 µm é considerada nanocápsula e acima de 500 µm, macrocápsula.

Os propósitos gerais da microencapsulação incluem: viabilizar o uso de líquidos em sistemas desidratados; separar materiais reativos, evitando interações entre eles; reduzir a toxidez do material ativo; controlar a liberação do material; aumentar a vida de prateleira por prevenir evaporação e oxidação e mascarar propriedades indesejáveis como sabor, odor e susceptibilidade ao pH (GOODWIN & SOMERVILLE, 1974; JACKSON & LEE, 1991; ARSHADY, 1993; SHAIDI & HAN, 1993; RÉ, 1998).

A microencapsulação tem sido utilizada com sucesso na indústria alimentícia para a proteção de substâncias sensíveis à temperatura, luz, oxigênio e umidade, para a diminuição da taxa de transferência do recheio ao meio em que se encontra

o produto e para a modificação de características físicas do material, facilitando o manuseio (DESAI & PARK, 2005).

Algumas características das microcápsulas podem ser alteradas para atender necessidades específicas de aplicação, tais como: composição, mecanismo de liberação, tamanho de partícula, morfologia e custo. Quando as microcápsulas são utilizadas em outros processos industriais, há uma série de questões que devem ser levadas em consideração na escolha do processo de microencapsulação: a funcionalidade que o ingrediente a ser microencapsulado deve fornecer ao produto final; o tipo de material de parede que pode ser utilizado; as condições de processamento às quais o material microencapsulado deve resistir sem liberar seu conteúdo; a concentração ótima de material ativo na microcápsula; o mecanismo de liberação do recheio; necessidades de tamanho de partícula, densidade e estabilidade e o limite de custo do material microencapsulado (DESAI & PARK, 2005).

1.4.1. Técnicas de microencapsulação

Existem várias técnicas que podem ser utilizadas para microencapsulação de ingredientes alimentícios, sendo que a seleção do método é dependente da aplicação que será dada à microcápsula, tamanho desejado, mecanismo de liberação e propriedades físico-químicas tanto do material ativo, quanto dos agentes encapsulantes (JACKSON & LEE, 1991; RÉ, 2000, AZEREDO, 2005). Essas técnicas podem ser classificadas em químicas, como por exemplo, a inclusão molecular, físicas como o caso do *spray drying, spray colling* e extrusão ou ainda físico-química onde se incluem as técnicas de coacervação simples, coacervação complexa e lipossomas (JACKSON & LEE, 1991).

1.4.1.1. Coacervação complexa

A coacervação, também chamada de separação espontânea de fases, foi desenvolvida no início do século passado e, devido à formação de partículas bem pequenas com recheio uninuclear, o processo de coacervação é considerado por muitos como a técnica original e verdadeira de microencapsulação. O termo é oriundo do latim “co” e “acervus”, que significa união e agregação de partículas (SHAHIDI & HAN, 1993; THIES, 1995; DUCEL et al., 2004; STRAUSS & GIBSON, 2004).

Esta técnica consiste em uma separação espontânea de fases, pela formação de um complexo insolúvel entre dois ou mais polímeros. Na maioria dos casos, entre os polímeros empregados há uma proteína e um polissacarídeo. Para que o processo aconteça, duas condições devem ser atendidas: os biopolímeros devem estar juntos em solução e as cargas opostas entre as suas cadeias devem estar em quantidades estequiométricas. A composição e concentração dos polímeros de parede, condições do meio como pH e força iônica estão diretamente relacionados com a eficiência de produção das microcápsulas e com características variadas de estrutura, tamanho e porosidade (YEO et al., 2005).

Alguns exemplos de agentes encapsulantes passíveis de serem utilizados no processo de coacervação são: gelatinas, alginatos, albuminas, caseína, ágar, gomas e pectinas (VANDERGAER, 1974; THIES, 1995). Diversos sistemas já foram avaliados no processo de coacervação complexa, sendo o sistema gelatina e goma arábica, o par mais estudado e compreendido (GOUIN, 2004).

Diversas aplicações industriais foram desenvolvidas para as microcápsulas coacervadas, entre elas, a purificação de macromoléculas, substituição de gordura em produtos *light*, embalagens biodegradáveis e biomaterial para a utilização na área médica. O método também é utilizado por diversos segmentos, incluindo o farmacêutico, alimentício, químico e cosmético para a veiculação de diversos tipos de material ativo (aromas, enzimas, fármacos e tintas) com aplicações variadas

(TOLSTOGUZOV, 1991; SCHMITT *et al.,* 1998; KRUIF et al., 2004; STRAUSS & GIBSON, 2004).

De um modo geral, o processo de microencapsulação por coacervação complexa envolve três etapas: formação de um sistema trifásico quimicamente imiscível (solvente, agente encapsulante e núcleo); deposição de um segundo agente encapsulante e solidificação da cobertura (DESAI & PARK, 2005). A coacervação complexa é normalmente utilizada para microencapsular compostos hidrofóbicos, devido à elaboração de uma emulsão oléo/água (o/a) na primeira etapa do processo. Todavia, alguns estudos recentes demonstraram ser possível encapsular núcleos hidrofílicos por meio de uma emulsão dupla água/óleo/água (a/o/a) no início do processo (MENDANHA et al.,2009; COMUNIAN et al.,2013).

1.4.2. Microencapsulação de edulcorantes

Os edulcorantes são listados entre os compostos passíveis de serem microencapsulados (FÁVARO-TRINDADE et al., 2008), objetivando diminuir a higroscopicidade, aumentar a resistência a altas temperaturas e prolongar a sensação de doçura (DZIEZAK, 1988; JACKSON & LEE, 1991).

A partir dos anos 80, começaram a surgir patentes para a encapsulação de edulcorantes, sendo o aspartame o mais estudado, devido ao seu uso limitado pela instabilidade a altas temperaturas. Edulcorantes, em geral, são protegidos por encapsulação em gordura, acetato polivinílico (PVA), amido, gomas, entre outros (POTHAKAMURY & BARBOSA-CANOVAS, 1995). Em 1987 um grupo de pesquisadores obteve a patente de uma goma de mascar com aspartame microencapsulado. Três anos depois outro grupo desenvolveu e patenteou um processo de produção de goma de mascar com aspartame microencapsulado em um filme composto por PVA e plastificante (PEGG & SHAHIDI, 2007).

Haja vista a escassez de trabalhos científicos envolvendo o estudo da microencapsulação de edulcorantes, um dos objetivos do presente trabalho foi estudar a microencapsulação dos edulcorantes aspartame, sucralose e estévia.

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ARTIGO DE PESQUISA 1

ROCHA-SELMI, G.A.; BOZZA, F. T.; THOMAZINI, M; BOLINI, H. M. A.; FÁVARO-TRINDADE, C. S. Microencapsulation of aspartame by double emulsion followed by complex coacervation aiming to provide protection and prolong the sweetness. Food Chemistry, 139, 72-78 (2013).

Microencapsulation of aspartame by double emulsion followed by complex coacervation aiming to provide protection and prolong the sweetness

ABSTRACT

The objective of this work was to microencapsulate aspartame by double emulsion followed by complex coacervation, aiming to protect it and control its release. Six treatments were prepared using sunflower oil to prepare the primary emulsion and gelatin and gum Arabic as the wall materials. The microcapsules were evaluated structurally with respect to their sorption isotherms and release into water (36ºC and 80ºC). The microcapsules were multinucleated, not very water soluble or hygroscopic and showed reduced rates of equilibrium moisture content and release at both temperatures. FTIR confirmed complexation between the wall materials and the intact nature of aspartame. The results indicated it was possible to encapsulate aspartame with the techniques employed and that these protected the sweetener even at 80ºC. The reduced solubility and low release rates indicated the enormous potential of the vehicle developed in controlling the release of the aspartame into the food, thus prolonging its sweetness.

Keywords: microcapsules, controlled release, sweetener, FTIR, isotherm, solubility.

1. INTRODUCTION

Aspartame is a dipeptide composed of L-aspartic acid and the methyl ester of phenylalanine, both amino acids found naturally in foods. It is about 160 to 220 times sweeter than sucrose and its flavour profile is described as clean and sweet like sucrose, without the bitter or metallic aftertastes normally associated with certain sweeteners such as acesulfame-K, cyclamate and saccharin (Butchko, Stargel, Comer, Maybel & Andress, 2001). It is inadequate for use in applications involving drastic heating for prolonged periods, such as baking, sterilization and frying, since under such conditions, part of the molecule may undergo hydrolysis leading to a loss of sweet taste (Nabors, 2002; Salminen & Hallikainen, 2001).

The main objectives of microencapsulating sweeteners are to increase their fluidity and resistance to high temperatures and prolong the sensation of sweetness by controlling their release (Fávaro-Trindade, Pinho & Rocha, 2008; Gouin, 2004). Thus this technology could facilitate the application of aspartame to products in which high processing temperatures are used, and also provide a gradual release of the sweetener when chewing, prolonging perception of the sweet taste of many products such as sweets and chewing gum.

A good wall material should present the following characteristics: good emulsifying property and film-forming, low viscosity, low hygroscopicity, good drying properties, stability, absence of flavors and good protection to the core (Shahidi & Han, 1993; Ré, 1998). Gelatin, a water soluble protein of high molecular weight and gum Arabic, a long chain polymer of high molecular weight is one of the most common and extensively utilized pair in complex coacervation (Qv, Zeng & Jiang, 2011). Microcapsules produced by complex coacervation are insoluble in water, resistant to high temperatures and show excellent characteristics for controlled release (Dong, Ma, Hayar, Jia, Xia & Zhang, 2011). These characteristics are desirable for the encapsulation of sweeteners, although the complex coacervation technique is appropriate for the encapsulation of hydrophobic compounds, which is not the case of aspartame. Mendanha, Ortiz, Fávaro-Trindade, Mauri, Monterrey-Quintero &

Thomazini (2009) were successful in encapsulating a casein protein hydrolysate, which is also highly water soluble, by adding a double-emulsion step at the start of the complex coacervation process.

A single paper was found in the literature whose objective was to study the stability of aspartame encapsulated in high melting point fat during the baking of cakes (Wetzel & Bellt, 1998). No other papers studying the microencapsulation of sweeteners were found in the literature, although it is a subject that stimulates great interest in the industries, since there are numerous patents involving this subject. Thus the present paper could provide an impulse for the divulgation of new research on the technique of microencapsulating sweeteners.

Hence the objective of the present work was to study the microencapsulation of aspartame by double emulsion followed by complex coacervation, structurally evaluate the microcapsules obtained, and also examine their physicochemical properties and rate of release into water.

2. MATERIAL AND METHODS

2.1. Materials

The sweetener aspartame (AS) (Ajinomoto do Brasil, Brazil) was used as the core material, and swine gelatin (GE) (Gelita, Brazil) and gum Arabic (GA) (Synth, Brazil) as the wall materials. Sunflower oil (Cargill, Brazil) and soybean lecithin (Caramuru, Brazil) were used to prepare the primary emulsion.

2.2. Preparation of the coacervated microcapsules

The methodology used to prepare the microcapsules was an adaptation of that of Mendanha et al. (2009), who encapsulated a protein hydrolysate by complex coacervation, adding a double emulsion step at the start of the process. Three concentrations of AS solution were prepared based on preliminary tests: 3.75, 5

and 10 g/100g of total solution, and were then emulsified at 50ºC with soybean oil in an Ultraturrax homogenizer (Turratec, TE-102, Tecnal, Brazil) for 3 minutes at 18.000 rpm, using soybean lecithin as the emulsifier (5 g/100 g of the total amount of AS). The ratios of sweetener:oil were 1:1, 1:2 and 1:3. The primary emulsion was emulsified in GE, and the GA solution subsequently added plus twice the total volume in distilled water. The pH value was adjusted to 4.0 and the system then cooled to 10ºC with constant stirring, and maintained in the refrigerator for 24h to complete particle precipitation. The concentrations of the wall material were 2.5 and 5 g/ 100g of total solution and the ratio of GE:GA maintained at 1:1 for all formulations. The amounts of core material were 50, 75 and 100 g/100 g of the total wall material used.

To increase the stability and facilitate handling, the coacervated microcapsules were freeze-dried. For this process, the excess water was removed and the coacervate frozen in a freezer at a temperature of -18ºC for 24h, followed by freeze-drying in a bench-scale freeze dryer (Terroni LC 1500 – São Carlos, Brazil).

2.3. Morphological evaluation

The morphology of the microcapsules was studied using an optical microscope (BEL Photonics®, Milan-Italy) equipped with a camera, using the BEL View v. 62 software, and by scanning electronic microscopy (SEM) using the Hitachi TM 3000 Tabletop Microscope (Tokyo, Japan) with the TM 3000 program. For SEM the microcapsules were placed on strips of double-faced carbon tape (Ted Pella, Inc., Redding- USA), which were then fixed to aluminium stubs. The images were captured with voltage acceleration of 5kV and a current of 1750 mA.

2.4. Physicochemical characterization of the coacervated microcapsules

2.4.1. Moisture content

The moisture contents of the freeze-dried material and of the non-encapsulated AS were determined automatically in a moisture analysis equipment (OHAUS - MB45, USA).

2.4.2. Solubility

A 1 g sample of each formulation was added to recipients containing 100 mL of distilled water, and stirred at 110 rpm for 30 min using a bench stirrer (Tecnal, Brazil), before centrifuging at 4000 rpm for 5 min. Aliquots of each supernatant was then removed with the aid of volumetric pipettes, transferred to previously weighed porcelain dishes, and dried to constant weight in an incubator at 105ºC. The dishes were weighed and the solubility calculated from the difference in weight (Cano-Chauca, Stringheta, Ramos & Cal-Vidal, 2005).

2.4.3. Hygroscopicity

The analyses were carried out in triplicate, weighing approximately 1 g of each sample into circular plastic containers (diameter 40 mm x height 10 mm). The microcapsules were placed in hermetic pots containing a saturated sodium sulphate solution (relative humidity of 81%) and weighed again after 7 days. The hermetic pots were kept at 25ºC in an incubator with controlled temperature. The hygroscopicity was expressed as grams of water absorbed by 100 grams of sample (Cai & Corke, 2000).

2.4.4. Particle size

The size and size distribution of the solid lipid particles were evaluated using a Shimadzu Sald-201V laser diffraction particle analyzer (Kyoto, Japan). The particles were dispersed in isopropanol (Synth, Brazil) and stabilized for 5 minutes before the analysis (Fávaro-Trindade, Santana, Monterrey-Quintero, Trindade & Netto, 2010).

2.5. Encapsulation yield

The encapsulation yield (EY) was calculated according to Jun-xia, Hai-yan & Jian (2011) as shown in equation 1. To determine the total AS present in the microcapsules, 0.1 g of freeze-dried microcapsules were weighed into a falcon tube and 5 mL of a NaCl solution (1g/ 100g of solution) added plus 5 mL acetonitrile. The tubes were shaken in a tube shaker, placed in an ultrasound bath for 5 min, centrifuged for 5 min at 4000 rpm and an aliquot of the supernatant removed for quantification of the AS.

The AS was quantified by external standardization in a liquid chromatograph (Shimadzu, Prominence, Japan), equipped with a spectrophotometric detector (214 nm), C18 column (250 mm; 4.6 µm) and a mobile phase of acetonitrile:methanol:phosphate buffer pH 3.5 in proportions of 1:1:8, injecting a sample volume of 10µL. The standard curve was prepared using concentrations of AS between 4.25 and 21. 25 mg per 100 mL.

$$EY = \text{total AS} / \text{AS used in the production} \quad (1)$$

2.6. Fourier transformed infrared spectroscopy (FTIR)

The pure ingredients (gelatine, gum Arabic and aspartame) and the microcapsules (six formulations) were characterized by infrared spectroscopy in the region from

4000 to 600 cm^{-1}, using a Perkin Elmer FT-IR spectrometer (Massachusetts/USA), with the aid of the Spectrum One (version 5.3.1.) software.

2.7. Evaluation of the sorption isotherms of the powdered microcapsules

The sorption isotherms were determined by a gravimetric method. About 1 g of each sample, previously dried by exposure to P_2O_5, were weighed and placed in desiccators for 3 weeks at 25ºC, with relative humidities varying from 11 to 84%. The moisture content was calculated from the weight gain, and the experimental equilibrium moisture content obtained from the difference between the amount of water absorbed divided by the mass of the dry sample.
The sorption isotherms were better described by the GAB model (eq 2), compared to BET and Peleg models.

$$Meq = (X_m C K a_w)/(1-Ka_w)(1-Ka_w+CKa_w) \quad (2)$$

Where: Meq – equilibrium moisture content (% dwb); a_w – water activity; X_m – moisture content of the molecular monolayer; C and K – parameters depending on the temperature and nature of the product.

2.8. Release of aspartame from the microcapsules into water at 36 and 80ºC

The release was analyzed according to the methodology of Dong et al. (2011) with some modifications with respect to the equipment used. Falcon tubes containing suspensions with 5% (mass basis) of capsules were placed in a water bath with orbital shaking at temperatures of 36 (human body temperature) and 80ºC (to simulate heat treatment). Aliquots were removed after 0, 20, 40, 60, 80 and 100

minutes for quantification of the AS using the same methodology used to determine the encapsulation yield.

2.9. Statistical analysis

The experiments were carried out in triplicates. Differences between mean values were determined using the analysis of variance (ANOVA) utilising the statistical software SAS version 8.0.

3. RESULTS AND DISCUSSION

3.1. Preparation of the microcapsules and their morphology

Numerous preliminary trials were carried out in order to define the concentrations of the ingredients and conditions for the production of the emulsions. Of the different emulsion formulations tested, those prepared with a 10% AS solution were shown to be unstable, since they visibly separated into phases about one hour after preparation. However the emulsions formed with the other concentrations were stable for 4h with the three sweetener:oil ratios used. Thus the emulsion with 5% AS and a sweetener:oil ratio of 1:1 was chosen for the production of microcapsules. Six formulations of microcapsules were produced, differing from one another with respect to the concentrations of the GE and GA solutions and the ratio of the core material (emulsion of AS in oil) in relation to the total mass of wall material. The formulations were denominated as follows: **A**: 2.5% GE & GA and 50% of core material; **B**: 2.5% GE & GA and 75% of core material; **C**: 2.5% GE & GA and 100% of core material; **D**: 5.0% GE & GA and 50% of core material; **E**: 5.0% GE & GA and 75% of core material; **F**: 5.0% GE & GA and 100% of core material.

Microcapsules were obtained with all the formulations tested, and as can be seen in Figure 1 (A, B and C), were multinucleated (which confers characteristics of

matrix and reservoir), with droplets of AS emulsion distributed at the centre of the microcapsules and not in the wall, which confers excellent controlled release characteristics to the capsules (Dong et al. 2011), which is one of the main objectives of encapsulating sweeteners. It can also be seen that as the amount of core material increased, so the microcapsules became less spherical, as also observed by Dong et al. (2011).

The electronic microscope images were similar for all the systems studied. Figure 1 (D) shows that the microcapsules had continuous walls showing no cracks or apparent porosity, which indicates the freezing and freeze-drying processes were adequate, since they did not damage the particles. Whole continuous walls are important for microcapsules, to assure greater protection and retention of the encapsulated material.

The microcapsules were connected one to another by solid bridges, an effect also observed by Prata, Zanin, Ré & Grosso (2008) when encapsulating vetiver oil by complex coacervation using GE and GA as the wall materials. These solid bridges can be attributed to agglomeration of the microcapsules caused by the process of freezing followed by freeze-drying.

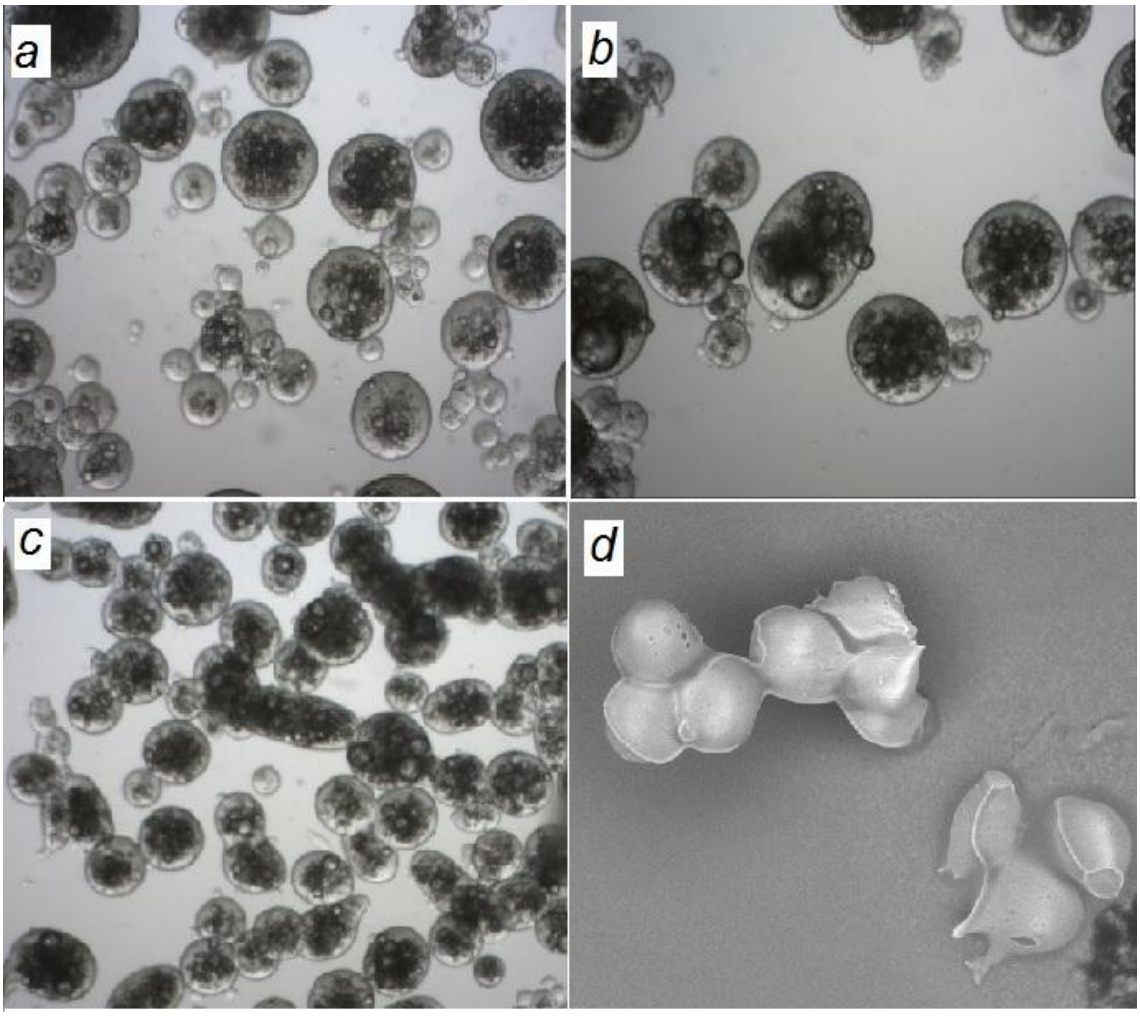

Figure 1: Micrographs obtained with the optical microscope (a, b, c), and scanning electronic microscope with x500 magnification (d), of the aspartame microcapsules: a,d: Formulation A (2.5% GE and GA and 50% core material), b: Formulation B (2.5% GE and GA and 75% core material), c: Formulation C (2.5% GE and GA and 100% core material)

Table 1: Analytical determinations of the freeze-dried coacervated aspartame microcapsules.

Formulation	H (g/100g)	M (%)	S (%)	PS (µm)	EY(%)
A	13.43 ± 0.94^{a}	5.86 ± 0.23^{ab}	9.46 ± 1.34^{c}	96.29 ± 0.06	45.15 ± 1.86^{d}
B	12.88 ± 1.42^{a}	7.42 ± 1.46^{a}	21.37 ± 0.95^{a}	99.76 ± 0.05	48.91 ± 2.22^{d}
C	12.29 ± 0.86^{a}	4.24 ± 0.11^{b}	18.95 ± 3.71^{ab}	102.38 ± 0.05	55.86 ± 1.35^{c}
D	10.73 ± 0.25^{a}	5.16 ± 0.42^{ab}	16.5 ± 3.19^{b}	95.40 ± 0.05	63.94 ± 0.23^{b}
E	12.23 ± 0.48^{a}	4.27 ± 0.21^{b}	13.81 ± 0.42^{b}	84.22 ± 0.11	71.70 ± 0.58^{a}
F	12.41 ± 2.63^{a}	3.99 ± 0.66^{b}	19.95 ± 1.93^{ab}	90.92 ± 0.05	64.11 ± 1.34^{b}
AS	10.86 ± 0.03^{a}	5.56 ± 0.01^{ab}	93.94 ± 0.57^{e}	-	-
GA	38.13 ± 1.73^{c}	9.17 ± 0.32^{c}	31.91 ± 1.71^{d}	-	-
GE	27.16 ± 0.06^{d}	11.99 ± 0.40^{d}	95.70 ± 0.62^{e}	-	-

^{a,b,c}: Means with different small letters in the same column differ ($p < 0.05$)

H = hygroscopicity; M = moisture, S= solubility; PS = particle size; EY = encapsulation yield; A = 2.5% GE & GA and 50% of core material; B = 2.5% GE & GA and 75% core material; C = 2.5% GE & GA and 100% of core material; D = 5.0% GE & GA and 50% of core material; E = 5.0% GE & GA 5,0% and 75% of core material; F = 5.0% GE & GA and 100% of core material.

3.2. Fourier transformed infrared spectroscopy (FTIR)

Since the spectra obtained for all the formulations were similar, only the spectra obtained for the non-encapsulated AS and for the AS encapsulated with formulation (A) are presented in Figure 2. Gum Arabic is a polysaccharide with free carboxyl groups, conferring a negative charge on the molecule. In the spectra obtained for the encapsulated samples, the large peak formed at about 2900 cm^{-1} was attributed to the presence of carboxyl groups from the gum Arabic, indicating that not all the carboxyl groups were involved in the coacervation process. Nevertheless, peaks characteristic of amides can be visualized at about 1500-1640

cm^{-1}, confirming the formation of a coacervate, since during the complex coacervation process, the carboxyl groups of the polysaccharides interact with the amine groups of proteins forming a complex that contains amides.

An observation of the spectra of the non-encapsulated and encapsulated AS shows that peaks characteristic of the AS molecule, such as, for example, the moderately intense peak between 1700 and 1800 cm^{-1}, coming from the ester present in the composition of the AS, were maintained in the composition of the AS, indicating that the coacervation process did not degrade the AS.

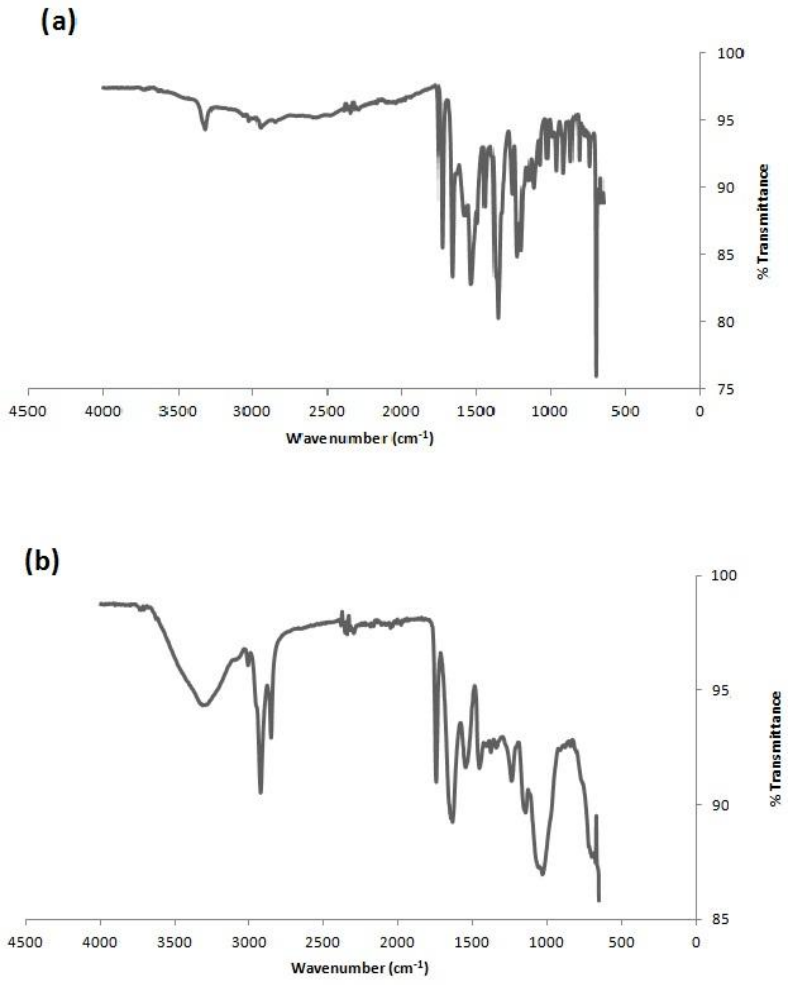

Figure 2: Spectra obtained in the infrared region for (a) non-encapsulated aspartame and for (b) aspartame microencapsulated in formulation A (2.5% GE & GA and 50% of core material)

3.3. Water sorption isotherms

The GAB, BET and Peleg mathematical models were evaluated for the fit of the data obtained, and the GAB model fitted them better. Table 2 shows that the GAB model was adequate to fit the experimental data due to the elevated values obtained for R^2. An evaluation of the parameters fitted showed little variation between the samples analyzed. For BET and Peleg models were found R^2 values lower than 0.95.

No papers were found in the literature reporting on studies of the sorption isotherms of microcapsules produced by complex coacervation, which made it difficult to compare the results, which were obtained using spray dried powders.

The parameter Xm (moisture content of the monolayer) corresponds to the amount of water strongly adsorbed to specific sites on the surface of the material, and is considered a critical value, above which the rates of the degradation reactions increase and the stability of the material is reduced. Low monolayer moisture contents were obtained, since the Xm varied from 6 to 11%. These values are similar to those obtained by Pérez-Alonso, Beristain, Lobato-Calleros, Rodriguez-Huezo & Vernon-Carter (2006), who also obtained low monolayer moisture contents for pure gum Arabic (8.11– 11.0%) at temperatures of 25, 35 and 40^{o}C.

With respect to the parameter K, which represents the correction factor for the properties of the multilayer molecules in relation to the product volume, low values were observed of between 0.6 and 0.8, within the values suggested by LEWICKI (1997) of $0.24 < K < 1$. Values for K below 1.0 are characteristic of food products.

Regarding the sorption constant C, which is due to interactions between the active sites of the product and the water molecules, it was observed that all the formulations showed values less than 200, being within the range commonly found in the literature (Alexandre, Figueiredo & Queiroz, 2007).

Figure 3 shows the sorption isotherms for the AS microcapsules, showing an increase in equilibrium moisture content with increasing water activity. No studies were found in the literature concerning the isotherms of microcapsules produced

by complex coacervation. However, the same behavior was reported by Comunian et al. (2011); Catelam, Fávaro-Trindade & Romero (2011) and Tonon, Brabet, Pallet, Brat & Hubinger (2009), who constructed isotherms for chlorofilida, passion fruit and acai puree powders, all obtained by spray drying with maltodextrin as the wall material. Nevertheless the results obtained for moisture equilibrium in the present study were much lower than those reported by Comunian et al. (2011), Catelam et al. (2011) and Tonon et al. (2009), for all the water activity values, conferring greater stability, and ease of handling, storage and application, corroborating the previously discussed results obtained for hygroscopicity.

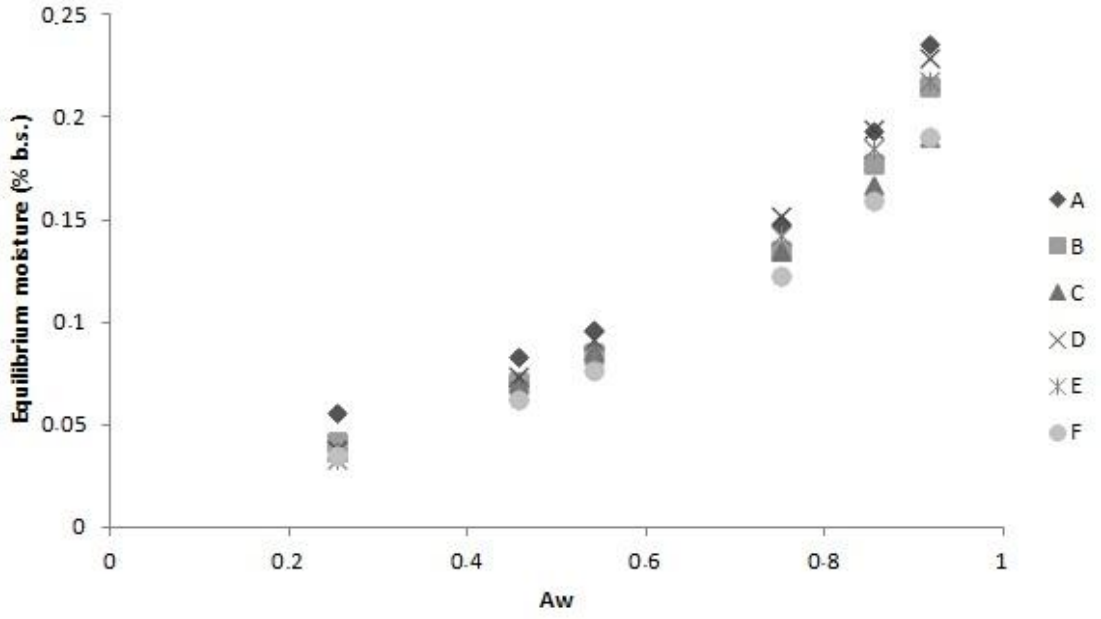

Figure 3: Sorption isotherms of the microencapsulated aspartame samples fitted to the GAB mathematical model

A = 2.5% GE & GA and 50% of core material; B = 2.5% GE & GA and 75% core material; C = 2.5% GE & GA and 100% of core material; D = 5.0% GE & GA and 50% of core material; E = 5.0% GE & GA 5,0% and 75% of core material; F = 5.0% GE & GA and 100% of core material

Table 2: Parameters for the fit of the GAB model to the isotherms of the aspartame microcapsules and the coefficients of determination (R^2)

Formulation	Xm	C	K	R^2
A	0.061	9.993	0.818	0.992
B	0.062	4.591	0.798	0.993
C	0.118	1.949	0.608	0.978
D	0.103	1.979	0.704	0.987
E	0.105	1.643	0.697	0.993
F	0.064	3.246	0.763	0.992

3.4. Release of aspartame from the microcapsules into water at 36 and 80ºC

Figure 4 shows the profile of the release of AS from the microcapsules into water at 36 and 80ºC for systems A, B and C, which all had the same concentration of wall material (2.5%), but differed with respect to the amount of core material.

The release profiles showed two phases, with the rate of release falling very quickly in the first phase, and then more slowly in the second phase. This behaviour was also observed by Dong et al. (2011), who analyzed the profile of the release of mint oil from microcapsules into hot water.

For both temperatures it can be seen that the smaller the amount of core material, the greater the rate of release. This could be attributed to the particle size, since according to the results for particle size, the smaller the amount of core material, the greater the mean diameter, leading to a greater surface contact with the water, making diffusion of the core material easier and thus increasing the rate of release.

Analyzing the two temperatures tested, it can be seen that an increase in temperature did not lead to an increase in the rate of release, showing that the

microcapsules were relatively resistant to high temperatures (80ºC). This behaviour was expected for microcapsules produced by complex coacervation, and is important for their application in products where high processing temperatures are used, as for example, chewing gum, where sweeteners are usually used.

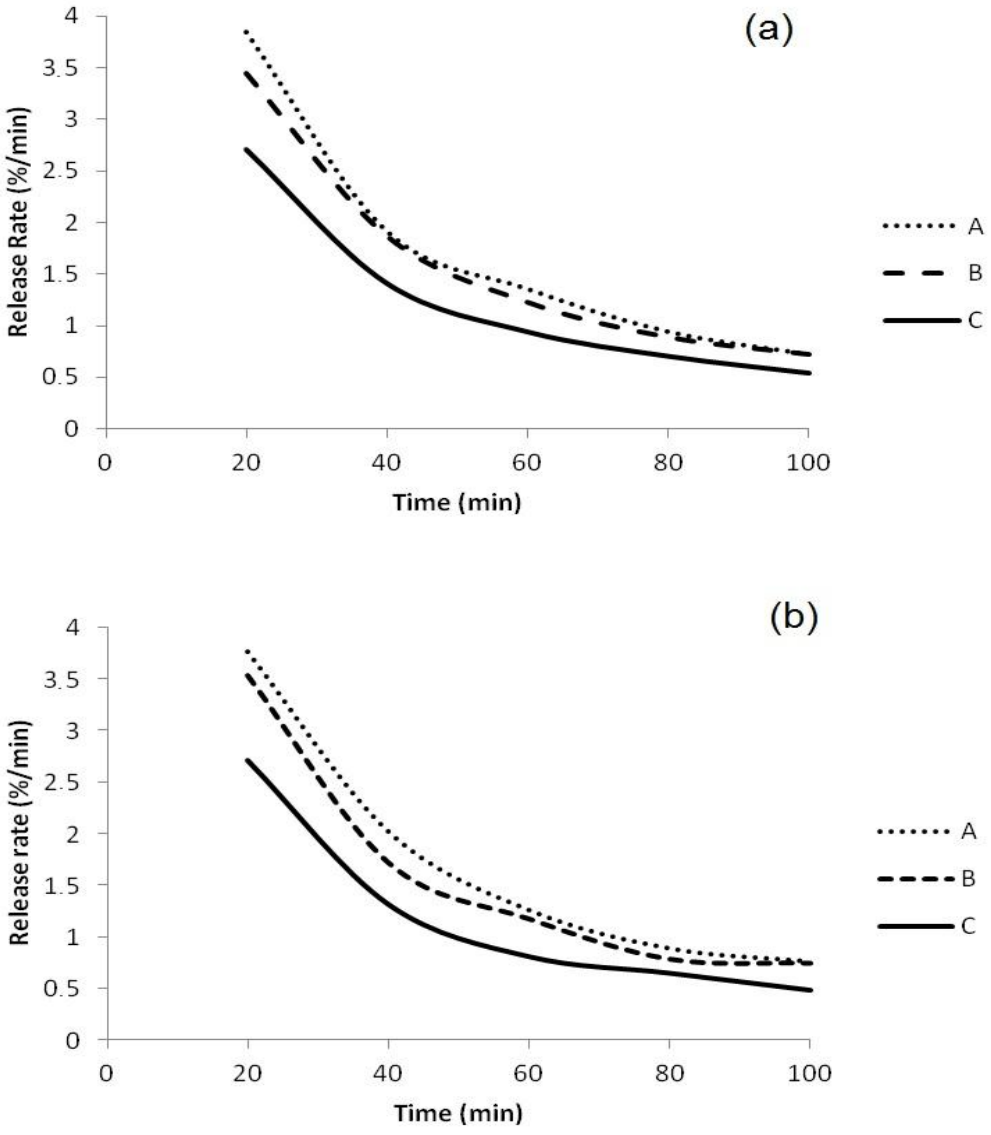

Figure 4: Rate of release of aspartame from microcapsules with different amounts of core material (A – 50%, B = 75% and C = 199%) into water at 36ºC and 80ºC (b)

4. CONCLUSIONS

Considering the proposed objectives and the results obtained, it can be concluded that the six formulations studied formed microcapsules with characteristics similar to those formed by complex coacervation, such as reduced solubility and heat resistance, indicating that the addition of a double emulsion to the process made it feasible to microencapsulate aspartame by this technique.

In addition, the powder obtained was only slightly hygroscopic, making its application easier.

All microcapsules studied in this research showed the potential for application in food, especially the formulations D, E and F, which showed higher values of EY. Then further studies should be carried out in order to evaluate its functionality in food products.

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Artigo de pesquisa 2

ROCHA-SELMI, G.A.; THEODORO, A.C.; THOMAZINI, M.; BOLINI, H. M. A.; FAVARO-TRINDADE, C. S. Double emulsion stage prior to complex coacervation process for microencapsulation of sweetener sucralose. Journal of Food Engineering, 119, 28-32 (2013)

DOUBLE EMULSION STAGE PRIOR TO COMPLEX COACERVATION PROCESS FOR MICROENCAPSULATION OF SWEETENER SUCRALOSE

ABSTRACT

Microencapsulation has proven viable for various industrial applications. In the case of sweeteners, microencapsulation can increase the fluidity and resistance to high temperatures and prolong sensation of sweetness. The aim of this study was to microencapsulate sucralose by double emulsion followed by complex coacervation. The microcapsules were evaluated by optical and scanning electron microscopy, hygroscopicity, solubility, moisture, water activity, particle size, encapsulation yield, potential ZETA, fourier transform infrared spectroscopy (FTIR) and thermal behavior. The microcapsules presented low hygroscopicity and solubility, and average size ranging from 81.04 to 113.49 μm. With FTIR, it was possible to observe the amide bond that confirmed the formation of coacervates. Zeta potential showed that two samples presented neutral charge, indicating complete coacervation. The Tg values were above room temperature (53.59 to 56.88 °C). Among the formulation studied, the one produced with 5% gelatin and gum Arabic and core material 75% presented the best characteristics.

KEYWORDS: microcapsules; zeta potencial; thermal behavior; FTIR; solubility.

1. INTRODUCTION

Sucralose is a synthetic sweetener derived from sucrose by selective replacement of three hydroxyl groups by chlorine atoms, resulting in substantial increase sweetness. It is soluble in water and ethanol and has sweetness intensity 400-1000 times sweeter than sucrose. The time-intensity profile is very similar to sucrose without bitter notes or metallic taste (GOLDSMITH & MERKEL, 2001; GRENBY, 1991; HOOD & CAMPBELL, 1990; WALLIS 1993).

Microencapsulation has shown great promise for incorporating some ingredients and additives in foods. In the case of sweeteners, this process is usually used with the purpose of increasing fluidity and resistance to high temperatures and extending the sensation of sweetness through the gradual and controlled release (GOUIN, 2004).

The complex coacervation (CC) consists of a spontaneous separation of phases by forming a complex that can be insoluble, between two or more polymers resulting from electrostatic interactions (YEO et al. 2005). However, this technique is suitable for encapsulating lipophilic materials. Since sucralose is a hydrophilic compound, to enable the use of this technique an adaptation was proposed in this study. Thus, a primary emulsion W / O was performed prior to CC, followed by double emulsion W / O / W.

Microcapsules produced by CC are water insoluble, temperature resistant and have excellent characteristics for controlled release (DONG et al., 2011). These characteristics are fundamental to achieve the aim of this study, which is to develop a vehicle for gradual release of sucralose during chewing.

No scientific paper discloses a microencapsulation method of sweeteners in the literature, but this topic has attracted great interest since there are many patents involving this matter. Given the above, the aim of this study was to encapsulate sucralose using a double emulsion technique followed by CC and characterize the microcapsules obtained.

2. MATERIAL AND METHODS

2.1. Materials

Sucralose sweetener (Techno Food Ingredients Co - CA, USA) was used as the core material. Bovine gelatin (GE) (Gelita - Cotia/SP, Brasil), and gum Arabic (GA) (Synth Diadema/SP, São Paulo, Brasil) were used as encapsulating agents. Lecithin (Gerbras Química Farmacêutica Ltda.) was used as emulsifier and soybean oil (Bunge-São Paulo/SP, Brasil) was used to produce the primary emulsion.

2.2. Production of microcapsules by complex coacervation

The methodology for production of microcapsules by complex coacervation (CC) used in this study was previously described by Mendanha et al. (2009). An emulsion was prepared with 30% aqueous sucralose solution, soybean oil (twice the volume of sucralose solution) and soy lecithin (3% of total solids concentration basis). Then, the GE solution at 50ºC (at 2.5 or 5%) was added under constant stirring (12000 rpm, 3 min) using Ultraturrax (IKA, T25, Germany). The same volume of GA solution (at 2.5 or 5%) and 4 times the volume of water were added to the emulsion, followed by adjusting the pH to 4.0 with HCl 0.1 M. The emulsion was cooled at 10 °C and stored at 7 °C for 24h for complete separation of phases. After this period, the solutions were frozen for 24h and freeze-dried. Six formulations with different concentrations of encapsulating agents (GE and GA) and different core material (sucralose in oil emulsion) as a function of the total content of encapsulating agents were obtained. The formulations were called as follows: A: 2.5% GE + GA and core material 50%; B: 2.5% GE + GA and core material 75%; C: 2.5% GE + GA and core material 100%; D: 5.0% GE + GA and core material 50%; E: 5.0% GE + GA and core material 75%; and F: 5.0% GE + GA and core material 100%.

2.3. Characterization of sucralose microcapsules

2.3.1. Morphological characteristics

The microcapsules were examined by optical microscopy (OM) and scanning electron microscopy (SEM). MO images were assessed by optical microscope (BEL photonics - Osasco / SP, Brasil) equipped with integrated 1.3 MP digital camera, and SEM images were assessed by scanning electron microscope (Hitachi TM-3000, Hiscope - New Jersey, USA), using 15 kV voltage.

2.3.2 Moisture content and Water activity (Aw)

The moisture content of microcapsules and unencapsulated sucralose was determined by Ohaus MB-35 moisture analyzer balance, and water activity was performed on Aqualab water activity analyzer (Series 3 TE Decagon Devices-USA)

2.3.3. Solubility

The solubility was determined by gravimetric method, according to Eastman and Moore (1984), cited by Cano-Chauca et al. (2005). The sample (0.5 g) was added in an Erlenmeyer flask containing 50 ml of distilled water and the system was homogenized at 110 rpm for 30 min, followed by centrifugation at 4000 rpm for 5 min. An aliquot of 25 mL of supernatant was transferred to a porcelain dish of known weight and kept in oven at 105 °C to constant weight. The mass of empty dish and dish containing the dried material were taken into account for calculation the solubility of the microcapsules.

2.3.4. Hygroscopicity

About 0.5 g of sample was weighed in plastic dishes and stored for 7 days in a closed container containing saturated solution of anhydrous Na_2SO_4 (81% RH). The hygroscopicity was expressed as mass of water absorbed per 100 g of sample (CAI & CORKE, 2000).

2.3.5. Particle size

The average particle size was assessed by a particle analyzer by laser diffraction (SALD - 201V, Shimadzu -Japan) with a measurement range between 0.5 to 500 micrometers. The particles were dispersed in isopropanol and stabilized for 5 minutes before the analysis.

2.3.6. Zeta potential measurements

The Zeta potential measurements were performed for the microcapsules, encapsulating agents, and unencapsulated sucralose by a Zeta Potential Analyzer (BTC - Brookhaven Instrument Corporation - USA) in 10 runs of three cycles each, by diluting the samples in 1 mM KCl solution.

2.3.7. Fourier transform infrared Spectroscopy (FTIR)

Analyses of sucralose, ingredients and microcapsules were obtained in the spectral wavelength range from 600 to 4000 cm^{-1}, by Perkin Elmer FT-IR Spectrometer with the aid of software Spectrum one v 5.3.1.

2.3.8 Encapsulation yield

The encapsulation yield (EY) was calculated according to Jun-xia et al (2011) as the ratio of the total sweetener present in the capsule (E_{total}) and the amount of sweetener used to produce the microcapsules ($E_{production}$), as shown in Equation 1.

To determine the total content of sweetener present in the microcapsules, 5 ml of 1% saline solution and 5 mL acetonitrile were added to falcon tubes containing 0.1 g of freeze-dried microcapsules. The tubes were shaken in a shaker tube and exposed to ultrasonic for 5 min, followed by centrifugation (4000 rpm) for 5 min. Then, an aliquot of the supernatant was removed for analysis, which was performed by external standardization in a liquid chromatograph (Shimadzu Prominence, Japan) equipped with a quaternary pump, auto injector (SIL - 10AF), reverse phase column (Shim-pack VP-ODS; 250 x 4.6 mm), diode array detector

(210 nm) and data software (LC solution), according to the methodology described in IAL (2008).

$$EY\ (\%) = \frac{Etotal}{Eproduction}\ x\ 100 \qquad (1)$$

2.3.9. Differential Scanning Calorimetry

Samples (~1 g) were equilibrated in air over dry silica gel at 25 °C. After equilibration (about 2 weeks), aliquots were taken for DSC analysis. Phase transitions were determined by differential scanning calorimetry using a DSC TA2010 controlled by a TA5000 module (TA Instruments, Newcastle, USA). Samples of about 10 mg, conditioned at TA aluminum pans were heated between -20 and 200 °C at a rate of 10°C/min, in N_2 inert atmosphere (45 mL/min). An empty pan was used as reference. Liquid nitrogen was used for sample cooling before the runs. Samples showing a devitrification peak after the first run were annealed at the devitrification peak temperature (Td) for 30 min before the second DSC run. Phase properties were determined by the DSC thermograms using the Software Universal Analysis V1.7F (TA Instruments, Newcastle, USA).

2.4 Statistical Analysis

The data were evaluated by ANOVA ($p < 0.05$) and Tukey's comparison test ($p < 0.05$) using the Statistica software (Statsoft, USA).

3. RESULTS AND DISCUSSION

Figure 1A shows one of the emulsions obtained after the addition of soybean oil in the aqueous solution of sucralose and lecithin. The presence of droplets and their movement (Brownian motion) on the microscope slide indicated the formation of the emulsion, which remained stable without phase separation for at least two

hours. Figure 1B presents the MO image of one of microcapsule formulations, which confirms that encapsulation of the sucralose was successful. All formulations exhibited similar OM and SEM images, with spherical shape and defined walls. Also in Figure 1B, it can be seen that microcapsules are multinucleate and the sucralose emulsion droplets were distributed through the center of the capsules but not on its walls, like a reservoir system, which, according to Dong et al. (2011), gives excellent characteristics of controlled release, which is a major goal to encapsulate sweeteners.

In Figure 1C is shown a SEM image of the dried capsules after the lyophilization process. The microcapsules had continuous walls with no cracks or apparent porosities, indicating that the lyophilization process was adequate, since it did not cause damage to the particles. These characteristics are important to ensure greater protection and retention of the encapsulated material. The capsules are cross-linked by solid bridges, which were also observed by Prata et al. (2008) in the encapsulation of vetiver oil by CC using GE and GA as encapsulating agents. These solid bridges can be attributed to the lyophilization process, which clusters the microcapsules. After rehydration, the lyophilized microcapsules exhibited the shape shown in Figure 1B.

Table 1 shows the values obtained for particle size, moisture content, aw, solubility, hygroscopicity, zeta potential, encapsulation yield and glass transition temperature.

The average particle size of the microcapsules ranged from 81 to 86 µm for formulations D, E and F (5% GE + GA) and from 100 to 113 µm for formulations A, B and C (2.5% GE +GA). As observed by Mendanha et al (2009), varying the core material did not cause major changes in particle size, however it was observed that the concentration of encapsulating agents have influenced the particle size, since the mean size of the microcapsules of formulations A, B and C were higher as compared to other formulations. This influence is cited in several studies that associate the size of the microcapsules with production parameters such as ratio of polymer wall, concentration of polymers used, stirring speed, cooling rate, and

drying process (LAMPRECHT et al. 2001; MENGER et al. 2000; NAKAGAWA et al. 2004).

The moisture content and aw are within both the expected range for powder products and recommended to guarantee the microbiological stability. With respect to solubility, there was a significant reduction (4-10 times) as comparing the encapsulated samples with unencapsulated sucralose. The low solubility is a characteristic expected for the microcapsules obtained by CC and it is desirable in the encapsulation of sweeteners, since possibly contributes to the gradual release of the sweetener during chewing, prolonging sensation of sweetness.

The hygroscopicity values of the microcapsules ranged from 5.16 to 16.11 g water absorbed / 100g sample. These values were considered low, thus it may favor packaging and material handling. The values obtained in this study were lower (up to 6 times) than those determined by Nori et al. (2011) in propolis microcapsules obtained by CC using soy protein isolate and pectin as encapsulating agents.

With regard to the zeta potential values, at pH 4, positive electric charges predominated in GE solution while negative electric charges predominated in GA, which is important to promote coacervation of these polymers. Concerning the microcapsules, samples E and F presented zeta potential toward zero, which means that the charges were neutralized as expected in CC process. The remaining samples presented negative zeta potential values, which indicate that negative charged groups remained, probably from GA, since GE is an amphoteric substance thus in acid medium positive charges may predominate. This result shows that these negative charged groups were not involved in electrostatic bonds to promote coacervation.

The EY ranged from 43.04 to 89.44%, thus it can be considered a good result concerning the hydrophilic material of this study, which has been previously emulsified in oil and encapsulated by CC. For both samples with 2.5% or 5.0% of encapsulating agents, the higher the core material, the lower the EY. This result may be due to the limited emulsifying capacity of GE in the interface O/W in the presence of excessive concentration of primary emulsion (water in oil). Possibly, if

less core material had been used the results for this parameter would have been even more satisfactory because GE would have acted more effectively in the interface between primary and secondary emulsion, avoiding the loss of core material. This result was similar to that reported by Jun-Xia et al. (2011), who encapsulated orange essential oil by CC using soy protein isolate and GA as encapsulating agents. These authors varied the core material from 10 to 70% as a function of the encapsulating agent and found the highest yield in the formulation with core material 10%.

Furthermore, similar core material showed slightly higher EY in the microcapsules produced with 2.5% encapsulating solution. According to Thies (1995), the increased viscosity caused by the high polymer concentration may interfere with capsules formation, since the mobility of the macromolecules may decrease and consequently increase the competition for solvent molecules.

The capsules showed similar and relatively high Tg values (53 to 56°C). Obtaining Tg values above room temperature is a very positive result, as it ensures physical stability of the capsules when stored at room temperature, whereas, according to Roos (1995), physical changes such as agglomeration and collapse of the structure may occur when the Tg is below the storage temperature.

Figure 2 shows the spectra of a formulation of encapsulated and unencapsulated sucralose and GA is a polysaccharide having free carboxyl groups that confer negative charge to this molecule, while GE is a positively charged protein in acidic medium, due to amine groups. During CC, carboxyl groups from polysaccharides interact with amino groups of proteins to form a complex containing amide. FTIR analysis verified the formation of amides in the samples, confirming the occurrence of CC. In the spectra of encapsulated samples, elongated peaks appeared around 2900 cm^{-1}, which are characteristic of carboxyl groups. There were also peaks between the wavelengths 3400 and 3550 cm^{-1}, which are characteristic of amine groups. The presence of these peaks indicates that not all GA carboxyl groups interacted with GE amino groups, confirming the zeta potential results. However, the presence of peaks characteristic of amides, which appear around 1500-1640

cm $^{-1}$ confirms the coacervate formation.

According to the thermograms shown in Figure 3, the microcapsules showed similar thermal behavior, as their thermograms did not differ. This result allows us to infer that changes in the core material and encapsulating materials did not alter the thermal behavior of the microcapsules. The microcapsules exhibited two melting peaks, at around 60 and 105 °C, which corresponded to melting peaks of gum Arabic and gelatin, respectively, as shown in the thermograms of these materials (Figure 4). Sucralose showed a crystallization peak at about 115°C and Tg at 113°C, proving to be a material quite stable. Once no crystallization peak appeared in the thermograms of microcapsules, it suggested that sucralose remained in the amorphous form.

This study shows the viability of producing microcapsules of sucralose by double emulsion followed by CC. Concerning the six formulations studied, according to the particle size, it is suitable to use the formulations D, E or F for food applications, once microcapsules are smaller than the others and can be easily incorporated into food products. Moreover, these microcapsules showed the lowest values for hygroscopicity, which facilitates transportation, storage and implementation. Regarding production parameters, formulation D showed higher EY than formulations E and F, so it may provide a better-controlled and gradual release of the sweetener, which is the main objective of this study. Therefore, formulation D is considered the best formulation for sucralose encapsulation as compared to the other formulations studied in the present paper.

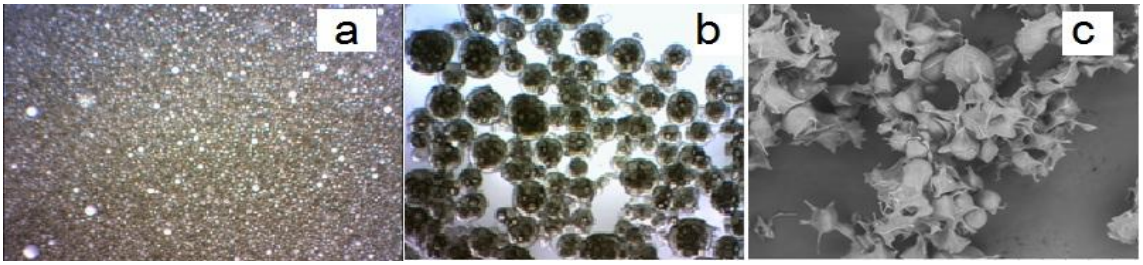

Figure 1 - Optical microscopy of formulation E (5% GE + GA and core material 75%) with 40 and 10x magnification (a and b) and scanning electron microscopy with 200x magnification (c)

Table 1 - Means and standard deviations obtained for the physicochemical properties of sucralose microcapsules, GE, GA, and unencapsulated sucralose

Sample	PS (µm)	M (%)	Aw	S (%)	H (g/100g)	ZP	EY(%)	Tg(ºC)
A	113.49 ± 0.02	11.19 ± 2.35^{adg}	0.51 ± 0.18^{ae}	20.91 ± 1.60^{ae}	16.11 ± 2.78^{a}	-3.76 ± 6.48 ^{a}	89.44^{a}	53.59
B	100.15 ± 0.05	11.4 ± 1.19^{adg}	0.63 ± 0.05^{adg}	9.93 ±0.93^{b}	9.98 ± 0.42^{c}	-4.16 ± 6.51 ^{a}	67.63^{b}	55.88
C	104.15 ± 0.05	6.68 ± 0.53^{bc}	0.35 ± 0.04^{ce}	9.66 ± 2.01^{b}	7.21 ± 0.40^{bd}	-18.78 ± 5.46 ^{b}	61.06^{c}	56.87
D	86.77 ± 0.08	13.97 ± 0.27^{d}	0.68 ± 0.06^{af}	14.17 ± 3.04^{bd}	7.19 ± 0.31^{bd}	-5.14 ± 8.52 ^{a}	68.56^{b}	53.75
E	81.04 ± 0.08	13.06 ± 1.54^{ad}	0.63 ± 0.06^{abh}	23.75 ± 2.01^{a}	6.71 ± 0.11^{cd}	0 ± 0 ^{a}	51.04^{d}	56.61
F	83.92 ± 0.08	12.79 ± 1.81^{ade}	0.77 ± 0.14^{bdf}	16.89 ± 2.09^{ed}	5.16 ± 0.26^{cd}	0 ± 0 ^{a}	43.04^{e}	56.88
GE	-----	11.99 ± 0.40^{adf}	0.61 ± 0.02^{adg}	31.91 ± 1.71^{c}	27.16 ± 0.06^{e}	13.7 ± 0.97	----	73.87
GA	-----	9.17 ± 0.32^{ecfg}	0.41 ± 0.01^{egh}	95.70 ± 0.62^{f}	38.13 ± 1.73^{f}	-21.33 ± 0.82	----	56.06
Suc	-----	9.45 ± 1.41^{ac}	0.58 ± 0.05^{aed}	100.40 ± 0.79^{g}	0.08 ± 0.00^{g}	-----	----	113.58

The same letters in the same column do not differ ($p < 0.05$). PS = particle size; M = moisture; Aw = water activity; S = solubility; H = hygroscopicity; ZP = zeta potencial; EY = encapsulation yield; Tg = glass transition temperature; Suc = sucralose. A: 2.5% GE + GA and core material 50%; B: 2.5% GE + GA and core material 75%; C: 2.5% GE + GA and core material 100%; D: 5.0% GE + GA and core material 50%; E: 5.0% GE + GA and core material 75%; and finally F: 5.0% GE + GA and core material 100%.

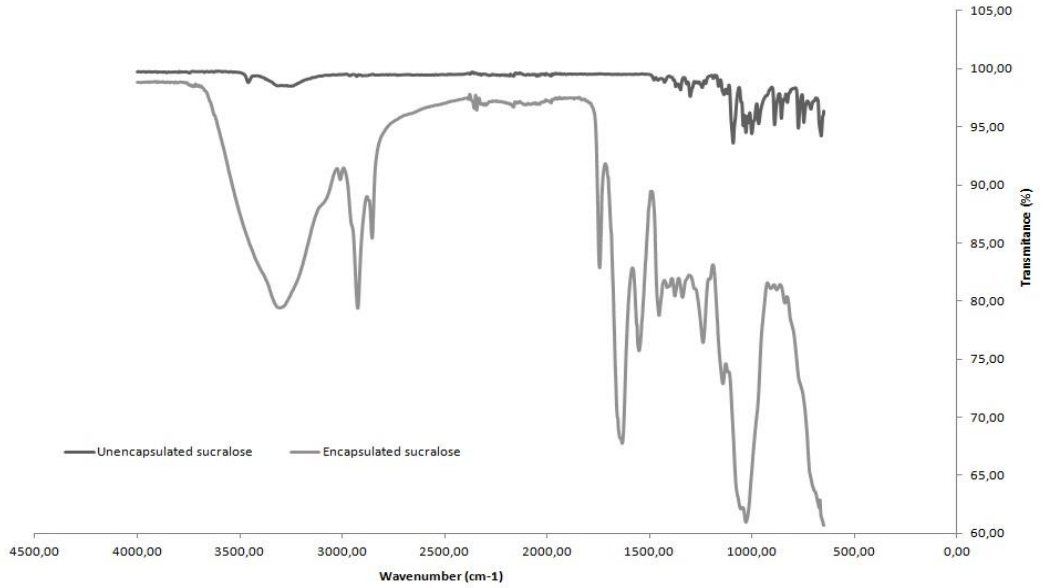

Figure 2 - FTIR of encapsulated and unencapsulated sucralose.

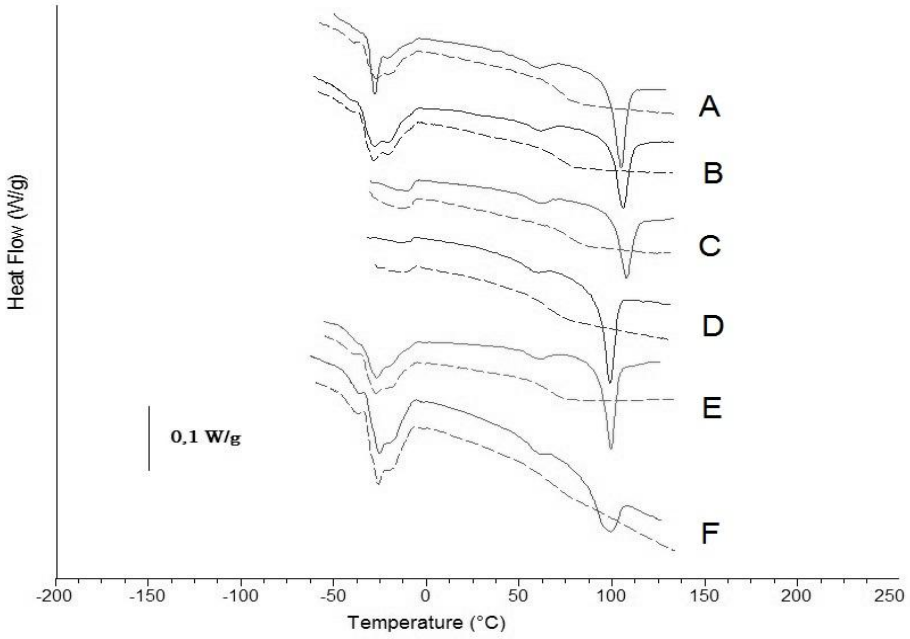

Figure 3 - Thermograms of the sucralose microcapsules. A: 2.5% GE + GA and core material 50%; B: 2.5% GE + GA and core material 75%; C: 2.5% GE + GA and core material 100%; D: 5.0% GE + GA and core material 50%; E: 5.0% GE + GA and core material 75%; and F: 5.0% GE + GA and core material 100%.

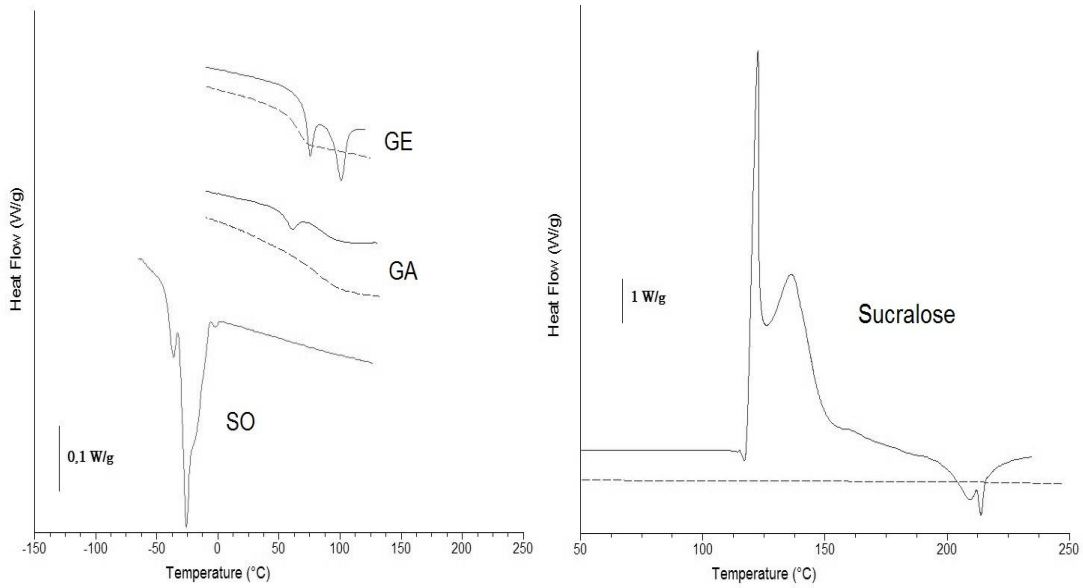

Figure 4 - Thermograms of the ingredients used to produce the microcapsules. GE = Gelatin, GA = gum Arabic and SO = soybean oil

4. CONCLUSION

Considering the aims and the results of this study, the proposed methodology of double emulsion followed by CC proved viable to encapsulate sucralose. Spherical and multinucleated microcapsules were formed, characteristics of complex coacervation technique, indicating that the double emulsion stage has been used successfully. The formulations have the potential to be applied in foods, especially formulation D, due to its most suitable characteristics. Future studies are required to the application of microcapsules in food products in order to study the release time and the effects on sensory characteristics of the products.

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Artigo de pesquisa 3

Functionality of sucralose microencapsulated by complex coacervation in chewing gum

Summary

Microencapsulation of sweeteners has attracted technological interest in recent years. One of the main reasons to encapsulate these compounds is to extend the sensation of sweetness, and the only way to evaluate the functionality of microcapsules on extending that sensation is through sensory analysis. Therefore, this study investigated the influence of the addition of the microcapsules of sucralose in texture and color of the chewing gum as well as the time-intensity profile, with the parameters maximum intensity, time to maximum intensity and total duration of the stimulus, and the acceptance of chewing gums made with sucralose in free and microencapsulated form. Microencapsulation process was effective in extending the duration of sweet stimulus in more than 2 minutes with no significant changes in the texture of the samples or sensory losses, demonstrating the potential use of this type of product.

1. Introduction

Sucralose, also known as trichlorogalactosacarose (TGS) is a compound derived from sucrose by selective replacement of three hydroxyl groups by chlorine atoms, resulting in a substantial increase of sweetness (WALLIS, 1993). The chlorination of sucrose molecule causes conformational changes that result in increased stability to acids and enzymes in relation to the original molecule (HOOD & CAMPBELL, 1990; WALLIS, 1993). This compound presents sweetness intensity 400-1000 times sweeter than sucrose and time-intensity sweetness profile very similar to that sugar, especially the nice sweet flavor without bitter notes or

metal waste (GRENBY, 1991; HOOD & CAMPELL, 1990; GOLDSMITH & MERKEL, 2001).

The perception of sweetness is a dynamic process that requires temporal evaluation for full characterization. In the assessment of food whose substitution of an ingredient alters the temporal properties, studies on the time-intensity profile are essential for investigating the factors that affect the processes (NOBLE et al., 1991). Chewing gums are an example of a product in which the flavor characteristics and texture change during consumption (McGOWAN & LEE, 2006). To evaluate the temporal behavior of sweeteners is important to predict their acceptance by the consumers, since each has specific characteristics of intensity, persistence of sweetness and presence or absence of aftertaste. These characteristics, in turn, may vary depending on the food to which the sweeteners are added (KETELSEN et al., 1993).

Microencapsulation is a process in which an encapsulating agent involves a core, in order to protect it from the harsh conditions of the environment, such as light, moisture, oxygen, and interactions with other compounds, stabilizing the product, increasing the shelf life and promoting controlled release of the microcapsule in pre-established conditions (SHAHIDI & HAN, 1993). Among the compounds that can be microencapsulated, sweeteners have emerged (FAVARO-TRINDADE et al. 2008) aiming at reducing its hygroscopicity, improving its fluidity and resistance to high temperatures and prolonging the sensation of sweetness (DZIEZAK, 1988; JACKSON & LEE, 1991, ROCHA-SELMI et al 2013a, ROCHA-SELMI et al 2013b). One of the main objectives of using microencapsulated ingredients in chewing gum is to enhance the controlled release once the ingredient is released gradually through the action of saliva and chewing (LEW, 2000). The quality of a chewing gum is mainly defined by the duration of its flavor (WONG et al., 2009). Since microencapsulation is a topic of technological interest, the objective of this study was to evaluate chewing gum produced with sucralose microcapsules regarding the perception of sweetness, overall acceptance and physicochemical characteristics.

2. Material and methods

2.1. Material

Six samples of chewing gum were investigated, three of which were produced with encapsulated sucralose, and three with sucralose in its free form. The microcapsules were produced by complex coacervation using gelatin and gum Arabic as encapsulating agents (ROCHA-SELMI et al. 2013b). The chewing gums were produced in a company of that segment in the state of São Paulo (Brazil).

2.2. Methodology

2.2.1. Production of chewing gums

The chewing gums were produced using the gum base provided by the company where they have been processed. For the production of gums, gum base was melted and sucralose (in free or encapsulated form) and mint´s aroma were incorporated. Then, gum base was shaped and diced to form the chewing gum. Three systems of sucralose microcapsules previously studied (ROCHA-SELMI et al. 2013b) differing in the core concentration (50, 75 and 100% in function of the total content of encapsulating agents) were used in the process. Each of these systems was applied to a sample of chewing gum, obtaining three samples containing the encapsulated sweetener. Equal amounts of microcapsules were added to each formulation in order to avoid possible interference on the results and to obtain different sweetener concentrations in the samples. For comparison purposes, three samples were produced with sucralose in free form in the same concentrations of the samples produced with the microencapsulated sweetener. Table 1 shows the nomenclature and the concentration of sucralose of the samples.

Table 1: Nomenclature and concentration of sucralose in the samples of chewing gum

Sucralose	Abbreviation	Sucralose concentration (mg) / 100g chewing gum
Microencapsulated – system A	SMA	40
Microencapsulated – system B	SMB	38
Microencapsulated – system C	SMC	35
Not Microencapsulated (Free) – A	SFA	40
Not Microencapsulated (Free) –B	SFB	38
Not Microencapsulated (Free) - C	SFC	35

System A, B and C = microcapsules produced with 50, 75 and 100%, respectively, of core material as a function of the total content of encapsulating agents.

2.2.2. Color and texture measurements

Color and texture of the samples were measured to evaluate whether the addition of the microcapsules has altered these parameters in chewing gum.

The apparent color was assessed by CieLab System by objective measures and direct reflection using a colorimeter (Hunter Lab - Color Quest II - Virginia, USA). The texture of the chewing gum was evaluated by compression test using a texture analyzer (TA-XT2 TA Instruments, Newcastle,EUA).

2.2.3. Sensory evaluation

The samples were served in individual booths with controlled lighting and temperature to ensure comfort and individuality for each assessor. The samples were served randomly coded with 3-digit numbers, unpacked, with rectangular shape nearly 2.5 cm x 1 cm.

2.2.3.1. Time intensity analysis

The time-intensity analysis was performed to evaluate the sweetness of the samples and to determine whether the microencapsulation increased the duration of sweetness when compared to samples containing sucralose in its free form. Twelve pre-selected trained panelists participated in the test.

Data collection for the time-intensity analysis was held on computers using the software Time-Intensity Analysis of Food and Tastes - TIAFT (UNICAMP, 2012) using a 9-cm scale. The panelists assessed the sweetness of the samples, recording the intensity of the attribute in accordance with the passage of time. The tests were performed in four sessions in complete balanced blocks and monadic presentation. The time-intensity curves allowed the analysis of the following parameters: maximum intensity recorded by the assessor (Imax), time to the maximum intensity (Timax) and total duration of the stimulus (Ttot) (PALAZZO & BOLINI, 2009).

2.2.3.2. Acceptance Test

The samples were evaluated by an affective test perfomed with 120 consumers aged between 18 and 65 years, who consumed chewing gum at least once a week. The samples were presented in complete and balanced monadic blocks. The attributes appearance, aroma, flavor, texture, and overall impression were evaluated using a 9 cm unstructured scale, anchored at the extremes between "extremely dislike" and "extremely like" (STONE et al. 2012).

2.2.4. Statistical Analysis

Data were analyzed by univariate statistical analysis (ANOVA) e Tukey´s mean test ($p \leq 0.05$).

3. Results and Discussion

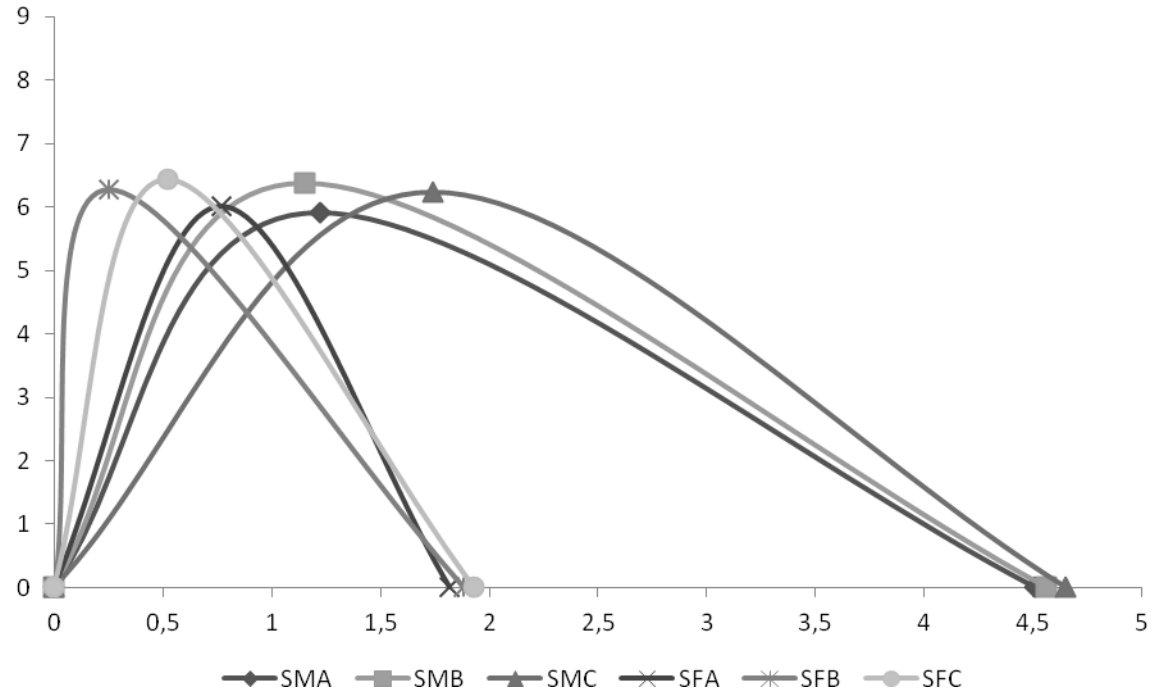
Table 2 shows the mean values for time-intensity analysis. Through these data, it was possible to construct the curves of sweetness of the samples (Figure 1). Samples with sucralose in free form had a faster release of sweet taste than the samples with encapsulated sucralose. This result indicates that the encapsulation process was able to promote a controlled release of the sweetener that was probably released during chewing. The maximum intensity profile was significantly

influenced by the concentration of sweetener present in the samples, once the higher the concentration of sucralose, the higher the maximum sweetness intensity perceived by the panelists. These results show that the microencapsulation process did not result in loss of sweetness, since no significant difference in the parameter Imax was observed for the samples containing the same concentration of free or encapsulated sucralose. Except for the samples with lower concentrations of sucralose (SMA and SFA), the samples with encapsulated sweetener reached the maximum intensity of sweetness, presenting times significantly higher as compared to the samples with sucralose in free form. A major goal of encapsulation of sweeteners is to extend sweetness. As shown by the parameter Ttot, the samples containing encapsulated sucralose presented total sweetness duration more than double the values obtained for the samples containing sucralose in free form, demonstrating the potential use of the encapsulation technique for this sweetener with the aim to promote its gradual release. Both samples containing sucralose in free form and encapsulated form showed a longer sweetness duration after the maximum intensity was reached, probably due to the sweet aftertaste of this sweetener, which was also observed in sensory studies with ice cream and chocolate (MELO, BOLINI, & EFRAIM, 2009; CADENA & BOLINI, 2011)

Table 2: Mean values for the time intensity analysis of sweetness of chewing gums containing microencapsulated sucralose and in its free form

	SMA	SMB	SMC	SFA	SFB	SFC
Imax	5,92^{b}	6,38^{a}	6,24^{a}	6,01^{b}	6,27^{a}	6,43^{a}
TImax (minutes)	1,22^{b}	1,15^{b}	1,74^{a}	0,77^{bc}	0,25^{c}	0,52^{c}
Ttot (minutes)	4,51^{a}	4,56^{a}	4,65^{a}	1,82^{b}	1,88^{b}	1,93^{b}

The same letters in the same row means no significant difference ($p < 0.05$). SMA, SMB and SMC: Microencapsulated – system A, B and C, respectively; SFA, SFB and SFC: Not Microencapsulated (Free) – A, B and C, respectively.



SMC: Microencapsulated – system A, B and C, respectively; SFA, SFB and SFC: Not Microencapsulated (Free) – A, B and C, respectively.

Figure 1: Time-intensity curves for the sweet taste stimulus of six samples of chewing gum

The acceptance test is important to check if the use of microcapsules affected the acceptance of the samples by the consumers. The mean values for the attributes appearance, aroma, taste, texture and global impression are presented in Table 3. In general, all samples were well accepted by consumers (mean scores above 4.5). For the attributes appearance and aroma, the samples SMA and SMB containing microencapsulated sucralose were significantly more accepted than the other samples. With respect to the attribute aroma, the sample containing encapsulated sucralose (SMB) and the one containing sucralose in free form (SLC) were the most accepted. These results allow the conclusion that, besides enabling

the gradual release of the sweetener, encapsulation promoted greater acceptance of the chewing gum, which may be considered very positive. The instrumental texture evaluation (Table 4) showed no significant difference in the compression force between the samples, but the addition of microcapsules significantly contributed to a better acceptance of texture by consumers. In sensory evaluation of texture of chewing gums containing microencapsulated acid citric (ABBASI et al. 2009), one sample had lower acceptance than the chewing gum produced with the acid in free form, while the other samples containing microencapsulated citric acid presented similar or better results than the sample containing the acid in free form, as occurred in the present study.

Table 4 also shows the values for the chromatic coordinates L*, a* and b*. The parameter L* varies from 0 to 100 and is associated with the luminosity of the samples. The higher this value, the clearer the sample is. The chromatic coordinate a* represents the green-red scale, where positive values indicate samples in the red region and negative values indicate the green region. The chromatic coordinate b* represents the blue-yellow scale, where positive values indicate samples in the yellow region, and negative values indicate the blue region. The chewing gum were whitish. No pigment was added to the samples, in order to do not mask possible color changes caused by the addition of the microcapsules.

The L* values in Table 4 indicate that the samples containing sucralose in free form were significantly clearer than the samples containing the microencapsulated sweetener, and the samples containing microencapsulated sucralose had higher a* values, while no correlation was observed for the coordinate b* for all samples. Perhaps the different values of the chromatic coordinates for the samples with free and encapsulated sucralose contributed to the greater acceptance of the encapsulated samples by consumers, since color influences the appearance of a product, which is an important factor for sensory acceptability of foods. For industrial application, these parameters can easily be changed by adding pigments, which is common in the production of chewing gum.

Table 3: Mean values of the acceptance test of six samples of chewing gum.

Sample	Appearance	Aroma	Taste	Texture	Global Impression
SMA	$7,7^{ab}$	$7,6^{a}$	$6,1^{c}$	$7,3^{a}$	$7,2^{a}$
SMB	$8,1^{a}$	$7,3^{a}$	$7,0^{a}$	$7,1^{a}$	$7,4^{a}$
SMC	$7,5^{bc}$	$6,3^{b}$	$6,5^{b}$	$7,5^{a}$	$6,4^{b}$
SLA	$7,2^{c}$	$6,4^{b}$	$6,2^{bc}$	$5,9^{c}$	$6,3^{b}$
SLB	$6,6^{d}$	$6,4^{b}$	$6,2^{bc}$	$6,0^{bc}$	$6,4^{b}$
SLC	$7,2^{c}$	$6,1^{b}$	$7,0^{a}$	$6,4^{b}$	$6,3^{b}$

The same letters in the same column means no significant difference ($p < 0.05$).

Table 4: Mean values of compression force and chromatic coordinates for the six samples of chewing gum.

	Texture	**Color**		
	Force (g)	**L**	**a**	**b**
SMA	$3037,66^{a}$	$82,96^{c}$	$1,00^{a}$	$9,54^{ab}$
SMB	$2961,32^{a}$	$85,29^{b}$	$0,79^{b}$	$8,78^{c}$
SMC	$3432,73^{a}$	$86,13^{b}$	$1,00^{a}$	$7,41^{d}$
SLA	$3263,40^{a}$	$88,25^{a}$	$0,24^{c}$	$9,89^{a}$
SLB	$3329,99^{a}$	$88,58^{a}$	$0,24^{c}$	$8,94^{bc}$
SLC	$3471,99^{a}$	$87,90^{a}$	$0,15^{d}$	$9,80^{a}$

The same letters in the same column means no significant difference ($p < 0.05$).

4. Conclusion

The results found in the present study proved that the addition of microcapsules of sucralose in chewing gum was effective in meeting the goal of extending the sensation of sweetness. The presence of these microcapsules did not change the texture of the samples when compared to the samples containing the sweetener in free form, and did not cause losses in the sensory acceptance, which are important parameters for the development of new products.

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Artigo de pesquisa 4

EFFECT OF MICROENCAPSULATION IN THE FUNCTIONALITY OF STEVIOSIDE

ABSTRACT

Microencapsulation may be an alternative form to reduce the hygroscopicity of stevioside, prolonging sweetness and masking bitterness. This study aimed at microencapsulating stevioside by double emulsion followed by complex coacervation using gelatin and gum Arabic as encapsulating agents and to evaluate the functionality of the microcapsules to prolong the sweet taste in chewing gum. The six treatments differed in concentration of encapsulating agents (2.5 and 5.0%), and core material load as a function of the content of encapsulating agent (50, 75 and 100 %). The microcapsules were relatively large (sizes ranging from 64.6 to 102.3 µm), slightly soluble (solubility ranging from 10.61 to 13.03 g of water absorbed / 100g powder) and slightly hygroscopic. FTIR analysis exhibited amide bonds that confirmed the formation of microcapsules by coacervation technique. Tg values were well above room temperature (55 to 59 °C). The microcapsules were effective to prolong sweetness on chewing gum.

Considering the results obtained in this study, it was concluded that the double emulsion before the process of complex coacervation, enables the encapsulation of hydrophilic materials such as stevia, by this technique. Also the capsules showed a promising use in chewing gum with the objective of increasing the duration of the sweet taste.

1. INTRODUCTION

Current demand revealed that besides pleasing flavor, appearance and texture, consumers want to combine these sensory characteristics with low-fat, low-sugar and reduced-calorie products. For these reason, sweeteners have received special attention, since obesity has become epidemic and the incidence of *Diabetes mellitus* has increased worldwide. Sweeteners are often used in diet and light products, which are developed for individuals with *diabetes* or those concerned to reduce or maintain weight. The sweeteners provide the desired level of sweetness without calories intake inherent to sucrose.

The steviosides are natural compounds extracted from the leaves of stevia (*Stevia rebaudiana*). The extract has white coloration and consists of stevioside and its anomers, the rebaudiosides, both responsible for the sweetness of the product (HIGGINBOTHAM, 1983; PARPINELLO et al., 2001).

The extract of stevia is very stable and provides sweet taste that lasts in the mouth for some time, but has pronounced bitterness and aftertaste, which limit its use (PARPINELLO et al., 2001; NEWSOME, 1993). Microencapsulation proved to be a viable alternative to mask or reduce the bitterness of casein hydrolysates (MOLINA et al. 2008; MENDANHA et al. 2009; ROCHA et al. 2009; FAVARO-TRINDADE et al. 2010)

There is much interest in the encapsulation of sweeteners, mainly aimed at delaying its release, as several patents have been dealing with the subject. However, there are very few scientific reports aimed to study the encapsulation of sweeteners. In previous studies of our group was demonstrated that is possible to encapsulate hydrophilic compounds by complex coacervation making a double emulsion as the first step (MENDANHA et al. 2009, COMUNIAN et al. 2013). It was useful including for the encapsulation of aspartame to protect it under high temperature and to provide control release (ROCHA-SELMI et al. 2013a) and for sucralose, to prolong the sweet taste and to reduce its hygroscopicity (ROCHA-SELMI et al. 2013b).

However, just a single paper was found in the literature whose objective was to make an application and to study the functionality of an encapsulated sweetener and it studied the stability of aspartame encapsulated in high melting point fat during the baking of cakes (WETZEL and BELLT, 1998). In view of this, the aim of this study was to microencapsulate stevioside by double emulsion method followed by complex coacervation using gelatin and gum arabic as encapsulating agents, characterize the microcapsules produced and to evaluate the duration of sweet taste in chewing gums produced with free and microencapsulated stevioside.

2. MATERIAL AND METHODS

2.1. Materials

Stevioside sweetener (Stevia Farma, Industrial S / A) was used as the core material load. Gelatin (GE) (Gelita) and gum Arabic (GA) (Synth) were used as encapsulating agents. Lecithin (Gerbras Química Farmacêutica Ltda.) and soybean oil (Bunge) were used as the surfactant and continuous phase, respectively, for formation of the primary emulsion (water in oil).

2.2. Methods

2.2.1. Preparation of coacervated microcapsules

The methodology used for production of microcapsules was the one described by Rocha-Selmi et al (2013b) with some modifications: The emulsion was prepared with 20% aqueous stevioside, soybean oil (three times the volume os stevioside solution) and soy lecithin (3% of total solids concentration). Six treatments with different concentrations of encapsulating agents (solutions 2.5 and 5% of GE and GA) and different core material concentration as a function of the encapsulating agents (50, 75 and 100%) were obtained. They were named as follows:

Treatments A, B and C with GE and GA 2.5% and core material 50, 75 and 100%, respectively. Treatments named D, E and F with GE and GA 5% and core material 50, 75 and 100%.

2.2.2. Analytical determinations

2.2.2.1 Hygroscopicity

Approximately 2 g of the lyophilized coacervates were placed in Petri dishes and kept in desiccators (250 mm diameter) containing 100 ml of saturated Na_2SO_4 at 25 ± 1 °C for a week. The weight of water absorbed by the sample was measured every hour during the first 5 h, then every 24 h during a week (CAI and CORKE, 2000). The result was expressed in grams of water absorbed per 100g of solids.

2.2.2.2 Solubility

The solubility index was determined as described by Cano-Chauca et al. (2005). The method consists of adding 1 g sample in a vessel containing 100 ml distilled water under magnetic stirring for 5 min, followed by centrifugation at 3000 x g for 5 min. Then, a 25 ml aliquot of supernatant was removed and dried at 105 °C until constant weight. The solubility index was calculated from the difference in weight.

2.2.2.3 Particle Size

The size and size distribution of the capsules were measured by laser diffraction using a Shimadzu SALD-201V particle size analyzer (Kyoto, Japan). The microcapsules were dispersed in isopropanol (Synth, Brazil) and stabilized for 5 min prior to analysis.

2.2.2.4. Morphology

The morphology of the microcapsules was observed by an optical microscope (OM) (BEL Photonics ® Microscope, Milan, Italy) equipped with camera and software BEL View v 62, and by scanning electron microscopy (SEM), using

Hitachi Tabletop Microscope (Tokyo, Japan) TM 3000, with TM 3000 program. For SEM, the microcapsules were placed on a double-sided carbon tape (Ted Pella, Inc., Redding, United States) which were fixed on aluminum stubs. The images were captured at an accelerating voltage of 5 kV and current of 1750 mA.

2.2.2.5 Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of the pure stevioside, ingredients and microcapsules were obtained by a FTIR spectrometer (Perkin Elmer Spectrum One, Shelton, CT, USA) using software Spectrum one v 5.3.1. The samples were placed on the measuring cell and the scans were performed in the spectral wavelength range of 600 to 4000 cm^{-1}.

2.2.2.6 Encapsulation Yield

The encapsulation yield was calculated according to Jun-xia et al (2011) as the ratio of the total sweetener present in the capsule (S_{total}) and the amount of sweetener used to produce the microcapsules ($S_{production}$), as shown in Equation 1.
To determine the total content of sweetener present in the microcapsules, 5 ml of 1% saline solution and 5 mL acetonitrile were added to falcon tubes containing 0.1 g of freeze-dried microcapsules. The tubes were shaken in a shaker tube and exposed to ultrasonic for 5 min, followed by centrifugation (4000 rpm) for 5 min. Then, an aliquot of the supernatant was removed for analysis, which was performed by external standardization in a liquid chromatograph (Shimadzu Prominence, Japan) equipped with a quaternary pump, auto injector (SIL - 10AF), reverse phase column (Shim-pack VP-ODS; 250 x 4.6 mm), diode array detector (210 nm) and data software (LC solution), according to the methodology described in IAL (2008).

$$EY(\%) = S_{(total)} / S(_{production}) \quad (1)$$

2.2.2.7 Differential Scanning Calorimetry

Samples (~1 g) were equilibrated in air over dry silica gel at 25 °C. After equilibration (about 2 weeks), aliquots were taken for DSC analysis. Phase transitions were determined by differential scanning calorimetry using a DSC TA2010 controlled by a TA5000 module (TA Instruments, Newcastle, USA). Samples of about 10 mg, conditioned at TA aluminum pans were heated between -20 and 200 °C at a rate of 10°C/min, in N_2 inert atmosphere (45 mL/min). An empty pan was used as reference. Liquid nitrogen was used for sample cooling before the runs. Samples showing a devitrification peak after the first run were annealed at the devitrification peak temperature (Td) for 30 min before the second DSC run. Phase properties were determined by the DSC thermograms using the Software Universal Analysis V1.7F (TA Instruments, Newcastle, USA).

2.2.3. Production of chewing gum and evaluation of the duration of sweet taste

Six samples of mint flavor chewing gum were produced, three with stevioside microencapsulated (with capsules of the treatments A, B and C) and three with free stevioside, corresponding to the concentrations obtained in the chewing gum with microcapsules. Table 1 shows the nomenclature and the stevioside concentration of each sample produced. To produce the chewing gums samples, the gum base provided by the company where they have been processed was used. This gum base was melted and stevia (in free or encapsulated form) and aroma of mint were incorporated. Then, gum base was shaped and diced to form the chewing gum samples.

Samples were subjected to a sensory test of time intensity of the sweet taste, which were served randomly coded with 3-digit numbers. Ten pre-selected and trained panelists participated in the test. Data collection was performed on computers using software Time-Intensity Analysis of Food and Tastes - TIAFT (State University of Campinas - UNICAMP, 2012) using a scale of 9 cm. The

panelists assessed the sweetness of the samples, recording the intensity of the attribute over time. The tests were conducted in four sessions with a monadic presentation using balanced complete blocks (Bolini and Palazzo, 2009).

Table 1: Concentration and nomenclature of chewing gum samples produced with microencapsulated and free stevioside.

Stevioside	Nomenclature	Stevioside concentration (mg) / 100g of chewing gum
Microencapsulated – treatment A	SMA	70
Microencapsulated – treatment B	SMB	75
Microencapsulated – treatment C	SMC	80
Free – A	SFA	70
Free –B	SFB	75
Free - C	SFC	80

Treatments A, B and C: GE and GA 2.5% and core material 50, 75 and 100%, respectively.

2.2.4. Statistical Analysis

Analyses were performed in triplicate and the data evaluated by ANOVA ($p < 0.05$) and Tukey's comparison test ($p < 0.05$) using the Statistica software (Statsoft, USA).

3. Results and Discussion

In previous studies of our group was demonstrated that is possible to encapsulate hydrophilic compounds by complex coacervation making a double emulsion as the

first step (Mendanha et al. 2009, Comunian et al. 2013; Rocha-Selmi et al. 2013a; Rocha-Selmi et al. 2013b).

So, as a hydrophilic active principle (stevioside) was encapsulated, a double emulsion was prepared, i.e. an aqueous solution of stevioside was emulsified in soybean oil in order to produce a primary emulsion water in oil (W / O), which was emulsified in GE (first step in CC processes) to form the secondary emulsion W / O / W.

Due to the need to prepare the double emulsion, we first studied the formation of droplets in the emulsion process and after we evaluated the microcapsules obtained when the anionic polymer (GA) was added and the pH decreased. Since GE is an amphoteric substance, reducing the pH allowed GE to change its charge to positive and bind GA.

Figure 1A shows one of the emulsions studied. Regardless of the concentrations of GE and sweetener solution, microscopic evaluation showed that all the formulations exhibited movement of droplets characteristic of Brownian Motion, indicating the formation of emulsion in all formulations.

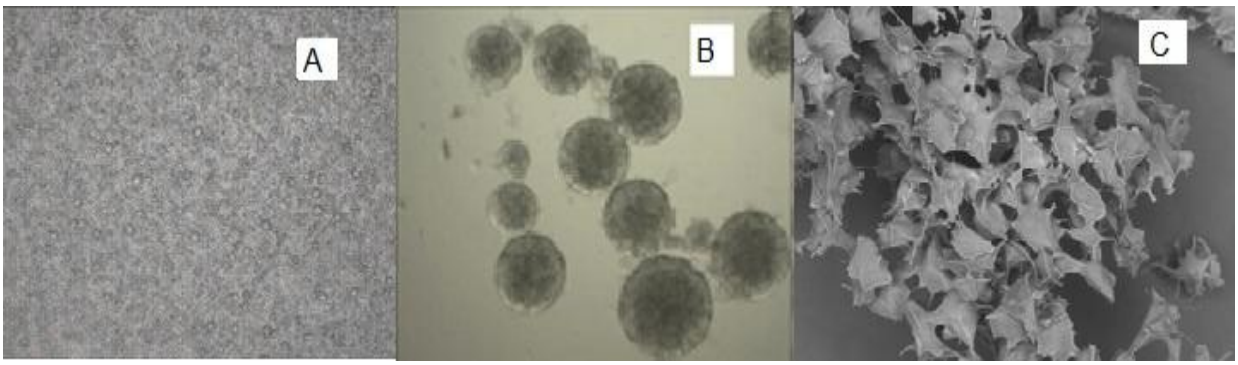
As the formulations prepared with the single emulsion remained visually stable (without phase separation) for at least 2 h and appeared to be similar by visual microscopic, all formulations were suited for addition of GA, changing pH and formation of microcapsules.

The images from OM and SEM were similar for all formulations. Figure 1B shows the MO of one formulation. All the formulations resulted in microcapsules with circular shape, which is suitable for facilitating the material flow. In addition, all microcapsules were polynucleated, unlike observed by Mendanha et al. (2009), who obtained mononuclear protein hydrolyzate microcapsules by using the same microencapsulation technique of this study. The difference can be attributed both to the higher stirring speed of the disperser, and the different polymers used to promote the coacervation.

According to Figure 1C, the microcapsules exhibited continuous walls with no apparent cracks or porosity, indicating that the processes of freezing and

lyophilization were adequate, since they did not cause damage to the particles. Microcapsules presented intact and continuous walls, which is essential to ensure greater protection and retention of the encapsulated material.

The microcapsules were cross-linked by solid bridges, which were also observed by Prata et al. (2008) in the encapsulation of vetiver oil by CC using GE and GA as encapsulating agents. These solid bridges may be attributed to agglomeration of



the microcapsules caused by the freezing process followed by lyophilization. However, after rehydration, the lyophilized microcapsules exhibited the shape shown in Figure 1B.

Figure 1 - Optical microscopy with 10x magnification (a and b) and scanning electron microscopy with 200x magnification (c) of the formulation A (2.5% gelatin and core material load 50%)

Table 2 shows the results (mean and standard deviation) for the parameters hygroscopicity, solubility and average particle size of the 6 samples. The encapsulated stevioside was less hygroscopic than its free form, thus the steps employed to promote encapsulation reduced the hygroscopicity of the sweetener, facilitating handling and storage. In addition, the values obtained in this study were up to 6 times lower than the results found by Nori et al. (2011) in propolis microcapsules obtained by CC using soy protein isolate and BTM pectin as encapsulating agents.

In general, the samples presented low solubility in cold water, as expected for microcapsules produced by CC, which are not water-soluble. These results are

very important as it may be possible to provide a gradual release of the sweetener when chewing, prolonging the perception of the sweet taste, which was one of the goals to encapsulate the stevioside.

The capsules produced in this study were relatively large, with mean sizes ranging from 64 to 102 μm. This result is similar to that obtained by Saravan and Rao (2010), who encapsulated drugs by CC, using GE and pectin or alginate as encapsulating agents.

Table 2 - Analytical determinations of lyophilized stevioside coacervates.

Samples	H (g water absorbed/100g powder)	S (%)	PS (μm)
A	7.08 ± 0.17^{a}	12.87 ± 0.24^{a}	89.3
B	4.96 ± 0.56^{ab}	11.89 ± 0.33^{ac}	97.1
C	3.77 ± 0.15^{bc}	11.33 ± 0.15^{bc}	102.3
D	5.01 ± 0.29^{ab}	13.03 ± 0.12^{a}	64.6
E	6.30 ± 1.44^{ab}	10.61 ± 0.27^{b}	96.5
F	2.21 ± 0.12^{c}	12.66 ± 0.47^{a}	89.3
Stevioside	21.93 ±0.02	94.16±0.69	-
GE	27.16 ± 0.06^{e}	31.91 ± 1.71	-
GA	38.13 ± 1.73^{f}	95.70 ± 0.62	-

The same letters in the same column do not differ ($p < 0.05$). H = hygroscopicity, S = solubility; PS = particle size,. A: 2.5% GE + GA and core material load 50%; B: 2.5% GE + GA and core material load 75%; C: 2.5% GE + GA and core material load 100%; D: 5.0% GE + GA and core material load 50%; E: 5.0% GE + GA and core material load 75%; and finally F: 5.0% GE + GA and core material load 100%.

GA is a polysaccharide having free carboxyl groups that confer negative charge to this molecule, while GE is a positively charged protein in acidic medium, due to amine groups. During CC, carboxyl groups from polysaccharides interact with

amino groups of proteins to form a complex containing amide. FTIR analysis verified the formation of amides in the samples, confirming the occurrence of CC.

The spectra of encapsulated samples (Figure 2) exhibited small peaks next to the elongated peaks that appeared around 2900 cm^{-1}, characteristic of carboxyl groups, indicating that not all the carboxyls groups are involved in the coacervation process. However, there were no peaks between 3400 and 3550 cm^{-1}, which are characteristic of amine groups, but peaks characteristic of amides appeared around 1500-1640 cm^{-1}, thus confirmed the formation of coacervates due to the reaction between GE amino groups and GA carboxylic groups.

As can be seen in Figure 2, all formulations exhibited peaks in the same bands and wavelengths. That is, variations in the core material load and encapsulating agents did not result in bonds beyond those already expected.

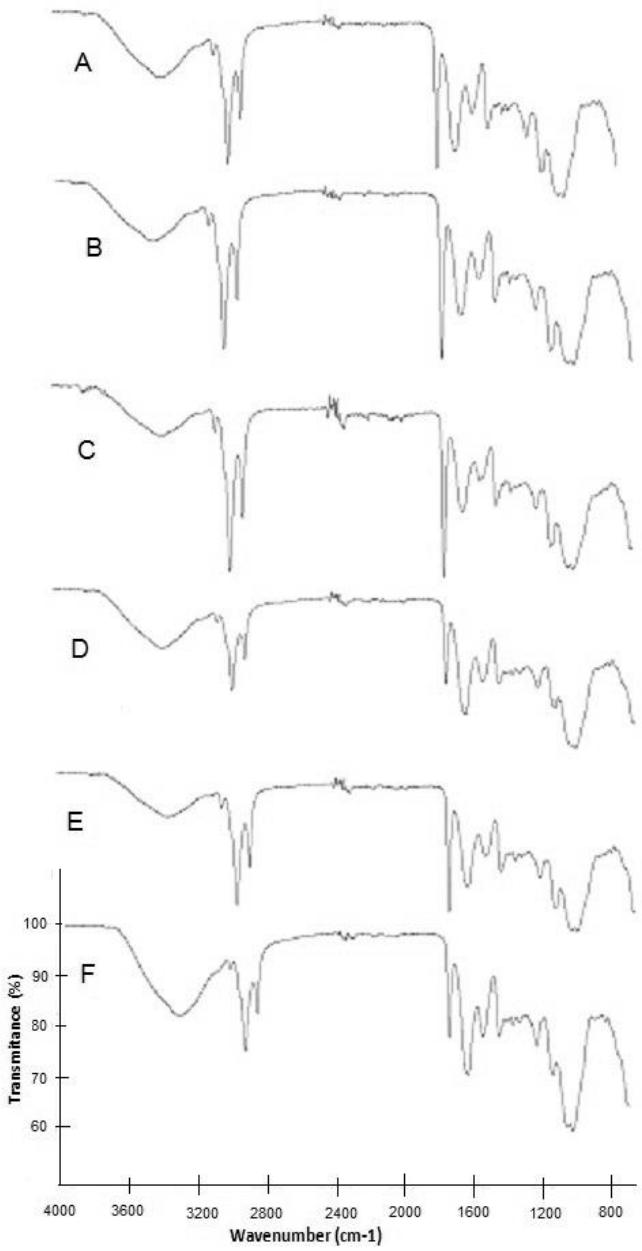


Figure 2 -Infrared spectra obtained for stevioside microcapsules produced using gelatin and gum Arabic, in the six formulations

Table 3 presents the EY and glass transition temperature. The EY was reasonable and ranged from 43 to 64.5%. For both concentrations of polymer, the higher the core material load, the lower the yield. Thus, treatments A and D may be the most suitable for encapsulating stevioside. Similar results were reported by Jun-Xia et al.

(2011), who encapsulated orange essential oil by CC using soy protein isolate and GA as encapsulating agents. This result may be due to the increased viscosity caused by the high polymer concentration that probably interfere with microcapsules formation, since the mobility of the macromolecules may decrease and consequently increase the competition for solvent molecules, as previously described by Thies (1995). Furthermore, the higher the core material load the higher the negative values of zeta potential, which means that some negative charged carboxyl groups from GA were not complexed with GE molecules, causing an ionic imbalance and decreasing the encapsulation yield. According to Yeo et al. (2005), the ionic strength is related to the stability of the microcapsules produced by complex coacervation.

The results obtained for EY were similar to those reported by Saravanan and Rao (2010), who encapsulated hydrophobic drugs, indomethacin and diclofenac sodium by complex coacervation, using GE and pectin or alginate as encapsulating agents. Moreover, the same authors found low EY for encapsulation of the drug metronidazole hydrochloride (approximately 5 %) by complex coacervation method using GE and pectin or alginate as encapsulating agents. According to these authors, the poor result was due to this drug is very water soluble, thus its molecules escaped during the coacervation process. Comparing the results obtained by Saravanan and Rao (2010) to the results of this paper, the initial stage using double emulsion may have been efficient to ensure higher encapsulation of water-soluble compound by complex coacervation, since the lower yield found in this study was 45%. Similar results were found for EY in encapsulation of sucralose (43 to 89%) and aspartame (45 to 71%) using the same methodology and encapsulating agents of this work (Rocha-Selmi et al., 2013a and Rocha-Selmi et al., 2013b). The microcapsules exhibited very similar and relatively high Tg values (Table 3). Obtaining Tg values well above room temperature can be considered a very important result because it ensures physical stability in this storage condition, as, according to Roos (1995), physical changes such as

agglomeration and collapse of the structure may occur when Tg is below the storage temperature.

Table 3 - Mean values of the parameters encapsulation yield and glass transition temperature (Tg).

Sample	**EY** (%)	**Tg** (^{o}C)
A	64,50± 1,66 [a]	59,39
B	59,56± 1,63 [b]	56,68
C	52,58± 3,74 [c]	55,35
D	64,40± 1,56 [a]	59,72
E	54,87± 3,15 [b]	57,29
F	43,35± 1,42 [c]	59,84
GE (2,5%)	-	73,87
GA (2,5%)	-	56,06

The same letters in the same column do not differ ($p < 0.05$). EY = encapsulation yield, Tg = Transition temperature, A: 2.5% GE + GA and core material 50%; B: 2.5% GE + GA and core material 75%; C: 2.5% GE + GA and core material 100%; D: 5.0% GE + GA and core material 50%; E: 5.0% GE + GA and core material 75%; and finally F: 5.0% GE + GA and core material 100%.

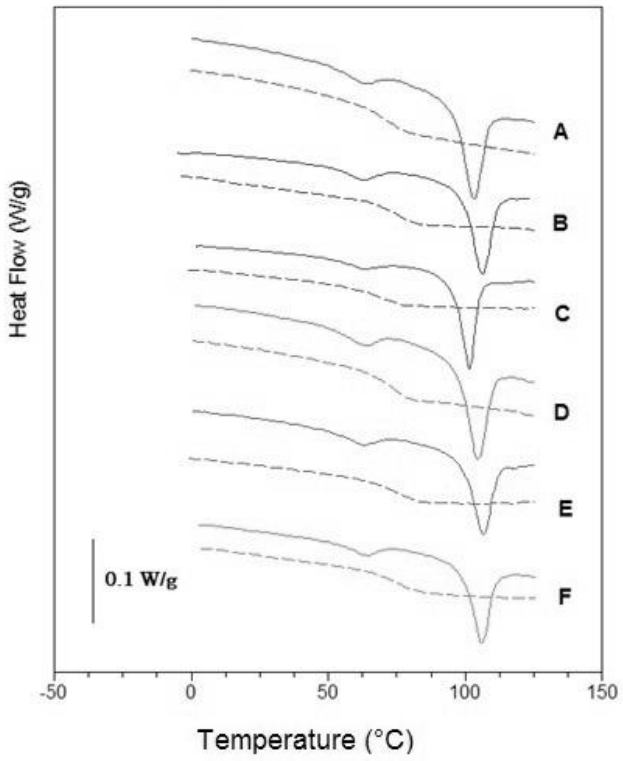


Figure 3 - Thermograms of stevioside microcapsules using gelatin and gum Arabic as wall materials in the six formulations.

According to the thermograms shown in Figure 3, the microcapsules showed similar thermal behavior, as their thermograms did not differ. This result allows us to infer that changes in the core material load and encapsulating agents did not alter the thermal behavior of the microcapsules. The microcapsules exhibited two melting peaks, at around 60 and 105 °C, which corresponded to melting peaks of gum Arabic and gelatin, respectively, as shown in the thermograms of these materials (Figure 4).

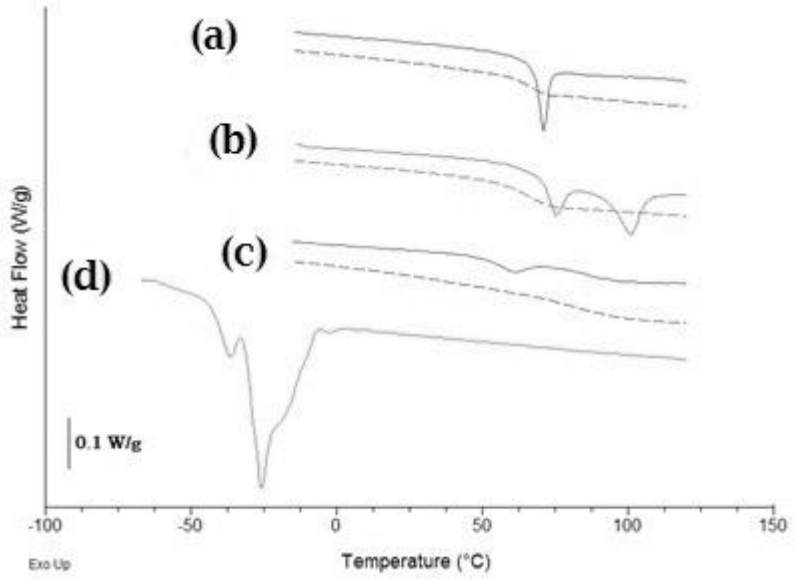


Figure 4: Thermograms of stevioside (a), gelatin (b), gum Arabic (c) and soybean oil (d).

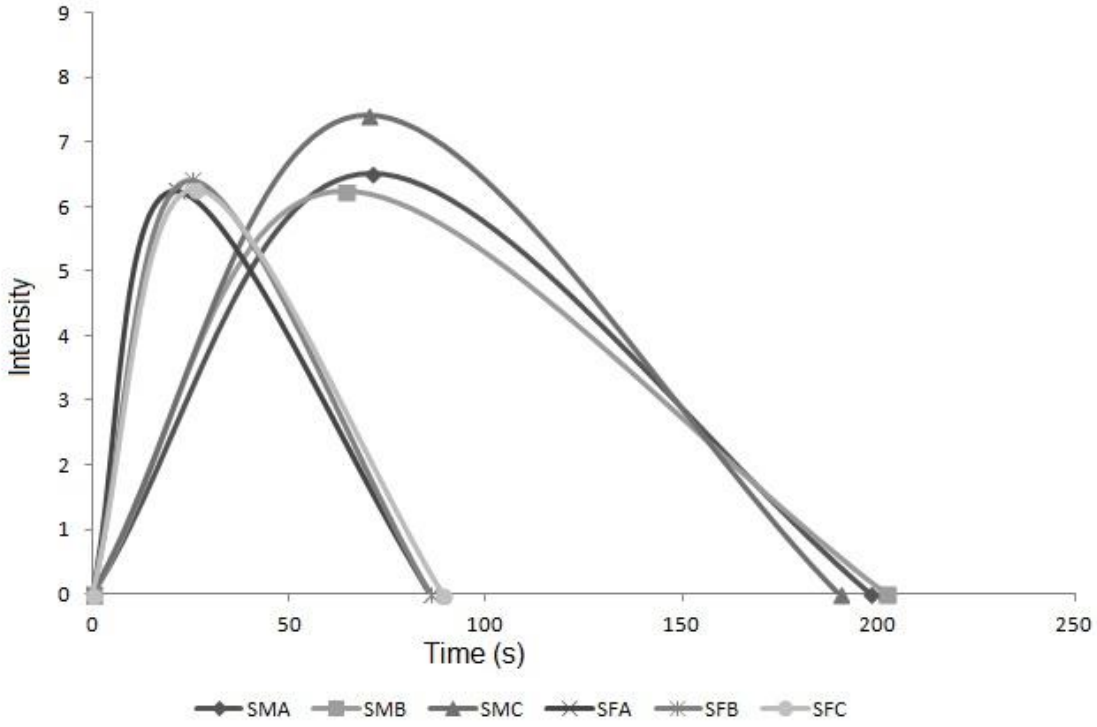
Table 4 shows the values obtained in the time-intensity samples of chewing gums. The parameters evaluated were: total time of sweet taste (Ttot), maximum intensity of sweetness (Imax) and time that occurred Imax (TImax). With these parameters, the curves were constructed for sweet taste profile for all samples, shown in Figure 5. It was observed that the total time duration of sweet taste was significantly higher for all samples with microencapsulated stevia and that these samples showed a sweet taste release profile over prolonged. With the presence of microcapsules, stevioside was being released gradually, contributing to the longer duration of sweetness. This result proved the efficiency of the microencapsulation process used to extend the sweetness of stevia.

Table 4: Means of the time-intensity parameters for sweetness of chewing gums samples

Sample	Ttot (s)	Imax	TImax (s)
SMA	198[a]	6.51[b]	71[a]
SMB	202[a]	6.23[b]	64[a]
SMC	190[a]	7.41[a]	70[a]
SFA	86[b]	6.25[b]	21[b]
SFB	86[b]	6.42[b]	25[b]
SFC	89[b]	6.27[b]	26[b]

The same letters in the same column do not differ ($p < 0.05$).

SMA, SMB and SMC: Microencapsulated – treatment A, B and C, respectively. SFA, SFB and SFC: Correspondent free A, B and C, respectively.



SMA, SMB and SMC: Microencapsulated – treatment A, B and C, respectively. SFA, SFB and SFC: Correspondent free A, B and C, respectively.

Figure 5: Time-intensity curves for sweetness of chewing gums samples

4. CONCLUSIONS

According to the results and considering the proposed objectives, we concluded that it is possible to obtain spherical microcapsules containing stevioside as core material. The suitable process parameters were as follows: double emulsion; complex coacervation using GE and GA at 2.5% or 5% and 50, 75 and 100% stevioside in relation to the total content of encapsulating agent; pH 4 to promote coacervation; and lyophilization process to stabilize the system. The double emulsion is a fundamental step to ensure higher yield in encapsulating water-soluble materials.

The microcapsules obtained in this study are relatively large, poorly soluble, little hygroscopic and physically stable at room temperature. The best formulations for encapsulating stevioside are those with core material load 50% as a function of total content of encapsulating agent, independent on the concentration of these agents (2.5 and 5%).

The application of microcapsules in chewing gum showed that encapsulation was able to prolong the sweet taste of stevia, which is one of the major objective to encapsulate sweeteners.

5. ACKNOWLEDGEMENTS

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Artigo de pesquisa 5

Evaluation of sweet taste in commercial chewing gums by time-intensity analysis and acceptance test

Summary

This study evaluated the time-intensity profile and the sensory acceptance of six samples of commercial chewing gum. Twelve panelists were selected to the time-intensity analysis, in which the parameters evaluated were Maximum Intensity (Imax), Time to Maximum Intensity (Timax), Total Time (Ttot) and Area under the curve (Area). In the acceptance test, 113 consumers assessed through an unstructured hedonic scale the attributes appearance, aroma, flavor, texture and overall acceptance. The results were analyzed by analysis of variance (ANOVA), Tukey's mean test and Internal Preference Mapping. The sample containing sucrose had the lowest values for Imax, Ttot and Area and obtained the lowest mean scores for all the attributes in the acceptance test.

Keywords: sensory; sweetener; aspartame; sucralose; acesulfame-k

1. Introduction

In recent years, along with a product with desired sensory characteristics such as taste, aroma, color and pleasing texture, consumers are increasingly choosing sugar-free or low-sugar products. This is one of the reasons sweeteners have received special attention. The replacement of sugar for sweeteners without loss of sensory characteristics has been the target of industry and researchers.

Chewing gums, defined as products comprising a gummy base, elastic, chewy and not swallowable follow this trend and play an important role in the segment of diet / light food. In the United States, about 60% of the chewing gum is sugar-free, a trend also accompanied by Brazil, once about 20% Brazilian market of diet / light was sugar free gums in 2002 (FADINI et al., 2005). These products have been

associated with oral health benefits, including the reduction of dental caries and plaque control (IMFELD, 1999).

To successfully substitute sucrose in food formulations, sweeteners must exhibit pleasing sensory properties with sweetness similar to sucrose, and the only way to evaluate the acceptance of a sweetener is by sensory analysis. As a large number of sweeteners are available, each sweetener can be used in the applications for which it is best suited and the limitations of individual sweeteners can be overcome by using them in blends (MEYER, 2002; NABORS, 2002). According to Lubbers and Guichard (2003), the sweeteners are the most important ingredients in the formulation of chewing gums.

The time-Intensity method is a descriptive analysis that measures the sensory perception of the intensity of specific attributes over time. This test was defined by Amerine et al. (1965) as the measure of the velocity, duration and intensity perceived by a single stimulus. The perception of sweetness is a dynamic process that requires time to review the complete characterization. Therefore, time-intensity studies strongly contribute to the evaluation of foods whose ingredient substitution alters the temporal characteristics (NOBLE et al. 1991). Regarding the chewing gums, this analysis is crucial, once it is a food chewed for a long period and thus sweetness and flavor may change over time.

The objective of the present study was to evaluate the time-intensity profile in relation to sweetness and sensory acceptance test of 6 samples of mint chewing gum.

2. Material and Methods

2.1. Materials

A total of six samples of minty chewing gum of two different brands, randomly chosen and purchased at a supermarket in the city of Campinas (Brazil) and stored at room temperature were studied. Five of these samples with different flavors, as

shown in Table 1, contained the sweeteners aspartame, sucralose, and acesulfame-k and one sample contained sugar, sucralose and acesulfame-k.

Table 1: Flavor, sweeteners and brands of the samples studied.

Sample	Flavor	Brand	Sweetners
A	Mint	1	aspartame, acesulfame-k and sucralose
B	Mint strong	1	aspartame, acesulfame-k and sucralose
C	Peppermint	2	sugar, acesulfame-k and sucralose
D	Peppermint	2	aspartame, acesulfame-k and sucralose
E	Peppermint	1	aspartame, acesulfame-k and sucralose
F	Mint	2	aspartame, acesulfame-k and sucralose

2.2. Methods

2.2.1. Time-intensity Analysis

Twenty-one candidates who consume chewing gum at least once a week were recruited and pre-selected by triangular tests using sucrose solutions with different concentrations by the Wald sequential approach (AMERINE et al, 1965). Fifteen candidates recruited were pre-selected and trained.

The time-intensity analysis was applied to evaluate the sweetness of the samples. The reference to maximum sweetness was determined by a consensus of all the pre-selected panelists, which were further trained with respect to the product attributes using as the reference a peppermint flavored chewing gum (Adams) and the six samples for the formation of sensory memory. The panel was trained in three sessions of one hour each.

Data collection for the time-intensity analysis was performed on a computer in individual booths with controlled temperature (22 °C) and white light, using a 9 cm-scale and the software Time-Intensity Analysis of Food and Tastes - TIAFT (Universidade Estadual de Campinas - UNICAMP, 2012). The panelists assessed the sweetness of the samples, and recorded the attribute's intensity changes over

time. The tests were conducted in four sessions with a monadic presentation using a balanced complete block design. The samples were offered at a rectangular shape of approximately 2.5 cm^2, coded with 3-digit number and unpacked. The time-intensity curves allowed the analysis of the following parameters: maximum intensity recorded by the candidate (Imax), time to maximum intensity (Timax), total time of stimulus (Ttot) and area of the time curve x intensity (Area) (PALAZZO and BOLINI, 2009).

2.2.2. Acceptance Test

The six samples previously described were evaluated by affective test with consumers. The panelists were 113 consumers of chewing gum between 18 and 52 years old, who consumed the product at least once week. The test was applied in individual sensory booths with controlled temperature and lighting. The samples coded with 3-digit numbers were served using a monadic balanced complete block design. The panelists evaluated the attributes appearance, aroma, flavor, texture and overall impression using a 9-cm linear hedonic scale (STONE and SIDEL, 2003), anchored at the extremes between "extremely dislike" and " extremely like ". The panelists also evaluated the ideal sweetness of the samples by hedonic scale anchored in the middle (ideal) and the left and right ends (extremely less sweet than ideal and extremely sweeter than ideal, respectively).

2.2.3. Statistical Analysis

Data were analyzed by univariate statistical analysis (ANOVA) and Tukey´s mean test ($p \leq 0.05$).

3. Results and Discussion

3.1. Time-intensity Analysis

Table 2 shows the means of the time-intensity parameters for all samples. The sample C (with sugar) obtained significantly lower values for maximum intensity of

sweetness, total time and Area among the samples studied ($p \leq 0.05$). The sample F presented the highest value for the parameter Timax, and no significant difference for this parameter was observed for the other samples.

The total duration of sweetness ranged from 3.88 to 4.96s, and can be considered short. There are many patents involving processing techniques aimed at prolonging total duration of sweetness and flavors in chewing gums. Among the techniques, the use of microencapsulation of sweeteners and flavors is predominant, targeting a controlled release and increased duration of sweetness and flavor, as the study of microencapsulation by complex coacervation of aspartame and sucralose (ROCHA-SELMI et al, 2013a; ROCHA-SELMI et al, 2013b).

The curves of time-intensity profile are shown in Figure 1. Sample means were used for constructing the curves. According to McGowan et al. (2005), the intensity of sweetness on chewing gum begins very high and will decrease as the gum is chewed. It is observed that the six samples of this study showed a similar curve profile, characterized by rapid release of the sweet taste, which reached its maximum intensity before the first minute of chewing. This rapid release of sweetness was also observed by Duizer et al (1996) in the evaluation of peppermint flavor chewing gum.

Table 2: Means of the time-intensity parameters for sweetness of commercial chewing gums

Sample	Imax	Timax(s)	Ttot(min)	Area
A	7.59^{a}	32.15^{b}	4.82^{a}	1178.01^{a}
B	7.38^{a}	31.67^{b}	4.96^{a}	1165.03^{a}
C	5.92^{c}	31.67^{b}	3.88^{b}	827.57^{c}
D	6.80^{b}	29.04^{b}	4.78^{a}	1052.24^{b}
E	7.16^{ab}	32.48^{b}	4.87^{a}	1138.68^{a}
F	7.31^{a}	36.26^{a}	4.78^{a}	1142.08^{a}

The same letters in the same column do not differ ($p < 0.05$). Imax = maximum intensity; Timax = time to maximum intensity; Ttot = total time of stimulus

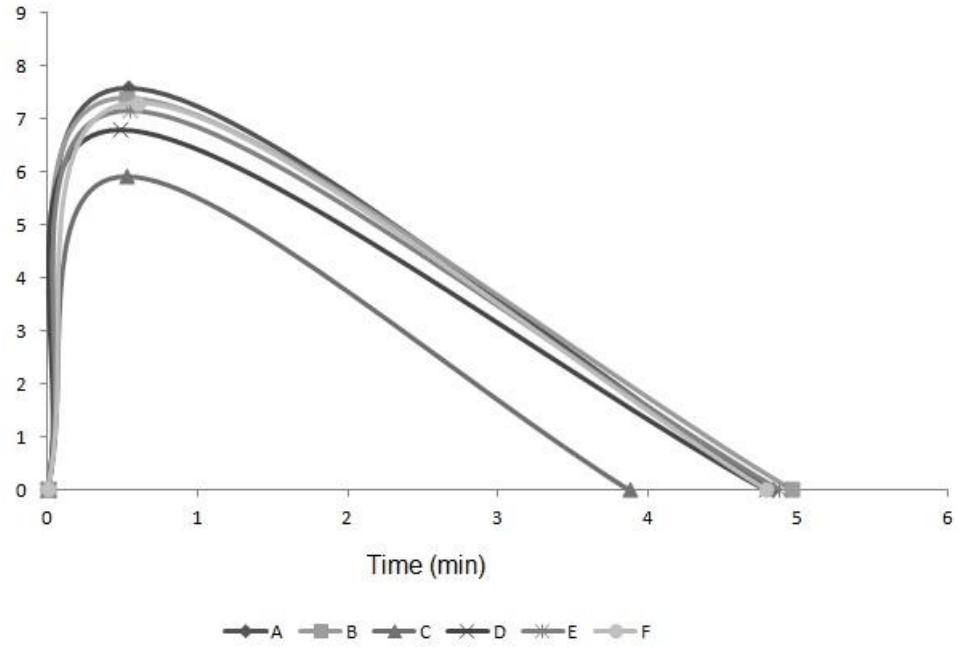

Figure 1: Time-Intensity curves for sweet taste stimulus of the six samples of chewing gums.

3.2 Acceptance Test

Table 3 shows ANOVA results for the parameters color, aroma, flavor, texture and global impression. All samples of chewing gum were accepted for appearance, flavor and overall impression, once they presented scores above 4.5 for these parameters. With respect to the taste, only sample C was rejected by consumers. For texture, the samples B and C presented scores lower than 4.5. Regarding the overall impression, sample C, which contains sugar in its formulation, had significantly lower score between the samples ($p \leq 0.05$). Sample A was significantly more accepted for the parameters flavor and overall impression. Figure 2 shows the results for the ideal sweetness. The six samples were considered very close to the ideal sweetness. This result is expected once it is a commercial sample, thus the test was carried out in order to verify if any sample

presented a concentration of sweetness very different from the others, which could affect the time-intensity profile and the acceptance of the product.

Figure 3 shows the Internal Preference Mapping for the samples. Axis 1 explains 43.3% and axis 2 explains 19% of the total variation between the samples, totaling 62.3%. The consumers are represented by squares in the vectorial space, which indicates the direction of preference for each consumer in relation to the samples, represented by circles. It is noted that the majority of consumers are allocated close to the samples A, D and E, confirming the results for global impression.

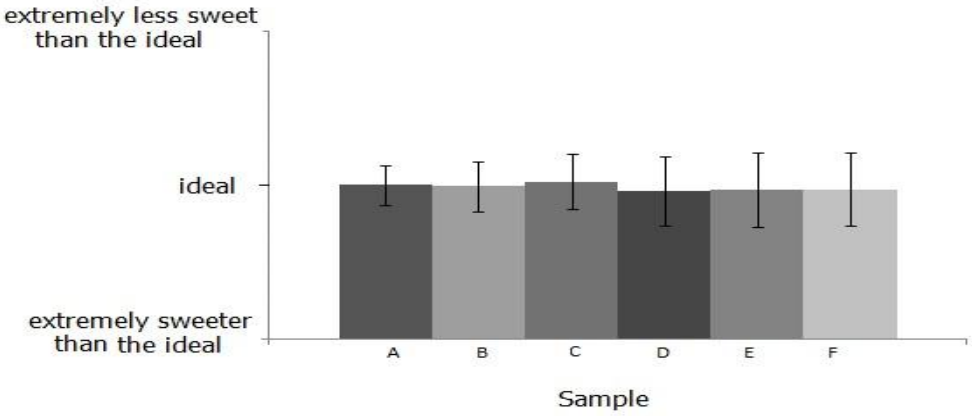

Figure 2: Responses of ideal sweetness of the six samples of commercial chewing gums.

Table 3: Means from acceptance test of commercial chewing gums

Sample	Appearance	Aroma	Flavor	Texture	Global Impression
A	6.52^{a}	7.21^{a}	7.15^{a}	6.55^{a}	7.09^{a}
B	5.71^{b}	5.30^{b}	5.24^{c}	4.42^{c}	5.33^{d}
C	5.65^{b}	5.20^{b}	4.18^{d}	4.45^{c}	4.54^{e}
D	6.45^{a}	5.58^{b}	5.77^{bc}	5.96^{ab}	5.97^{bc}
E	6.01^{ab}	5.53^{b}	6.46^{ab}	6.60^{a}	6.35^{b}
F	5.65^{b}	5.48^{b}	5.24^{c}	5.51^{b}	5.48^{cd}

The same letters in the same column do not differ ($p < 0.05$).

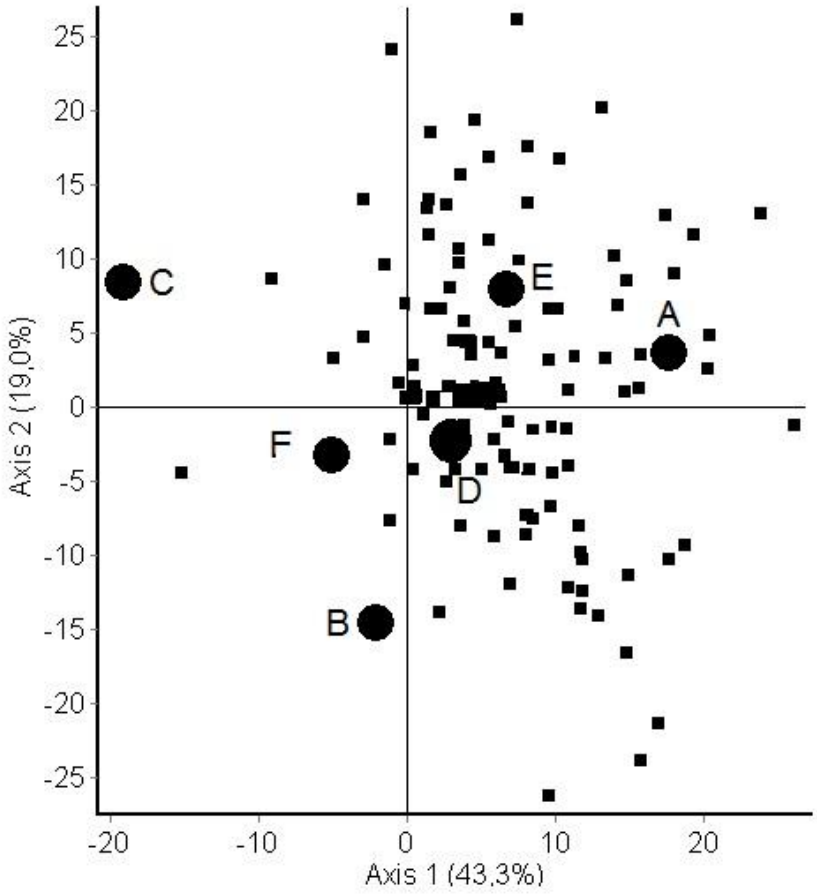

Figure 3: Internal Preference Mapping of commercial chewing gums.

4. Conclusion

The sweetness release profile of the commercial chewing gums showed rapid release of sweet taste, reaching its peak before the first minute of chewing, and presenting relatively short duration (less than 5 minutes). Furthermore, it was observed that the products containing sugar not always are the most sensorially accepted as compared to those containing sweeteners as sucrose substitutes. In the case of chewing gums of the present study, the sample containing sugar, acesulfame-k and sucralose was less accepted by the panelists and showed the lowest sweetness on time-intensity profile.

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CONCLUSÃO GERAL

Foi possível microencapsular aspartame, sucralose e estévia por coacervação complexa adicionando a etapa de dupla emulsão ao processo. As microcápsulas obtidas apresentaram formato esférico e recheio multinucleado. O processo de liofilização foi eficiente para a secagem das cápsulas, sem acarretar danos a elas. De uma maneira geral as microcápsulas apresentaram reduzida higroscopicidade e solubilidade e uma boa estabilidade em temperatura ambiente.

Por meio da avaliação da funcionalidade das microcápsulas de sucralose e estévia, é possível concluir que seu uso em gomas de mascar é promissor, visto que os tempos totais de duração da doçura foram maiores em relação às amostras com edulcorante na forma livre, corroborando com uma das principais razões para se encapsular edulcorantes, que é o aumento do tempo de percepção do gosto doce. Além disso, a avaliação sensorial de amostras comerciais mostrou que as gomas de mascar com edulcorantes obtiveram boa aceitação sensorial.