



UNIVERSIDADE ESTADUAL DE CAMPINAS  
Faculdade de Engenharia de Alimentos

BRUNA MARCACINI AZEVEDO

DESENVOLVIMENTO E PERFIL SENSORIAL DE CHOCOLATES MEIO AMARGOS  
CONTENDO INULINA E ADOÇADOS COM REBAUDIOSÍDEO A COMO  
SUBSTITUTO DA SACAROSE

DEVELOPMENT AND SENSORY PROFILE OF BITTERSWEET CHOCOLATES  
CONTAINING INULIN AND SWEETENED WITH REBAUDIOSIDE A AS SUCROSE  
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## RESUMO

Nos últimos anos, houve uma crescente demanda por produtos naturais e alimentos com perfil nutricional balanceado e com ingredientes saudáveis. Por isso, pesquisadores têm se concentrado na reformulação de produtos tradicionalmente ricos em gordura e açúcar, substituindo estes componentes por ingredientes com efeitos fisiológicos positivos, incluindo edulcorantes naturais e compostos prebióticos. Neste contexto, o objetivo deste estudo foi desenvolver e avaliar o perfil sensorial de chocolates meio amargos adicionados de inulina e estévias contendo diferente teores de rebaudiosídeo A (60, 80 e 97%). A primeira etapa consistiu em determinar a quantidade ideal de sacarose a ser adicionada em chocolates meio amargos (47,5% m/m), usando a escala Just-About-Right (JAR). A equivalência de doçura dos chocolates meio amargos sem açúcar foi determinada pelo método tempo-intensidade (0,16%). O perfil sensorial descritivo foi avaliada pela Análise Descritiva Quantitativa (ADQ) e método Tempo-Intensidade (TI) para os estímulos de doçura, amargor e derretimento na boca. O teste de aceitação foi realizado usando uma escala hedônica não estruturada de 9 cm. Cento e vinte consumidores avaliaram as amostras de chocolate meio amargo com relação à aparência, aroma, sabor, textura e impressão global. A correlação dos dados descritivos com os dados de aceitação foi determinada pelo mapa externo de preferência, usando os mínimos quadrados parciais (PLS). Medidas de textura e cor e composição centesimal aproximada foram determinadas. Os resultados da ADQ mostraram que os diferentes conteúdos de rebaudiosídeo A testados não interferiram nos atributos de gosto doce, gosto residual doce, gosto amargo e gosto residual amargo. Além disso, o amargor foi mais acentuado nas amostras com redução no teor de gordura. Verificou-se ainda que as amostras sem açúcar e com redução no teor de gordura apresentaram as maiores variações em relação à textura, diferindo significativamente da amostra convencional em relação aos atributos de dureza, derretimento e arenosidade. Embora algumas diferenças tenham sido detectadas entre as amostras no teste descritivo, o teste de aceitação e o mapa de preferência externo demonstraram que, em geral, todas as amostras apresentaram boa aceitação sensorial, incluindo aquelas com teor de gordura reduzido. O perfil de tempo-intensidade para o estímulo de doçura foi semelhante para todas as amostras de chocolate. As diferenças entre os conteúdos do rebaudiosídeo A não foram percebidas pelos assessores. Em relação ao estímulo de amargor, as amostras com baixo teor de gordura tiveram uma percepção mais acentuada desse atributo pelos avaliadores, com diferenças significativas para  $I_{max}$  e Área quando comparadas às amostras sem açúcar. O estímulo derretimento na boca foi mais afetado nas amostras com teor reduzido de gordura. Os chocolates com teor reduzido de gordura apresentaram valores de dureza mais elevados e foram significativamente diferentes das demais amostras. Em relação aos parâmetros de cor, os valores de  $L^*$  variaram de 27,5 a 29,6, caracterizando as amostras como chocolates meio amargos. Não houve diferença significativa nos valores de umidade e cinzas. As amostras com baixo teor de gordura apresentaram uma diferença significativa ( $p > 0,05$ ) nos valores de proteína, carboidratos e lipídios, o que era esperado, uma vez que estas formulações tinham metade da quantidade de manteiga de cacau (7,5%) e maior quantidade de leite desnatado e inulina. Em relação ao valor calórico dos produtos, foi verificado que as amostras de chocolate meio amargo com redução de gordura apresentaram, em média, uma redução de 27,62% das calorias, podendo ser considerados chocolates meio amargos *light*, segundo a legislação brasileira. Os resultados sensoriais obtidos neste estudo são úteis para a indústria de alimentos e pesquisadores que trabalham com edulcorantes e prebióticos em alimentos, especialmente na área de chocolates.

**Palavras chave:** análise sensorial; chocolate; inulina; edulcorantes.

## ABSTRACT

Recently, there is a growing demand for natural products and foods with nutritionally balanced profile and healthy ingredients. Therefore, researchers have been focused on reformulating food products traditionally rich in fat and sugar, by the replacement with natural ingredients with positive physiological effects, including natural sweeteners and prebiotic compounds. In this context, the objective of this study was to develop and evaluate the sensory profile of bittersweet chocolates added inulin and stevias with different rebaudioside A contents (60, 80 and 97%). The first step was to determine the ideal sucrose level in bittersweet chocolates (47.5% w/w) using the Just About Right (JAR) scale. The sweetness equivalence in sugar-free bittersweet chocolates was determined by the time-intensity method (0.16%). The descriptive sensory profile was determined by quantitative descriptive analysis (QDA) and time-intensity method (TI) for multiple stimuli (sweetness, bitterness and melting rate). The acceptance test was performed using a 9-cm linear hedonic scale (unstructured). One hundred and twenty consumers evaluated the samples of bittersweet chocolates with respect to appearance, aroma, flavor, texture and global impression. The correlation of descriptive and acceptance data was determined by external preference map, using partial least squares (PLS). Texture and color measurements and proximate composition were determined. The results for the QDA showed that the different contents of rebaudioside A tested did not interfere with the attributes of sweet taste, sweet aftertaste, bitter taste or bitter aftertaste. In addition, the bitterness was more accentuated in the samples with reduced fat content. It was also verified that the samples with no added sugar and with reduced fat contents showed the greatest variations in relation to texture, differing significantly from the conventional sample with respect to the attributes of hardness, mouth melting and sandiness. Although some differences were detected among the samples in the descriptive test, in general, the acceptance test and the external map of preference demonstrated that all the samples showed good sensory acceptance, including those with a reduced fat content. The time intensity profile for the sweetness stimulus was similar for all chocolate samples. The differences among the contents of rebaudioside A were not perceived by the assessors. In relation to the bitterness stimulus, the low-fat samples had a more accentuated perception of this attribute by the assessors, with significant differences for  $I_{max}$  and Area when compared to the sugar-free samples. The stimulus melting in the mouth was more affected in the low-fat samples. The low-fat chocolates presented higher hardness values, and were significantly different from the other samples. Regarding the color parameters, the  $L^*$  values ranged from 27.5 to 29.6, characterizing the samples as bittersweet chocolates. There was no significant difference in moisture and ash values. The low-fat samples had a significant difference ( $p > 0.05$ ) in protein, carbohydrate, and lipid values, which was expected since these formulations had half the amount of cocoa butter (7.5%) and higher amount of skimmed milk and inulin. Regarding the caloric value of the products, the low-fat bittersweet chocolate presented, on average, a reduction of 27.62% of the calories, being considered light bittersweet chocolates, according to the Brazilian legislation. The sensory results obtained in this study are useful for food industry and researchers working with sweeteners and prebiotics in food, especially in chocolate.

**Keywords:** sensory analysis; chocolate; inulin; sweeteners.



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## 1 INTRODUÇÃO

O desenvolvimento de novos produtos com alta qualidade sensorial e benefícios à saúde tem ganhado atenção nos últimos anos. Neste contexto, tem-se estudado muito sobre chocolates com maiores teores de cacau, por serem excelentes fonte de polifenóis, principalmente catequinas, epicatequinas e procianidinas (PLAZA et al., 2017). Estudos sugerem que estes compostos presentes no liquor do cacau podem contribuir na prevenção de doenças cardiovasculares, por meio de seus efeitos na redução da pressão arterial, melhora na função endotelial, elevação do óxido nítrico, redução da agregação plaquetária, diminuição da oxidação de lipídeos e lipoproteínas, bem como na diminuição da resistência à insulina (KERIMI; WILLIAMSON, 2015). Além disso, os polifenóis do cacau parecem agir de forma eficaz como agentes quimiopreventivos contra doenças crônicas, incluindo câncer e doenças neurodegenerativas (SHAHIDI; AMBIGAIPALAN, 2015).

Apesar dos possíveis benefícios à saúde e do sabor agradável do chocolate, a maioria dos chocolates comercialmente disponíveis são ricos em gordura e açúcar, e este excesso de calorias pode diminuir os benefícios potenciais deste produto (LATHAM et al., 2014).

A indústria de alimentos tem buscado alternativas para a substituição e/ou redução da sacarose e gordura nos alimentos, principalmente devido a alguns fatores: excesso de peso, obesidade e diabetes, decorrentes, dentre outros fatores, de uma alimentação inadequada. Em 2014, mais de 1,9 bilhões de adultos, com 18 anos ou mais, estavam acima do peso no mundo, sendo que dentre estes, mais de 600 milhões eram obesos (WHO, 2016a). Além disso, estima-se que o número de adultos que vivem com diabetes quase quadruplicou desde 1980, passando de 108 milhões em 1980 para 422 milhões, em 2014 (WHO, 2016b).

Recentemente, o desenvolvimento de novos produtos que contenham extrato de estévia como substituto da sacarose aumentou, visto que os consumidores estão buscando produtos de baixa caloria contendo adoçantes naturais em vez de compostos artificiais (PALAZZO et al., 2011). A estévia é um edulcorante natural, com intenso gosto doce, extraída das folhas da planta *Stevia Rebaudiana Bertoni*, nativa da América do Sul (TAVARINI; ANGELINI, 2013). Esta planta tem uma classe de compostos conhecidos como glicosídeos de esteviol, dentre eles, esteviosídeo, rebaudiosídeos A e C, dulcosídeo A, sendo que o esteviosídeo e rebaudiosídeo A estão presentes em concentrações mais elevadas (GARDANA; SCAGLIANTI; SIMONETTI, 2010). O rebaudiosídeo A é mais doce, estável e

menos amargo do que o esteviosídeo (GOYAL et al., 2010). Portanto, espera-se que quanto maior o teor de rebaudiosídeo A na estévia, melhor será o perfil sensorial deste edulcorante.

O desenvolvimento de chocolates com substitutos de gordura também tem sido muito investigado. A inulina é um polímero composto principalmente por frutose, por este motivo pertence à classe de carboidratos conhecida como frutanos (MODZELEWSKA-KAPITULA, M., KLEBUKOWSKA, 2010). É utilizada na indústria de alimentos para inúmeras finalidades, incluindo a substituição de gordura dos alimentos (RODRÍGUEZ GÁRCIA; SALVADOR; HERNANDO, 2014). Essa fibra apresenta efeito bifidogênico, ou seja, estimula o crescimento das bifidobactérias, que melhoram a saúde humana (KARIMI et al., 2015).

O desenvolvimento adequado de chocolates meio amargos contendo substitutos de açúcar e gordura depende diretamente da Análise Sensorial, que é a única técnica capaz de avaliar a aceitação e as características sensoriais de um edulcorante, considerando ainda que, cada edulcorante apresenta características sensoriais distintas, que podem variar em função do alimento ou bebida ao qual foi adicionado. Esta ciência também é fundamental para avaliar o comportamento da inulina como substituta de gordura no chocolate meio amargo, já que o sabor, a textura e o derretimento devem ter comportamento semelhante ao da manteiga de cacau.

Neste sentido, o presente trabalho visou realizar testes sensoriais com chocolates meio amargos em barra, produzidos com substitutos de sacarose e de gordura, comparando seus comportamentos sensoriais entre si e em relação ao produto convencional, visando à obtenção de chocolates meio amargos alternativos com alta qualidade sensorial.



## 2 OBJETIVOS

### 2.1 OBJETIVO GERAL

O objetivo geral deste trabalho foi desenvolver e avaliar chocolates meio amargos em barra, sem açúcar e adoçados com estévias contendo diferentes teores de Rebaudiosídeo A, adicionados de inulina e contendo reduzido teor de manteiga de cacau.

### 2.2 OBJETIVOS ESPECÍFICOS

Os objetivos específicos deste trabalho foram:

- Determinar a concentração ideal de sacarose a ser adicionada no chocolate meio amargo;
- Produzir chocolates meio amargos adoçados com estévias contendo diferentes teores de rebaudiosídeos A com doçura equivalente à ideal;
- Produzir chocolates meio amargos *light* em calorias e *diet*;
- Definir os perfis sensoriais destes chocolates meio amargos;
- Avaliar a aceitação das amostras desenvolvidas;
- Correlacionar os dados descritivos com o teste de aceitação;
- Estudar o perfil Tempo-intensidade dos estímulos de gosto doce, gosto amargo e derretimento na boca;
- Realizar análises físico-químicas e químicas nos chocolates desenvolvidos;
- Correlacionar os dados físico-químicos e químicos com os dados sensoriais.

### 3 REVISÃO BIBLIOGRÁFICA

#### 3.1 Chocolate

Segundo a legislação brasileira (RDC 264), o chocolate é o produto obtido a partir da mistura de derivados de cacau (*Theobroma cacao* L.), massa (ou pasta ou *liquor*) de cacau, cacau em pó e ou manteiga de cacau, com outros ingredientes, contendo, no mínimo, 25% (g/100 g) de sólidos totais de cacau. O produto pode apresentar recheio, cobertura, formato e consistência variados (BRASIL, 2005).

O produto deve apresentar-se sólido à temperatura ambiente (20 a 25°C) e fundir rápido e completamente durante a degustação a 36-36,5°C (COHEN, 2003).

#### 3.2 Produção de cacau (*Theobroma cacao* L) e aspectos gerais do cacauero (*Theobroma cacao* L)

A estimativa da produção mundial de cacau em 2015/2016 foi de, aproximadamente, 3965 mil toneladas (Organização Internacional de Cacau - ICCO, 2017). De acordo esta Organização, os maiores produtores mundiais de cacau são a Costa do Marfim com 1570 mil toneladas na safra 2015/16, seguida por Gana (820 mil toneladas), Indonésia (330 mil toneladas), Camarões (250 mil toneladas), Equador (230 mil toneladas), Nigéria (190 mil toneladas) e Brasil (135 mil toneladas) (ICCO, 2016).

O Brasil, até a chegada da vassoura-de-bruxa (*Moniliophthora perniciosa*), em 1989, era o segundo maior produtor de cacau do mundo (LOPES et al., 2011), caindo para a sétima posição e ficando responsável por 3,38% do total mundial (ICCO, 2016).

O cacauero pertence à ordem Malvales, família *Malvaceae* e gênero *Theobroma* (LOPES et al., 2011). Existem mais de 16 espécies de cacau, mas a mais comum no Brasil é *Theobroma cacao* L (criollo venezuelano) (ASSOCIAÇÃO BRASILEIRA DA INDÚSTRIA DE CHOCOLATES, CACAU, AMENDOIM, BALAS E DERIVADOS - ABICAB, 2011).

O fruto do cacauero tem forma oval com 15 a 20 centímetros de comprimento do eixo maior, e cor amarela quando maduro. O cotilédone e um pequeno gérmen de planta embrionária são recobertos por uma película denominada testa, e a semente é revestida por uma polpa branca com tons rosados, mucilaginosos e adocicada (BATALHA, 2009).

### 3.3 Pré-processamento do cacau

O cacaveiro produz frutos que contêm polpa e sementes. Após a abertura do fruto, a casca é removida e as sementes, envolvidas pela polpa mucilaginosa, são submetidas ao processo de fermentação. Nesta etapa, ocorre a formação de muitos compostos químicos precursores de sabor e aroma que definirão as características sensoriais dos chocolates produzidos a partir dessas amêndoas (BECKETT, 2009).

A secagem é uma etapa que deve ser iniciada imediatamente após a fermentação. O teor de umidade das sementes deve ser reduzido de 60% para 8% (ALMEIDA; LEITÃO, 1995), para garantir uma melhor conservação. O resultado é um produto comercializado no mercado mundial, designado como amêndoas de cacau fermentadas e secas (BECKETT, 2009).

Devem ser retiradas das amêndoas de cacau as impurezas, como pó, pedras, paus, sementes defeituosas, restos de polpas, entre outros (VERÍSSIMO, 2012).

A etapa seguinte, denominada Torração, consiste em um tratamento térmico que visa complementar o desenvolvimento das reações químicas responsáveis pelo desenvolvimento de cor e sabor do chocolate, iniciadas na fermentação (BECKETT, 2009).

O sabor do chocolate é o resultado de uma mistura complexa de um grande número de compostos, incluindo pirazinas, aldeídos, éteres, tiazóis, fenois, cetonas, alcoóis, furanos e ésteres, formados principalmente por meio da reação de Maillard, que envolve aminoácidos e açúcares redutores (FARAH; ZAIBUNNISA; MISNAWI, 2012).

Nesta etapa ocorre também a redução do teor de água, ácidos voláteis e outras substâncias presentes nas amêndoas que podem conferir amargor e acidez no produto final, inativação de enzimas que podem degradar a manteiga de cacau, além da redução no número de micro-organismos (BECKETT, 2009).

Os *nibs* (amêndoas quebradas em pedaços menores) devem ser pré-moídos em moinhos de martelos, pinos ou discos e, posteriormente, é realizada a moagem fina em moinhos de discos, de esferas ou de cilindros, até ser atingida a granulometria de 20 a 40 $\mu$ m. Os *nibs* são convertidos em uma massa fluida denominada liquor (NIEDIEK, 1994).

O liquor pode ser comercializado para a fabricação de chocolates e prensado para a obtenção de manteiga e torta de cacau (COHEN, JACKIX, SOUSA, 2004).

### 3.4 Processamento do chocolate

A Figura 1 representa o fluxograma do processamento tradicional do chocolate.

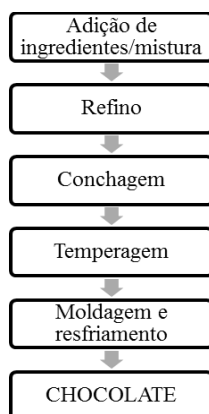


Figura 1 – Processamento do chocolate

#### 3.4.1 Adição dos ingredientes e mistura

Os ingredientes são adicionados em tanques encamisados a 40°C e misturados, com o objetivo de realizar a homogeneização dos ingredientes em pó (açúcar e leite em pó) com os ingredientes líquidos (manteiga de cacau e *liquor* de cacau fundidos), por tempo suficiente para se transformarem em uma massa plástica adequada para a próxima etapa (BECKETT, 1994).

#### 3.4.2 Refino

O refino garante que as partículas de cacau sólido, o açúcar e qualquer sólido do leite sejam quebradas, de modo que se tornem suficientemente pequenas para não serem percebidas pela língua (VERÍSSIMO, 2012).

O método mais comum de obtenção deste requisito é pela utilização de um refinador com cinco rolos (BECKETT, 1994).

Segundo Luccas (2001), o tamanho das partículas da massa refinada deve ficar entre 20-25µm, uma vez que tamanhos maiores que 25µm proporcionam areosidade na boca ao degustar o chocolate, e tamanhos inferiores a 20µm podem causar problemas tecnológicos, ao

causar aumento da viscosidade e do limite de escoamento, dificultando processos posteriores de transporte, dosagem e recobrimento.

### **3.4.3 Conchagem**

A conchagem constitui a última etapa de importância na formação do sabor característico e desejável do chocolate e envolve a redução da umidade, a volatilização dos ácidos graxos e aldeídos, o desenvolvimento da textura uniforme e a mudança da cor devido à emulsificação e oxidação de taninos. A volatilização reduz o amargor e desenvolve o sabor do chocolate. As partículas sólidas, tais como o açúcar e o cacau, são revestidas com gordura. Este envolvimento das partículas sólidas pela gordura, associado ao cisalhamento e à movimentação da massa de chocolate, contribuem para a textura do chocolate, que também exerce grande influência ao sabor global do chocolate. Além disso, ocorre a formação de compostos por meio da reação de Maillard (PRAWIRA; BARRINGER, 2009).

Para a realização da conchagem adequada, são necessários o cisalhamento, a agitação e o aquecimento da massa entre 50 e 70°C, dependendo do tipo de chocolate desejado (ao leite, branco ou amargo). Esta etapa pode levar de 8 a 96 horas, dependendo do tipo de produto que se deseja e do equipamento utilizado (AFOAKWA; PATERSON; FOWLER, 2007).

### **3.4.4 Temperagem, Moldagem e Resfriamento**

Devido à natureza polifórmica da manteiga de cacau, o chocolate deve ser temperado ou pré-cristalizado. A temperagem consiste em uma técnica de pré-cristalização controlada, necessária para induzir a formação de cristais estáveis (forma  $\beta$  ou V) na manteiga de cacau. Esta técnica envolve a redução da temperatura do chocolate para induzir à cristalização de ambas as formas polimorfas (estáveis e instáveis). Em seguida, a temperatura é elevada até o ponto onde as formas polimorfas instáveis fundem, gerando apenas cristais polimorfos estáveis que podem então “semear” a cristalização do chocolate como um todo na forma estável (TALBOT, 1994).

Este processo, responsável pela cristalização de apenas 2 a 4% de cristais da manteiga de cacau, é capaz de gerar características como brilho; contração do volume, facilitando a

desmoldagem; dureza e quebra à temperatura ambiente; rápida e completa fusão na boca e rápido desprendimento de aroma e sabor na degustação (COHEN; JACKIX; SOUSA, 2004).

A Figura 2 apresenta o processo de temperagem mais comumente utilizado. Primeiramente, a massa de chocolate é aquecida para completa fusão dos cristais de gordura, em temperaturas próximas a 45°C. Em seguida, é realizado o resfriamento, de forma controlada e sob agitação, para induzir a cristalização da gordura, removendo o calor sensível e formando os cristais instáveis e estáveis. A taxa de resfriamento deve ser próxima de 2°C/min até a temperatura de aproximadamente 27°C. Por fim, realiza-se o reaquecimento da massa para fusão dos cristais instáveis, ao redor de 30-32°C (BECKETT, 2009).

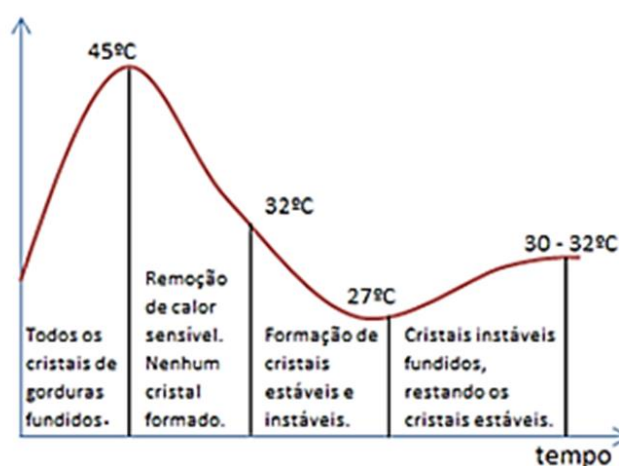


Figura 2 – Etapas do processo de temperagem (BECKETT, 2009).

Para a moldagem de chocolates em barra, o chocolate temperado é depositado dentro de moldes, que são então vibrados para assegurar a distribuição uniforme do chocolate e liberar bolhas de ar. Feito isso, o produto é resfriado e retirado da forma (FERRARO, 2005).

O objetivo da etapa de resfriamento é continuar a cristalização da manteiga de cacau na forma  $\beta$ . Esta forma é associada a altos níveis de brilho e bons resultados de contração, que leva a boas características para a retirada das formas (SUBRAMANIAN; MURPHY, 2001). A temperatura do ar utilizado em túneis de resfriamento durante a fabricação de chocolate é geralmente 10-15°C, sendo que a menor temperatura deve ser no centro do túnel para remover o calor de cristalização, e as maiores temperaturas na entrada e saída do túnel para, respectivamente, permitir a continuidade do crescimento dos cristais do tipo beta (cristalização em velocidade moderada) e evitar a condensação de umidade na superfície do

chocolate. Em média, é necessária uma redução de 10°C na temperatura do chocolate para a cristalização (KEIJBETS et al., 2010).

### **3.5 Principais ingredientes do chocolate meio amargo**

#### **3.5.1 Sacarose**

A sacarose é um dissacarídeo formado por uma molécula de glicose e uma molécula de frutose, unidas por ligação  $\alpha$  (1,2) (BECKETT, 2009). Trata-se do ingrediente mais popular na indústria de alimentos para obter o gosto doce (JAMIESON, 2008). É extraída da cana-de-açúcar ou do açúcar de beterraba e é usada industrialmente em produtos de panificação e confeitaria, bebidas, geleias e doces, chocolate, etc (BECKETT, 2009).

A sacarose apresenta gosto doce sem residual, com o mínimo de persistência na boca. É um ingrediente usado como agente de corpo, modificador de textura, intensificador de sabor e conservante (AFOAKWA; PATERSON; FOWLER, 2007).

#### **3.5.2 Líquor de cacau e Manteiga de cacau**

O líquido é produzido a partir da moagem dos *nibs* de cacau (BECKETT, 2009). Por sua vez, a manteiga de cacau é obtida pela prensagem das amêndoas de cacau. Trata-se de uma das gorduras mais estáveis, contendo antioxidantes naturais (polifenóis) que a impedem de ficar rançosa, dando-lhe um prazo de validade de dois a cinco anos (VERÍSSIMO, 2012). É responsável por diversas características de qualidade do chocolate, como dureza e quebra à temperatura ambiente, fusão rápida e completa na boca, brilho, contração durante o desmolde e rápido desprendimento de aroma e sabor na degustação. Sua natureza polimórfica define as condições de processamento e está diretamente relacionada à estabilidade do produto durante o armazenamento (QUAST, 2008).

#### **3.5.3 Leite em pó**

Do ponto de vista nutricional, o leite é considerado um alimento com uma composição completa, sendo uma boa fonte de proteínas (aminoácidos essenciais), gorduras, lactose,

vitaminas e minerais. Por esta razão, o leite, como um ingrediente, aumenta o valor nutricional dos produtos alimentícios (CLAEYS et al., 2013).

Leite em pó é o produto obtido por desidratação do leite de vaca, mediante processo tecnologicamente adequado. A classificação deste alimento é feita pelo conteúdo de gordura, sendo que o leite em pó desnatado possui menos que 1,5% deste nutriente (ENGETECNO, 1996).

### **3.5.4 Emulsificantes - Lecitina de soja e Polirricinoleato de Poliglicerol (PGPR)**

O chocolate possui ingredientes hidrofílicos e hidrofóbicos. Como a interação entre estes grupos não ocorre espontaneamente, o agente emulsificante torna-se o responsável por essa interação (SOKMEN; GUNES, 2006).

A lecitina de soja é o agente emulsificante mais aplicado na produção de chocolates, e pode reduzir em até 10 vezes a quantidade de manteiga de cacau de uma formulação. As suas principais funções são: possibilitar uma homogeneização perfeita entre gordura e água; estabilizar a emulsão retardando a separação da gordura do produto; proporcionar uma sensação de maior quantidade de gordura no produto; proporcionar plasticidade, suavidade e antiaderência ao produto e distribuir melhor o aroma (WEYLAND; HARTEL, 2008).

O PGPR também pode ser adicionado ao chocolate derretido, melhorando a sua fluidez e favorecendo a remoção de bolhas de ar (SCHANTZ; ROHM, 2005).

### **3.5.5 Aromas**

São substâncias ou misturas de substâncias com propriedades capazes de conferir ou intensificar o aroma e/ou sabor dos alimentos. Para efeitos do Regulamento Técnico RDC nº 2, de 15 de janeiro de 2007, os aromatizantes classificam-se em naturais ou sintéticos. Os aromatizantes naturais são os obtidos exclusivamente por métodos físicos, microbiológicos ou enzimáticos, a partir de matérias-primas aromatizantes naturais, como, por exemplo, óleos essenciais. Por sua vez, os aromatizantes sintéticos são os compostos quimicamente definidos obtidos por processos químicos (BRASIL, 2007).

O aroma de baunilha, denominado vanilina, é obtido a partir da planta *Vanilla planifolia* na forma de gluco-vanilina, na proporção de 2% em peso (BERGER, 2000). Existe



também a vanilina artificial, comumente derivada de licores de sulfito, produzidos durante o processamento da polpa de madeira para a fabricação de papel. Porém, o extrato sintético de vanilina fornece apenas a nota sensorial principal do sabor de baunilha (CLARK, 1990).

Para a produção de chocolate, é comum a utilização de vanilina em pó.

### **3.6 Benefícios do consumo de chocolate meio amargo para a saúde**

O cacau é um ingrediente usado na produção de chocolate e que é rico em substâncias com propriedades antioxidantes, como flavonoides, catequinas, epicatequinas e procianidinas (LOFFREDO et al., 2017). Tais substâncias têm atraído muita atenção, por serem compostos que inibem ou retardam os processos oxidativos no organismo, causados pelo excesso de radicais livres, devido ao estilo de vida e situações patológicas. Existe a relação entre o consumo destes compostos e possíveis implicações benéficas à saúde humana, particularmente em relação a alguns tipos de câncer, doenças cardiovasculares e outras doenças crônicas (AFOAKA, 2016). Pesquisas indicam que os flavonoides encontrados no cacau podem diminuir a oxidação da Lipoproteína de Baixa Densidade (LDL), além de reduzir a agregação plaquetária e a pressão sanguínea (LOFFREDO et al., 2017; ISHAQ; JAFRI, 2017).

Outras pesquisas têm demonstrado também a influência do cacau no sistema imunológico. Tzounis e colaboradores (2011) demonstraram que o consumo diário de uma bebida com alto teor de cacau durante 4 semanas modificou a composição da microbiota e resposta imune a nível intestinal, aumentando significativamente as populações de bifidobactérias ( $p < 0,01$ ) e lactobacilos ( $p < 0,001$ ).

Abril-gil e colaboradores (2012) verificaram a importância do consumo dos flavonoides do cacau no tratamento de alergias. Neste estudo, ratos induzidos à alergia receberam uma dieta de cacau (contendo 0,2% de polifenóis, p / p) ou uma dieta padrão. Os animais alimentados com uma dieta padrão apresentaram concentrações elevadas de IgG1, IgG2a, IgG2b anti-OVA e concentrações elevadas de IgE anti-OVA, que são anticorpos envolvidos na resposta alérgica. Em contraste, os animais alimentados com uma dieta rica em cacau apresentaram concentrações significativamente mais baixas de anticorpos anti-OVA IgG1 e IgG2a.

Efeitos neuroprotetores do cacau foram recentemente revisto por Nehlig (2012). Estudo sugeriu que o cacau ou seus derivados pode ter um potencial terapêutico para a doença de Alzheimer (SCHINDOWSKI; BELARBI; BUE´E, 2008).

Chocolates com alto teor de cacau possuem em sua composição o neurotransmissor serotonina, além de o seu precursor, denominado triptofano (GUILLÉN-CASLA et al., 2012). Por ser capaz de aumentar a produção deste neurotransmissor, o chocolate é um alimento capaz de ativar os centros do bem estar no cérebro humano, combatendo os sintomas de estresse (WALCUTT, 2009). O consumo de chocolate pode desempenhar também um papel na prevenção de hiperglicemia, resistência à insulina e obesidade. No estudo de Min e colaboradores (2013), animais alimentados durante 5 semanas com dieta rica em gordura e extrato de polifenóis do cacau tiveram menor acúmulo de lipídeos e maior regulação do receptor de insulina.

Além de todos os efeitos benéficos citados anteriormente, o chocolate é considerado um alimento com boa qualidade nutricional. Isto porque o cacau presente em sua formulação contém vários minerais essenciais, incluindo magnésio, cobre, manganês, potássio, cálcio, ferro, fósforo e zinco, que desempenham papéis importantes na fisiologia do corpo humano. Além disso, o cacau também contém vitamina A e E, além de algumas vitaminas do complexo B (tiamina, riboflavina e niacina) (ICCO, 2005).

### **3.7 Substitutos de sacarose no chocolate**

O chocolate sempre foi conhecido por ser um produto rico em sacarose. Uma das formas de diminuir o seu valor calórico é reduzir a concentração de açúcar da formulação (NEBESNY; ZYZELEWICZ, 2005), que varia, normalmente, de 30 a 55% no chocolate tradicional (URBANSKI, 2003).

Diante deste contexto, a indústria de alimentos passou a investir nos chocolates dietéticos adicionados de edulcorantes, direcionados a pessoas que necessitam de uma dieta com ingestão controlada de açúcares.

O desenvolvimento de chocolates com baixo teor calórico e com características sensoriais agradáveis requer a utilização de ingredientes que oferecem tecnologias para substituir açúcares. Estes ingredientes incluem edulcorantes de alta intensidade e agentes de corpo com reduzido teor calórico (KOPCHIK, 1995).

### 3.7.1 Edulcorantes

A elevada incidência de obesidade, diabetes e doenças cardiovasculares resultou na crescente preocupação, dentre outras coisas, com o conteúdo de açúcar e calorias dos produtos alimentícios, o que tem aumentado a busca por alimentos isentos e/ou reduzidos de sacarose (YE et al., 2014), adicionados de edulcorantes.

Os edulcorantes são substâncias de gosto doce intenso, com poder de dulçor de centenas a milhares de vezes superior ao da sacarose (AIDOO et al., 2013), com baixo ou inexistente valor energético. Estas substâncias são consideradas não calóricas por não serem metabolizadas pelo organismo ou pela sua utilização em quantidades muito pequenas, tornando o aporte calórico insignificante (CAVALLINI; BOLINI, 2005).

Para serem utilizados em alimentos, os edulcorantes devem: ter propriedades funcionais e sensoriais semelhantes às da sacarose; possuir baixa densidade calórica; ser fisiologicamente inertes e aceitáveis sensorialmente; auxiliar na manutenção ou redução do peso corpóreo, no controle de diabetes e ser comercialmente viáveis (MALIK; JEYARANI; RAGHAVAN, 2002).

Os edulcorantes naturais avaliados neste estudo serão: estévias contendo diferentes teores de rebaudiosídeo A e o maltitol, permitidos para o consumo, segundo a legislação brasileira (BRASIL, 2008a).

#### 3.7.1.1 Estévia

Estévia é um edulcorante de alta intensidade, originada do extrato das folhas de *Stevia rebaudiana* Bertoni, planta originária da Serra do Amambay, na fronteira do Brasil com o Paraguai (BARBET-MASSIN et al., 2015). Trata-se de um ingrediente natural, que possui gosto doce e é ausente de calorias, podendo ser utilizado como um substituto da sacarose ou como uma alternativa para edulcorantes artificiais (ANTON et al., 2010).

Os adoçantes naturais das folhas de Estévia, chamados glicosídeos de esteviol, são diterpenos, isolados e identificados como esteviosídeo, esteviolbiosídeo, rebaudiosídeos (A, B, C, D, E, F, M) e dulcosídeo A, os quais são 200 a 300 vezes mais doces que a sacarose (GARDANA et al., 2010).

Os principais glicosídeos de esteviol são o rebaudiosídeo A e esteviosídeo (Figura 3), sendo que o Rebaudiosídeo A é considerado superior em relação à qualidade de doçura e sabor, enquanto o Esteviosídeo é conhecido pelo seu gosto residual amargo (LEMUS-MONDACA et al., 2012). As concentrações dos glicosídeos presentes na estévia determinarão o nível de doçura da mesma (VARANUJ; CHATCHAI, 2009).

O rebaudiosídeo A tem o perfil de sabor mais desejável e é o mais estável de todos os glicosídeos de esteviol (DUBOIS, 2000). Estudos permitiram o desenvolvimento de um novo extrato, com uma maior proporção de rebaudiosídeo A (até 97 %), que fornece um produto mais agradável que os primeiros extratos utilizados (HOUGH, 1998; RAMESH et al., 2006; RAJASEKARAN; GIRIDHAR; RAVISHANKAR, 2007; YADAV et al., 2014).

No Brasil, a regulamentação do uso dos edulcorantes, incluindo a estévia, é de responsabilidade do Ministério da Saúde, por meio da Agência Nacional de Vigilância Sanitária (ANVISA), com base em laudos fornecidos pelo *Joint Expert Comitee on Food Additives* (JECFA), cabendo a esta aceitar ou não a recomendação do Comitê. O uso da estévia foi regulamentado pela Portaria n°14 desde 1988 (BRASIL, 1988).

O valor da Ingestão Diária Aceitável (IDA) para o edulcorante estévia é de 0,06 g/100 g (BRASIL, 2008a).

Alguns estudos têm avaliado a aplicabilidade da estévia em chocolates (SHAH; JONES, VASILJEVIC, 2010; PALAZZO et al., 2011; AIDOO; AFOAKWA; DEWETTINCK, 2015; MORAIS-FERREIRA et al., 2016).

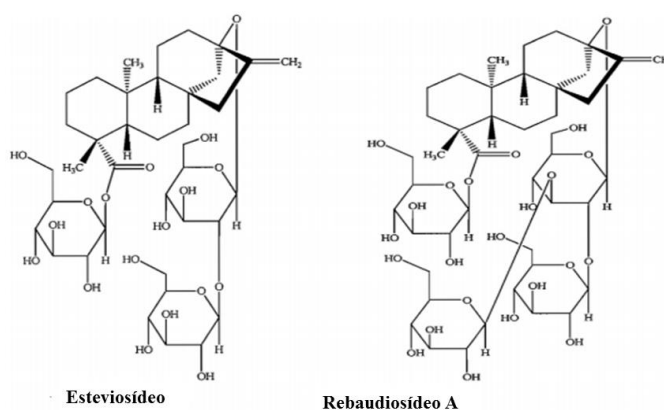


Figura 3. Estrutura da molécula de esteviosídeo e rebaudiosídeo A.

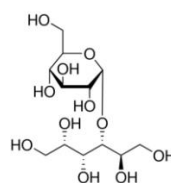
Fonte: Carakostas et al. (2008).

### 3.7.1.2 Maltitol

O maltitol é um dissacarídeo composto por uma unidade de glicose ligada a um único sorbitol através de uma ligação  $\alpha$ -1,4. Este carboidrato não ocorre na natureza e sua produção é realizada industrialmente através da hidrogenação catalítica da maltose, produzida pela hidrólise do amido (MARIE; PIGGOTT, 1991).

Este poliól é não higroscópico, de ação refrescante, podendo ser utilizado como substituto de gordura e açúcar e como melhorador de cremosidade no chocolate (URBANSKI, 2003).

Seu valor calórico é reduzido (2,4 kcal/g), além de possuir Índice Glicêmico (IG) de 35-52 (CHATTOPADHYAY; RAYCHAUDHURI; CHAKRABORTY, 2014), considerado baixo ( $\leq 55$ ) (SAMPAIO et al., 2007). O IG é definido como a relação entre a área abaixo da curva de resposta glicêmica duas horas após o consumo de uma porção de alimento-teste contendo 50 gramas de carboidrato, e a área abaixo da curva de resposta glicêmica correspondente ao consumo de uma mesma porção de carboidrato do alimento-referência (glicose ou pão branco) (SARTORELLI; CARDOSO, 2006).



**Maltitol**

Figura 4 - Estrutura da molécula de Maltitol

Fonte: Arraez, S. R. (2015).

### 3.8 Substitutos de gordura no chocolate

O chocolate também é bastante conhecido por ser um produto rico em gordura (NEBESNY; ZYZELEWICZ, 2005), dessa forma, uma das alternativas para tornar este alimento mais saudável é a utilização de um substituto de gordura.

### 3.8.1 Inulina

A inulina é um carboidrato do grupo de polissacarídeos denominados frutanos, composto por uma glicose terminal e subunidades de frutose ligadas entre si, apresentando um grau de polimerização (DP) de 10 ou mais unidades. A inulina convencional tem DP médio de 10 e contém em torno de 6 a 10% de açúcares livres, representados pela glicose, frutose e sacarose (NINESS, 1999). A inulina denominada de “alta performance” (HP) consiste de misturas de frutanos tipo inulina exclusivamente com  $DP \geq 10$  tendo, portanto, maior massa molecular (ROBERFROID, 2005).

Trata-se de uma fibra solúvel alimentar não digerível, presente naturalmente em mais de 36 mil plantas, porém, sua extração mais comum é feita a partir das raízes de chicória (GONÇALVES; ROHR, 2009). É vastamente aplicada como ingrediente em uma série de alimentos, tais como sorvete feito com leite de ovelha, chocolates, pães e bolos (BALTHAZAR et al., 2017; AIDOO; AFOAKWA; DEWETTINCK, 2015; ZIOBRO et al., 2013; VOLPINI-RAPINA et al., 2012).

A aplicação da inulina na indústria de alimentos deve-se, principalmente, às suas propriedades que a torna capaz de substituir o açúcar e, principalmente, a gordura, com a vantagem de não resultar em aumento calórico. O potencial de substituição de gordura pela inulina foi descoberto e patenteado em 1992 pela empresa Beneo Orafiti (COUSSEMENT, 1999). Em altas concentrações ( $> 25\%$  para inulina convencional e  $> 15\%$  para inulina HP), a inulina tem propriedade de formação de gel quando misturada à água ou leite, resultando em estrutura cremosa que pode ser incorporada em alimentos para substituir até 100% da gordura (FRANCK, 2002). O gel formado apresenta textura espalhável, sensação tátil oral homogênea, típica da presença de gordura, aspecto brilhante e liberação equilibrada de compostos de sabor na boca (ROBERFROID, 2005). Isto se deve à habilidade da inulina de formar microcristais quando misturada à água ou leite. Esses microcristais não são percebidos na boca, mas interagem para formar textura finamente cremosa que promove a sensação de gordura (FRANCK, 2002).

A inulina é considerada um dos prebióticos mais utilizados na indústria de alimentos. Os prebióticos, quando presentes na formulação de um alimento, tornam este produto funcional, por afetar benéficamente uma ou mais funções do organismo, além da finalidade nutricional, como aumento da absorção de cálcio (ROBERFROID, 2005). De acordo com a

Organização das Nações Unidas para Alimentação e Agricultura (FAO/AGNS) (2007), os prebióticos podem ser definidos como componentes alimentares não digeríveis que conferem benefícios à saúde do hospedeiro, associados à modulação de sua microbiota.

A Agência Nacional de Vigilância Sanitária (ANVISA) permite a seguinte alegação para a inulina presente ou adicionada ao alimento: “Contribui para o equilíbrio da flora intestinal. Seu consumo deve estar associado a uma alimentação equilibrada e hábitos de vida saudáveis.” Esta alegação pode ser utilizada desde que a porção do produto pronto para consumo forneça, no mínimo, 2,5 g de inulina (BRASIL, 2016).

A dose diária aceitável para inulina é estabelecida em 40 gramas. Não existem evidências de toxicidade ou distúrbios gastrointestinais associados ao consumo de inulina (VAN LOO et al., 1995). Após a ingestão, a inulina não é quebrada no sistema digestivo humano, não resultando, portanto, em contribuição calórica neste processo. Apenas no cólon ocorre a degradação de inulina por fermentação de bactérias, que produzem ácidos graxos de cadeia curta. Dependendo do grau de sua fermentação colônica, o valor calórico da inulina pode variar entre 0 e 2,5 kcal/g, mas com o objetivo de padronizar a rotulagem dos alimentos, recomenda-se atribuir à inulina o valor calórico de 1,5 kcal/g (ROBERFROID, 1999). Alguns de seus benefícios comprovados são: melhoria do trânsito gastrintestinal, redução de compostos tóxicos, como a amônia, diminuição da incidência de câncer de cólon, auxílio na redução dos níveis de colesterol (GONÇALVES; ROHR, 2009), além de aumentar a absorção de minerais, como o cálcio (COSTA; ROSA, 2016).

A ingestão de inulina também resulta em um significativo incremento dos benefícios das bifidobactérias (COSTA; ROSA, 2016). As bifidobactérias exercem atividades antimicrobianas, prevenindo ou aliviando desordens gastrointestinais; têm a capacidade de modular o sistema imune; melhoram a resposta alérgica e exercem importante papel no estabelecimento de microbiota saudável (PATEL; SHUKLA; GOYAL, 2015).

### **3.9 Análise Sensorial**

A Análise Sensorial é uma ciência utilizada para evocar, medir, analisar e interpretar reações às características de alimentos ou outros materiais da forma como são percebidos pelos sentidos da visão, olfato, gosto, tato e audição (STONE; BLEIBAUM; THOMAS,

2012), por meio de elementos da psicofísica (psico = resposta comportamental; física = estímulos) (MEILGAARD; CIVILLE; CARR, 2007).

Atualmente, esta ciência é considerada uma ferramenta indispensável para a indústria de alimentos para auxiliar na identificação e adequação de atributos sensoriais (MININ; DELLA LUCIA; CARNEIRO, 2006). Isso porque a indústria de alimentos vem desenvolvendo inúmeros produtos com baixo teor calórico e com propriedades funcionais, e essas alterações na formulação podem causar alterações sensoriais, muitas vezes consideradas negativas, comparadas aos produtos tradicionais (BAIXAULI et al., 2008).

### **3.9.1 Análise Descritiva Quantitativa (ADQ<sup>®</sup>) e Análise Tempo-Intensidade**

A Análise Descritiva Quantitativa (ADQ<sup>®</sup>) é uma metodologia completa e sofisticada que proporciona a obtenção de uma descrição detalhada das propriedades sensoriais de um produto. A ADQ proporciona uma base para o mapeamento das similaridades e diferenças do produto desenvolvido em relação aos similares e fornece também dados para a determinação dos atributos que são importantes para sua aceitação (STONE; BLEIBAUM; THOMAS, 2012).

Apesar de ser um dos métodos descritivos mais utilizados, a aplicação da ADQ<sup>®</sup> requer que os provadores sejam recrutados e treinados (ROUSSEAU, 2004).

A Análise Tempo-Intensidade consiste na medida da velocidade, duração e intensidade percebidas por um único estímulo, através da associação da percepção humana com recursos da informática (LAWLESS; HEYMANN, 2010). Uma vez que a percepção do aroma, do gosto e da textura em alimentos é um processo dinâmico que apresenta mudanças perceptíveis em intensidade durante todo o tempo de contato com a mucosa oral (LEE; PANGBORN, 1986), torna-se fundamental aplicar este tipo de técnica no desenvolvimento de um novo produto.

Nesse contexto, considera-se a Análise Tempo-Intensidade como um complemento da análise sensorial clássica, já que fornece informações sobre a sensação percebida ao longo do tempo (CLIFF; HEYMANN, 1993).



### 3.9.2 Testes Afetivos

Os testes afetivos não requerem treinamento dos provadores e são importantes por expressar a opinião dos consumidores sobre o produto a ser testado. É uma importante ferramenta, pois determina diretamente a opinião do consumidor em relação a ideias, características específicas ou globais de determinado produto, sendo, por isso, também denominado de teste de consumidor (MININ; DELLA LUCIA; CARNEIRO, 2006).

O Teste de Aceitação é um tipo de método afetivo que avalia o quanto o consumidor gostou ou não de um determinado produto. A escala hedônica de nove centímetros é a mais aplicada para medir a aceitação de um grupo de provadores, devido à sua simplicidade, confiabilidade e validade de seus resultados (STONE; BLEIBAUM; THOMAS, 2012).

### 3.9.3 Doçura Ideal e Estimativa de Magnitude

A escala do ideal é o método afetivo mais aplicado para se medir a quantidade ideal de um determinado componente para provocar a melhor aceitação e preferência de um grupo de provadores, devido à confiabilidade e validade de seus resultados e à simplicidade em ser utilizado pela equipe (MEILGAARD; CIVILLE; CARR, 2007). Na determinação da Doçura Ideal, os consumidores avaliam as amostras e registram em uma escala específica o quão ideal estas amostras encontram-se em relação ao atributo que se deseja avaliar, no caso, doçura, conforme o método de Vickers (1988).

Para a substituição adequada da sacarose por algum edulcorante no alimento, deve-se aplicar alguma técnica sensorial que determine a doçura equivalente deste composto, ou seja, que determine a quantidade do edulcorante necessária para fornecer a mesma doçura ideal obtida com a sacarose.

Várias metodologias podem ser utilizadas com o objetivo de determinar a equivalência de doçura: teste do limiar absoluto (FABIAN; BLUM, 1943), teste de ordenação, comparação pareada, estimativa de magnitude, comparação de um padrão de glicose com edulcorantes por meio da escala de intensidade e análise tempo-intensidade (CARDELLO, 1996).

Larson-Powers e Pangborn (1978) encontraram dificuldade para determinar a equivalência de doçura do ciclamato e da sacarina através do teste de comparação pareada, o

que gerou a necessidade de uma estimaco indireta e sensvel das propriedades sensoriais mltiplas destes compostos, utilizando-se, ento, a tcnica tempo-intensidade.

Portanto, o mtodo tempo-intensidade est se tornando bastante utilizado, porque possibilita a comparao da percepo da doura da sacarose com a doura de outros edulcorantes. Morais-Ferreria e colaboradores (2016) aplicaram a anlise tempo-intensidade para determinar as douras equivalentes de sucralose e rebaudiosdeo A, em relao à sacarose, em chocolates branco adicionados de *goji berry*.

## ESTRUTURA DA TESE

A tese foi dividida em artigos em conformidade com a descrição a seguir:

- **Artigo 1:** “The influence of the rebaudioside A content of stevia (*Stevia rebaudiana* Bertoni) on the determination of sweetness equivalence in bittersweet chocolates, using the time-intensity analysis” – apresenta como foi realizada a determinação da concentração ideal de sacarose para o chocolate meio amargo e as concentrações equivalentes à sacarose de cada edulcorante estudado;
  
- **Artigo 2:** “The importance of the Rebaudioside A content of Stevia (*Stevia rebaudiana* Bertoni) in the sensory profile of bittersweet chocolates with no added sugar and with prebiotic and relationship with consumer expectations” – mostra o perfil sensorial descritivo das amostras de chocolates meio amargos desenvolvidas e sua relação com os dados obtidos por consumidores;
  
- **Artigo 3:** “Bittersweet chocolates containing prebiotic and sweetened with stevia (*Stevia rebaudiana* Bertoni) with different Reubaudioside A contents: Multiple time-intensity analysis and physicochemical characteristics” – apresenta a realização da análise tempo intensidade para os atributos doçura, amargor e derretimento na boca e sua associação com os parâmetros físico-químicos.

Os artigos publicados (1 e 3) tiveram autorização para publicação na presente tese de Doutorado (anexos B e E). O comprovante de submissão do artigo 2 está no anexo C.

**ARTIGO 1 – Artigo publicado no Journal of Food Science, 81, p. S3006–S3014.**

*The influence of the rebaudioside A content of stevia (Stevia rebaudiana Bertoni) on the determination of sweetness equivalence in bittersweet chocolates, using the time-intensity analysis*

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**The influence of the rebaudioside A content of stevia (*Stevia rebaudiana* Bertoni) on the determination of sweetness equivalence in bittersweet chocolates, using the time-intensity analysis**

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**Abstract**

The consumption of diet products has increased greatly in recent years. The objectives of the study were to develop a bittersweet chocolate added inulin and stevias with different rebaudioside A contents (60, 80 and 97%). Five chocolate samples were formulated with different sucrose concentrations to determine the ideal sucrose concentration for bittersweet chocolate. The use of just-about-right scale identified an ideal sucrose concentration of 47.5% (w/w). The sweetness equivalence in sugar-free bittersweet chocolates was determined by the time-intensity method by fourteen selected and trained judges. The data collected during each session of sensory evaluation furnished the following parameters in relation to the sweet stimulus: I<sub>max</sub> (maximum intensity recorded); T<sub>max</sub> (time at which the maximum intensity was recorded); Area (area of time x intensity curve); and T<sub>tot</sub> (total duration time of the stimulus). The time-intensity analysis indicated that the percentages of rebaudioside A did not interfere with the sweetness intensity of the sweetener stevia in bittersweet chocolate and there was no significant difference in the concentrations tested (0.16%; 0.22%; 0.27%) of each stevia, in relation to the parameters evaluated. In addition, the reduction in fat content did not alter the perception of the sweetness intensity of the samples. These results showed important information to research and development of chocolate products. Therefore, the use

of the lowest stevia concentration tested (0.16%) is the most indicated for use, since this quantity was sufficient to reach the ideal sweetness of the product, so there was no point in adding more.

### **Practical Application**

This study aimed to show important information to research and development of food technology areas, especially to development of chocolate products. Sensory tests were applied to development of a bittersweet chocolate added prebiotic (inulin) and natural sweeteners. Studies of this nature are essential in order to develop sugar-free bittersweet chocolates with functional properties, considering the product quality and the production costs and benefits.

### **Introduction**

Currently consumers are demanding healthier food products with the addition of functional compounds. Thus an increasing number of new food formulations are being developed to satisfy this need (Icoz and others 2005). The confectionary industry, especially that of cocoa and chocolate, has also been influenced by this growing search for healthier products.

Since bittersweet chocolate contains larger amounts of polyphenolic compounds (epicatechins, catechins, anthocyanins and flavonoids) than traditional milk chocolate, it could have health benefits associated with its consumption, amongst which cardiovascular benefits such as an improvement in blood pressure, and improvements with chronic diseases (Pereira and others 2014; Loffredo and others 2014). However, despite containing antioxidant substances, bittersweet chocolate has high sugar and fat contents in its formulation (Afoakwa 2010). Thus the development of a sugar-free functional bittersweet chocolate with a reduced fat content would be an excellent alternative for the food industry, in order to improve the nutritional quality of this food.

Sweeteners are commonly used as sugar substitutes in chocolate. These compounds provide texture and a sweet taste to the products and greatly reduced calorie contents (Kato and Moskowitz 2001). The polyols such as isomalt, maltitol and xylitol (Sokmen and Gunes 2006; Olinger 1994), lactitol (Melo and others 2009), sorbitol and mannitol (Afoakwa and others 2007) are frequently used in the production of sugar-free chocolates. However since

they present reduced sweetness, the polyols are more and more used in association with high-intensity sweeteners (Melo and others 2009).

Recently stevia-based sweeteners containing steviol glycosides derived from the plant *Stevia rebaudiana* Bertoni, have received much attention and been widely used as natural high-intensity sweeteners, substituting the added sugar in foods (Guggisberg and others 2011; Puri and others 2011). This plant contains various glycosides such as steviosides, rebaudiosides (A, B, C, D and E) and dulcoside A, which are about 200-300 times sweeter than sucrose (Gardana and others 2010). Rebaudioside A is considered to be superior in relation to its sweetness quality and flavor, whereas stevioside is known for its marked bitter aftertaste (Lemus-Mondaca and others 2012). The concentration of the extracted glycosides will determine the level of sweetness of the sweetener stevia (Varanuj and Chatchai 2009).

Stevia is normally consumed by individuals who suffer from diabetes or other diseases related to carbohydrate metabolism disorders (Lemus-Mondaca and others 2012; Puri and others 2011), and also by individuals wishing to consume reduced calorie foods.

On using a high intensity sweetener as a sugar substitute, the viscosity and thickening properties of the food are reduced. For this reason, inulin is frequently used to improve the texture of sugar-free chocolate (Rad and others 2012). Inulin is also widely used as a fat substitute since it simulates the mouthfeel and mouth-coating properties of fat (Ramawat and Me´rillon 2015).

Inulin is a poly-dispersed fructan with a single glycosidic unit at the end of each oligomeric and polymeric chain (Ramawat and Me´rillon 2015), which satisfies the requisites for consideration as a functional ingredient denominated prebiotic. The International Scientific Association of Probiotics and Prebiotics (ISSAP) defines prebiotics as ‘Selectively fermented ingredients that allow specific changes in the composition and/or activity of the intestinal microbiota; thus conferring health benefits and wellbeing on the individual’ (Gibson and others 2010), such as bifidogenic and immune-stimulant actions and pathogenic intestinal bacteria-suppressing action. In addition inulin shows hypolipidemic action (Kaur and Gupta 2002) and increases the dietary fiber content (Roberfroid 2005).

The development of functional, sugar-free bittersweet chocolate with a reduced fat content requires the application of sensory studies to correctly evaluate the amount of each sweetener that should be used to obtain the desired sweetness. This occurs because the sweeteners may have particular characteristics in different types of foods and beverages

(Rodrigues and others 2015), and also because the intensity of the perception of the sweetness could be affected by an alteration in the product formulation, such as a reduction in the fat content (Paixão and others 2014).

The time-intensity analysis has become a useful tool in the food industry, since it makes it possible to compare the perception of sweetness in the food containing sucrose with the perception of sweetness in that with added sweeteners, with time. Palazzo and others (2011) applied the time-intensity analysis to determine the equivalent concentrations of sweetness of sucralose, stevia and neotame as sucrose substitutes in milk chocolate.

Thus the objectives of the present study were to establish the ideal sucrose concentration for bittersweet chocolate and the equivalent concentrations of stevia samples with different rebaudioside A contents (60, 80 and 97%) in sugar-free bittersweet chocolate with reduced fat content, using the time-intensity analysis.

## **Material and Methods**

### ***Material***

The ingredients used in the manufacture of all the samples were: stevia with different rebaudioside A (Reb A) contents (60, 80 and 97%) (Steviafarma Industrial, Maringá, Brazil); Orafit GR<sup>®</sup> inulin (Beneo, Mannheim, Germany); maltitol (Sweet Pearl<sup>®</sup> P90 Roquette, Roquette Freres, France); icing sugar (Mais Doce, Limeira, Brazil); cocoa mass (Barry Callebaut, Extrema, Brazil); deodorized cocoa butter (Barry Callebaut, Extrema, Brazil); skimmed milk powder (Embaré, Lagoa da Prata, Brazil); Solec CH soy lecithin (Solae, Esteio, Brazil); Grinsted<sup>®</sup> - PGPR 90 polyglycerol polyricinoleate (Danisco, Cotia, Brazil); and artificial vanilla flavor (Synergy Aromas, Vinhedo, Brazil).

### ***Methods***

This study was carried out in two steps. The first step was to determine the ideal sucrose concentration to be added to bittersweet chocolate. The second step aimed to determine the equivalent concentration of the different sweeteners (stevia with different rebaudioside A contents) in sugar-free bittersweet chocolates. It is important to emphasise that half of the samples also had a reduced fat content in addition to being sugar free.



### ***Preparation of the bittersweet chocolates***

The bittersweet chocolates were produced in the Cereals and Chocolate Technology Center (CEREAL CHOCOTEC) of the Food Technology Institute (ITAL) located in Campinas, SP, Brazil.

The samples were produced by the conventional method. The dry ingredients were weighed and homogenized in a Kitchen Aid model K5SS planetary mixer (Kitchen-Aid, St. Joseph, MI, USA). The melted cocoa liquor was then added plus approximately 20% of the total mass of cocoa butter, in order for the mixture to have an ideal plastic consistency for the next stage of refining. After homogenization, the mixture was transferred to a Draiswerk GMBH three-roll refiner (Draiswerke GmbH, Mannheim Waldo, Mahweh, NJ, USA), and the rolls adjusted such that the particle size was between 20 and 25  $\mu\text{m}$  (Luccas and others 2014). This was measured using a manual micrometer (Mitutoyo brand), taking 10 readings for each sample batch. After refining, the rest of the cocoa butter was added to the chocolate mass and the whole mass submitted to conching at a temperature of 65°C for 16 hours in an Inco longitudinal conch (Inco, Avaré, SP, Brazil). The chocolate mass was then tempered on a marble table. The tempering index was measured using a Temperindex Sollich (Bad Salzflun, Germany) equipment to check if the tempering process was adequate. According to the equipment manual, correctly tempered chocolate should present a temperindex value between 4 and 6. The tempered chocolate was poured into polycarbonate molds and submitted to vibration on a Jafinox (BR) vibratory table to remove air bubbles for approximately 10 minutes. The criterion used to ensure the removal of bubbles was the appearance. All samples which have bubbles were not used in sensory tests. The molds were then passed twice through a SIAHT cooling tunnel (Siaht, Jundiaí, SP, Brazil), with entrance and exit temperatures of 18°C and a temperature of 12°C in the center. The chocolates were removed from the molds, wrapped in aluminum foil and stored for fifteen days at 20°C in a BOD incubator (Novatecnica, BR, Brazil).

### ***Preparation of the bittersweet chocolate samples for the ideal sweetness test***

Five chocolate samples were formulated with different sucrose concentrations to determine the ideal sucrose concentration for bittersweet chocolate as shown in Table 1.

**Table 1** – Recipes used to determine the ideal sweetness of bittersweet chocolate.

Ingredients (%)	Recipes				
	A	B	C	D	E
Sucrose	40	43	46	49	52
Cocoa butter	18	17.5	17	16.5	16
Cocoa mass	30	29	28	27	26
Skimmed milk powder	11.4	9.9	8.4	6.9	5.4
Soy lecithin	0.3	0.3	0.3	0.3	0.3
Polyglycerol Polyricinoleate (PGPR)	0.2	0.2	0.2	0.2	0.2
Vanilla flavor	0.1	0.1	0.1	0.1	0.1

***Preparation of the bittersweet chocolate samples for the equivalent sweetness test***

To determine the equivalent concentration of sweetener for the diet bittersweet chocolates, three types of sugar-free chocolates were produced, sweetened with stevia samples varying only in their rebaudioside A content (60, 80 and 97%). In addition, three types of diet bittersweet chocolates were produced using the same sweeteners, but with reduced fat contents. Each sample was produced with three concentrations (0.16%, 0.22% and 0.27%) of each sweetener, giving a total of 18 formulations of diet chocolate, as shown in Table 2.

**Table 2** – Recipes used to determine the Equivalent Sweetness.

Ingredients (%)	Chocolate samples					
	Stevia 60% Reb A	Stevia 80% Reb A	Stevia 97% Reb A	Stevia 60% Reb A <sup>1</sup>	Stevia 80% Reb A <sup>1</sup>	Stevia 97% Reb A <sup>1</sup>
Cocoa mass	30	30	30	30	30	30
Cocoa butter	15	15	15	7.5	7.5	7.5
Maltitol	32.5	32.5	32.5	32.5	32.5	32.5
Inulin	15	15	15	20	20	20
Skimmed milk powder	6.9	6.9	6.9	9.4	9.4	9.4
Soy lecithin	0.3	0.3	0.3	0.3	0.3	0.3
PGPR	0.2	0.2	0.2	0.2	0.2	0.2
Vanilla flavor	0.1	0.1	0.1	0.1	0.1	0.1

Stevia	(0.16; 0.22; 0.27)	(0.16; 0.22; 0.27)	(0.16; 0.22; 0.27)	(0.16; 0.22; 0.27)	(0.16; 0.22; 0.27)	(0.16; 0.22; 0.27)
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<sup>1</sup> Samples with reduced fat contents.

In the elaboration of the diet bittersweet chocolate formulations containing maltitol, the researchers were concerned that the concentrations used should not cause any digestive discomfort. Since the recommended portion for chocolate consumption is of 25 g, according to the manufacturers, each individual would consume 8.12 g of maltitol/portion. According to Koutsou and others (1996), a 30 g dose of maltitol in chocolate-flavored milk did not significantly increase digestive discomfort as compared to chocolate-flavored milk sweetened with sucrose. In addition the inulin content in the formulation was sufficient to guarantee the prebiotic activity of this fiber, since Brazilian legislation alleges that inulin can only be considered prebiotic when a minimum of 3 g are added to each solid food portion (25 g in the case of chocolate) (Brasil 2008).

### ***Sensory analysis***

The tests were carried out in individual acclimatized (22°C) booths in the Sensory Science and Consumer Studies Laboratory of the Food Engineering Faculty (FEA) of the State University of Campinas (UNICAMP), Brazil.

The samples were presented to the judges on disposable napkins codified at random with three-digit algorithms (Meilgaard and others 2004) and accompanied by water at room temperature and a water cracker. All the computers in the laboratory were equipped with FIZZ Network Sensory Software (Biosystemes, Couternon, France) and with the Time-Intensity Analysis of Food and Tastes (TIAFT) software (UNICAMP 2012), developed in the Sensory Analysis Laboratory of the Food Engineering Faculty/UNICAMP, applied with successfully in several studies to analysis in different foods (Rodrigues and others 2015; Morais and others 2014; Palazzo and others 2011).

### ***Ideal sweetness determination***

The ideal sucrose concentration (%) for the bittersweet chocolate was initially determined by an affective test using the Just-About-Right (JAR) scale (Vickers 1988).

The samples were presented in a sequential monadic way using a balanced complete block design (Stone et al., 2012) with five sucrose concentrations (40; 43; 46; 49 and 52% w/w) (Melo and others 2007).

The tasters received 4 g of each sample and were instructed to evaluate the samples using a nine centimeter non-structured scale, anchored at the left and right extremities by “extremely less sweet than ideal” and “extremely sweeter than ideal”, respectively. The central point of the scale (marked in scale) represented the ideal concentration to be added to the product.

One hundred non-trained individuals took part in the test, between the age of 18 and 60 years (mean age = 25.4; SD = 6.2), all being students and workers at the Food Engineering Faculty of UNICAMP, recruited via posters and e-mail invitations. The most of the tasters were women (72%). The exclusion criterias included lactose intolerant individuals and tasters who didn't appreciate bittersweet chocolate.

The results were analyzed by a simple linear regression between the intensity values and the sucrose concentration (Guinard and others 1997).

#### ***Determination of the sweetness equivalence by way of the time-intensity analysis***

The pre-selection consisted of applying triangular difference tests between two commercial bittersweet chocolate samples with different cocoa contents, using Wald's sequential analysis (Meilgaard and others 2004). The two samples used were significantly different at a level of 1%, previously determined using a paired test with 30 tasters. 18 individuals were pre-selected at this point, between the age of 18 and 60 years (mean age = 27.3; SD = 3.8). The most of the tasters were women (66.67%).

#### ***Training session and selection***

The pre-selected individuals were rigorously trained before evaluating the sweet taste of the samples, using a bittersweet chocolate sample containing 49% sucrose as the maximum sweetness reference intensity. The training was carried out by direct contact of the individuals with this reference of maximum intensity, in three 0.5h training sessions. The objective was for all the tasters to form a sensory memory and understand in a similar way what the maximum sweetness on the scale would be.

In the selection stage, the tasters evaluated the sweet taste of the sample containing sucrose at the level chosen in the ideal sweetness test and also the diet samples, with and without fat reduction. At the start of the analysis, the tasters were instructed to place the whole sample in the mouth (4 g). They were then instructed to indicate the intensity of the attribute as a function of the passage of time using the mouse on the ten-point scale on the monitor. The mouse cursor slides freely so that the tasters can continuously register the perceived intensity as a function of time. The continuous scale is horizontal, and it has 10 vertical lines indicating the numbers 0 through 9 (0 corresponds to none, 4.5 corresponds to moderate and 9 corresponds to strong) (Palazzo and Bolini 2014). The test conditions were previously standardized using TIAFT software: (i) initial waiting time, 10s, (ii) time with sample in mouth, 15s and (iii) time after ingestion, 1 min and 30s. The samples were presented in a monadic way with three repetitions.

At the end of the test, the data were continuously collected and the time intensity curve obtained. The parameters of the curves analyzed statistically were: maximum intensity (**I<sub>max</sub>**, the maximum sweetness intensity of each sample), the time for maximum intensity (**T<sub>I<sub>max</sub></sub>**, the maximum intensity time), the area under the curve (**Area**, the total area under the time-intensity curve), total time (**T<sub>tot</sub>**, the time for the perception of sweetness from the first to the last perception).

14 tasters were selected. All being students and workers at the Food Engineering Faculty of UNICAMP and the most of the tasters were women (65%), between the age of 18 and 60 years (mean age = 24.6; SD = 2.6)

### ***Evaluation of the sweet taste by the time-intensity analysis***

The tasters evaluated the sweet taste of all the bittersweet chocolate samples presented in a monadic way with three repetitions, registering the intensity of the attribute with the passage of time. The samples were presented according to a randomized balanced order (Wakeling and MacFie 1995). At the end of the test the data were collected and the time-intensity curves obtained. The equivalent concentrations were determined by verifying which time-intensity curve of each sweetener was most similar to the sucrose curve.

Since the total number of samples was high (19 samples), the tasters evaluated only two to three samples in each session and crackers and taste-free water were provided for palate cleansing, to eliminate fatigue between samples. All participants were employees or

students at the Food Engineering Faculty of UNICAMP, therefore, it was possible to make more than one session per day, depending on the time availability of each taster, with an interval of two hours between them at least. The test took approximately three months. The taster participated voluntarily in this survey and signed a consent.

### ***Statistical analysis***

The opinions of the tasters, provided by ideal sweetness test, were transformed into numerical data (-4 to +4) in order to analyze the results, the ideal sweetness corresponding to the value of 0. The ideal amount of sucrose was calculated using the regression equation.

The parameters of the curves (Imax, TImax, Area, Ttot) were analyzed statistically by the Univariate analysis of variance (ANOVA) and Tukey's means test.

The tasters selected were those showing ability in the interactive test with the computer and also based on their powers of discrimination, repeatability and team concordance (Damásio and Costell 1991), as verified by the two-factor variance analysis (sample and repetition) for each taster in relation to each parameter of the time-intensity curve. 14 tasters who obtained significant  $F_{\text{sample}}$  values ( $p < 0.30$ ) and non-significant  $F_{\text{repetition}}$  values ( $p > 0.05$ ) were selected.

## **Results and Discussion**

### ***Determination of the ideal sweetness***

The ideal sweetness test made it possible to define the ideal amount of sucrose to add to bittersweet chocolate.

The ideal amount of sucrose was calculated using the regression equation (Figure 1) and the value obtained was 47.5%, with a value for  $R^2$  of 99.64% indicating an optimum correlation (Callegari-Jacques 2003).

Various studies have determined the ideal sucrose concentration for different food matrixes, such as that carried out by Paixão and others (2014), who found an ideal sweetness of 7% w/w for chocolate flavored milk at 6°C, and another study with chocolate flavored milk enriched with chia oil, which determined an ideal sweetness of 8% w/w (Rodrigues et al., 2015). Morais and others (2014) determined the ideal sucrose concentration for a chocolate dessert as being 8.13% and Melo and others (2007) evaluated the ideal sweetness of milk

chocolate bars as being 43%, a value inferior to that found in the present study with bittersweet chocolate. This probably occurred because the consumers tried to compensate the bitterness of the bittersweet chocolate by increasing its sweetness. When different gustatory stimuli are mixed, the intensity of one is frequently decreased by the perception of the other (Keast 2008), and the suppression of bitterness by an increase in sweetness is an example of this (Ares and others 2009).

These different studies demonstrate that it is fundamental to carry out this sensory analysis for each type of product and even for different varieties of the same product, as for example, in chocolate (milk and bittersweet chocolates).

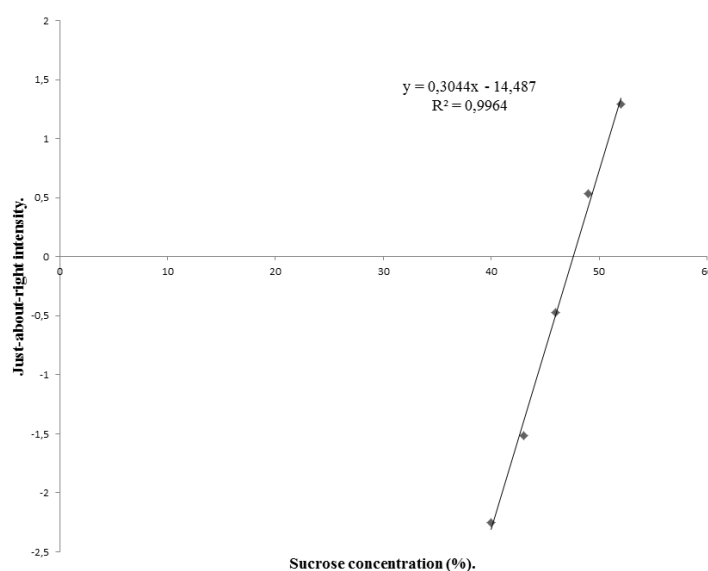


Figure 1: Ideal sucrose concentration to be added to bittersweet chocolate, obtained with the ‘just about right’ scale.

### ***Determination of sweetness equivalence by way of the time-intensity analysis***

Table 3 shows the parameters for the time-intensity curves of the bittersweet chocolate sample sweetened with sucrose, and of those sweetened with different types of stevia containing 60%, 80% and 97% of rebaudioside A (Figures 2, 3 and 4).

**Table 3** - Parameters for the time-intensity curves of the bittersweet chocolate sample sweetened with sucrose, and of those sweetened with different types of stevia.

Samples	Tlmax	Imax	Area	Ttot
Sucrose	22.1 <sup>bc</sup>	6.6 <sup>ab</sup>	206.01 <sup>abc</sup>	56.28 <sup>a</sup>
Stevia 60% Reb A 0.16%	21.8 <sup>c</sup>	6.26 <sup>ab</sup>	212.8 <sup>abc</sup>	62.6 <sup>a</sup>
Stevia 60% Reb A 0.22%	20.42 <sup>c</sup>	6.8 <sup>a</sup>	223.07 <sup>a</sup>	61.66 <sup>a</sup>
Stevia 60% Reb A 0.27%	24.2 <sup>abc</sup>	6.2a <sup>b</sup>	198.67 <sup>abc</sup>	62.48 <sup>a</sup>
Stevia 60% Reb A <sup>1</sup> 0.16%	22.5 <sup>bc</sup>	5.63 <sup>b</sup>	176.47 <sup>abc</sup>	60.59 <sup>a</sup>
Stevia 60% Reb A <sup>1</sup> 0.22%	24.1 <sup>abc</sup>	5.99 <sup>ab</sup>	197.94 <sup>abc</sup>	62.65 <sup>a</sup>
Stevia 60% Reb A <sup>1</sup> 0.27%	23.29 <sup>abc</sup>	5.84 <sup>ab</sup>	162.3 <sup>c</sup>	55.76 <sup>a</sup>
Stevia 80% Reb A 0.16%	20.96 <sup>c</sup>	6.49 <sup>ab</sup>	202.3 <sup>abc</sup>	60.08 <sup>a</sup>
Stevia 80% Reb A 0.22%	21.12 <sup>c</sup>	6.85 <sup>a</sup>	221.46 <sup>ab</sup>	62.45 <sup>a</sup>
Stevia 80% Reb A 0.27%	24.37 <sup>abc</sup>	6.18 <sup>ab</sup>	195.1 <sup>abc</sup>	61.23 <sup>a</sup>
Stevia 80% Reb A <sup>1</sup> 0.16%	21.45 <sup>c</sup>	5.81 <sup>ab</sup>	171.05 <sup>abc</sup>	57.2 <sup>a</sup>
Stevia 80% Reb A <sup>1</sup> 0.22%	26.35 <sup>ab</sup>	5.7 <sup>b</sup>	181.59 <sup>abc</sup>	61.43 <sup>a</sup>
Stevia 80% Reb A <sup>1</sup> 0.27%	21.66 <sup>c</sup>	6.06 <sup>ab</sup>	169.31 <sup>bc</sup>	56.41 <sup>a</sup>
Stevia 97% Reb A 0.16%	21.58 <sup>c</sup>	6.28 <sup>ab</sup>	197.41 <sup>abc</sup>	58.65 <sup>a</sup>
Stevia 97% Reb A 0.22%	22.05 <sup>bc</sup>	6.52 <sup>ab</sup>	207.91 <sup>abc</sup>	57.8 <sup>a</sup>
Stevia 97% Reb A 0.27%	22.52 <sup>bc</sup>	6.57 <sup>ab</sup>	217.64 <sup>bc</sup>	60.83 <sup>a</sup>
Stevia 97% Reb A <sup>1</sup> 0.16%	21.99 <sup>bc</sup>	5.9 <sup>ab</sup>	193.31 <sup>abc</sup>	59.64 <sup>a</sup>
Stevia 97% Reb A <sup>1</sup> 0.22%	23.03 <sup>abc</sup>	5.83 <sup>ab</sup>	190.72 <sup>abc</sup>	60.74 <sup>a</sup>
Stevia 97% Reb A <sup>1</sup> 0.27%	23.05 <sup>abc</sup>	6.11 <sup>ab</sup>	198.42 <sup>abc</sup>	60.56 <sup>a</sup>
Standard error (SE)	5.33	1.25	64.12	9.75
Minimum Significant Difference (MDS)	4.45	1.04	53.46	8.12

<sup>1</sup> Samples with reduced fat contents.



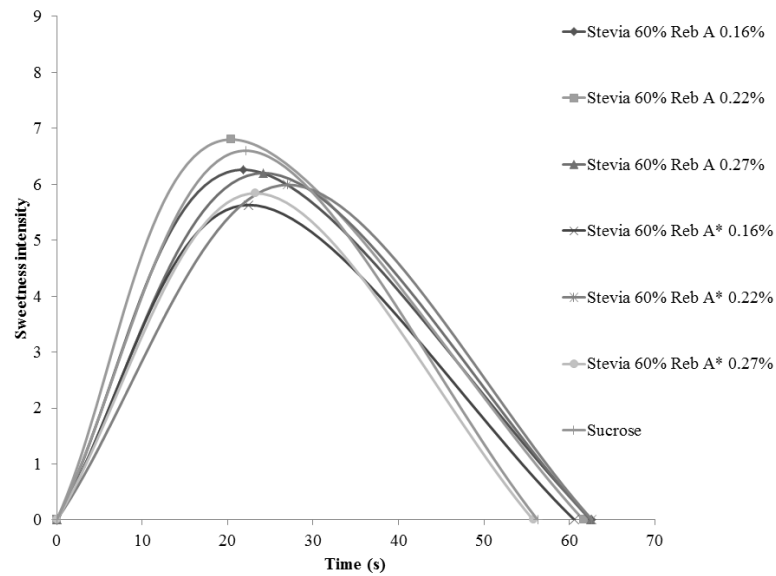


Figure 2: Time-intensity curves for sweet taste stimulus of bittersweet chocolate formulated with different stevia with 60% rebaudioside A concentrations (from 0.16% to 0.27%).

\* Samples with reduced fat contents.

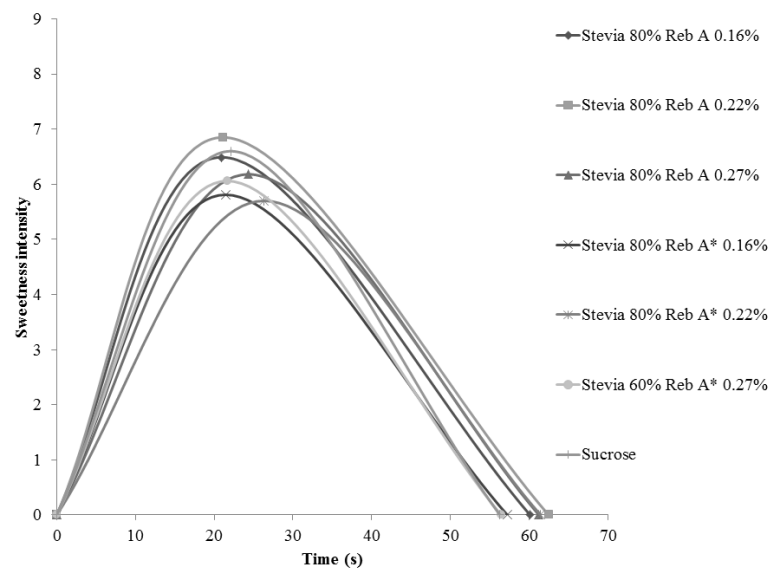


Figure 3: Time-intensity curves for sweet taste stimulus of bittersweet chocolate formulated with different stevia with 80% rebaudioside A concentrations (from 0.16% to 0.27%).

\* Samples with reduced fat contents.

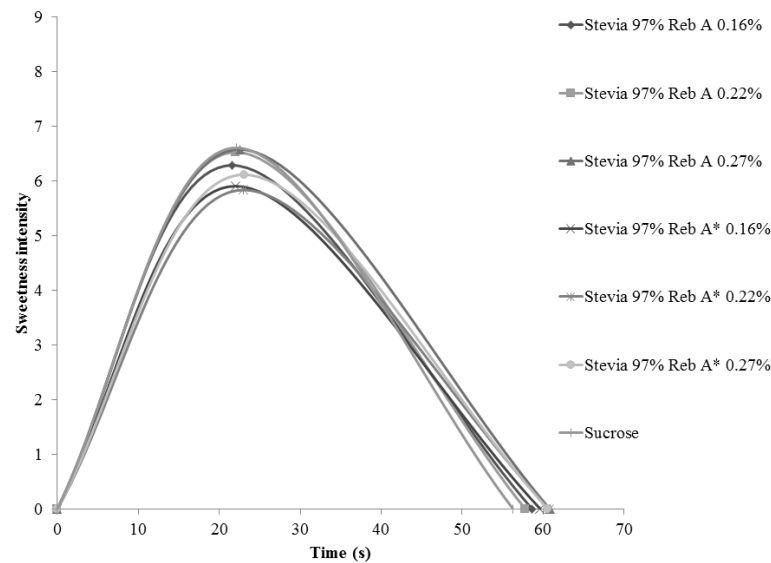


Figure 4: Time-intensity curves for sweet taste stimulus of bittersweet chocolate formulated with different stevia with 97% rebaudioside A concentrations (from 0.16% to 0.27%).

\* Samples with reduced fat contents.

On comparing the parameters of the bittersweet chocolate sweetened with sucrose with those of the other six samples, three important results should be discussed. The first and most important is that the percentage of rebaudioside A did not interfere with the sweetness intensity of the sweetener stevia in bittersweet chocolate, since the results showed no statistical difference ( $p < 0.05$ ) independent of the type of stevia used.

According to the literature, rebaudioside A is the glycoside that confers the sweet taste on stevia (Wang and others 2016), and thus it was expected that one would need to add more of the stevia containing only 60% of this glycoside to the bittersweet chocolate than of the stevia containing 97% of the glycoside, in order to attain the same perception of sweetness intensity. Possibly it was due to the synergistic effect of the sweeteners with the other ingredients used, such as polyol (maltitol). The polyols combine well and are synergistic with high intensity sweeteners, such as stevia (Nabors, 2011).

It is important to point out that the higher the rebaudioside A content of the stevia, the more expensive it is. Thus the fact that the stevia with 60% of rebaudioside A is cheaper, but presents the same behavior as the stevia with 97% of this glycoside with respect to the perception of sweetness intensity in relation to time, is a highly interesting result for the food

industry. Dutra and Bolini (2014) evaluated the sweetness equivalence of stevia samples containing different rebaudioside A contents when added to acerola juice, and also failed to find significant differences between the results. In the case of bittersweet chocolate, the bitter taste of the product could have made perception of the sweet taste of the product by the tasters difficult.

In addition, another point for discussion is the fact that the reduction in fat content did not alter the perception of the sweetness intensity of the samples. According to Wiet and others (1993), in study with pasteurized skimmed milk, the perception of the sweet taste increases as decreases the fat content. However, Melo and others (2009) also found that the reduction in fat content did not alter the perception of the sweetness intensity of the diabetic/reduced calorie milk chocolates, sweetened with sucralose and stevia. Possibly, the reduction in cocoa butter content of three samples of bittersweet chocolates in this study (only 7.5%) was insufficient to alter the perception of the sweetness intensity. Paixão and others (2014) also found no significant difference in the sweetening power of stevia containing 97% rebaudioside A in whole and skimmed chocolate flavored milks. This same study found a great difference in sweetening power for the same two types of chocolate flavored milks when sweetened with neotame, showing the importance of carrying out this type of study.

The last conclusion was that there was no significant difference in the concentrations tested (0.16%; 0.22%; 0.27%) of each stevia (60%, 80% and 97% of rebaudioside A), in relation to the parameters evaluated:  $I_{max}$ ,  $T_{I_{max}}$ ,  $T_{tot}$  and area. Palazzo and others (2011) also found no significant difference for these same parameters for the four different concentrations of stevia tested in chocolate milk. This could be due to the sweetness reaching a terminal threshold in terms of intensity of sweetness (Keast and Roper 2007). As the concentration of the chemical increases significantly, the intensity of sweetness mutually increases to a theoretical asymptote where concentration increases no longer cause subsequent increases in intensity (Keast and Breslin 2003).

PCA (figure 5) explained 76.91% of the variability in the samples in the time-intensity analysis for sweet taste. Although it is just a graphical representation of the results, it can be seen that, in general, all the samples were in the central region of the figure, indicating that they act in a similar way in the time-intensity analysis for sweetness.

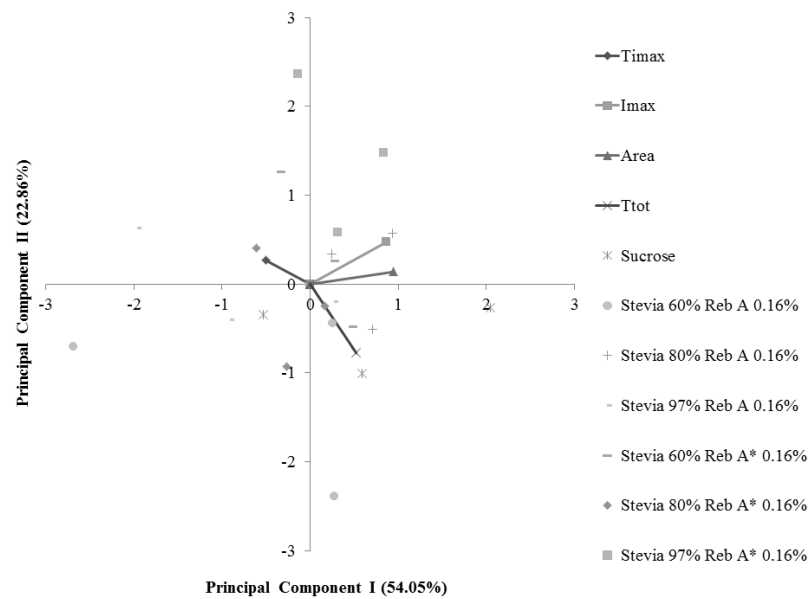


Figure 5: Principal component analysis of bittersweet chocolate samples for the attribute Sweetness, using time-intensity analysis.

The results of this study allow one to choose the best and most convenient stevia concentration for the food industry to add to bittersweet chocolate, considering its rebaudioside A content. Since there was no significant difference between the different concentrations used of each type of stevia, a concentration of 0.16% is the most indicated for use, since this quantity was sufficient to reach the ideal sweetness of the product, so there was no point in adding more. It should be mentioned that the use of the lowest stevia concentration tested would decrease the manufacturing costs of the product, and also probably reduce the bitterness of the samples, an attribute frequently associated with the use of the sweetener stevia.

This study investigated the concentration and type of stevia that provides similar results in terms of sweetness intensity. Additional studies should be carried out to determine the acceptability of the products, and the bitterness intensity that contributes to product acceptance should be identified.

## Conclusions

The ideal sucrose concentration for bittersweet chocolate was 47.5% w/w. With respect to sweetness equivalence, it was concluded that the rebaudioside A contents of the

types of stevia studied did not alter the perception of their sweetness intensities in the bittersweet chocolate. Studies of this nature are essential in order to develop sugar-free bittersweet chocolates with functional properties, considering the product quality and the production costs and benefits. The ideal sweetness and sweetness equivalence tests make up the initial stage in the correct development of diet bittersweet chocolates and of bittersweet chocolates with functional properties, it being fundamental to carry out descriptive studies and subsequently those of acceptance in order to obtain a complete well-backed sensory study.

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**ARTIGO 2 – Artigo submetido no Journal of Food Science and Technology.**

*The importance of the Rebaudioside A content of Stevia (Stevia rebaudiana Bertoni) in the sensory profile of bittersweet chocolates with no added sugar and with prebiotic and relationship with consumer expectations*

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**The importance of the Rebaudioside A content of Stevia (*Stevia rebaudiana* Bertoni) in the sensory profile of bittersweet chocolates with no added sugar and with prebiotic and relationship with consumer expectations**

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*Abstract*

The purpose of the study was to evaluate the influence of different rebaudioside A contents on the sensory profile of bittersweet prebiotic chocolates with no added sugar and with a reduction in the fat content, and correlate these descriptive data with the acceptance. The results for the QDA showed that the different contents of rebaudioside A tested did not interfere with the attributes of sweet taste, sweet aftertaste, bitter taste or bitter aftertaste. In addition, the bitterness was more accentuated in the samples with reduced fat content. It was also verified that the three samples with no added sugar and with reduced fat content showed the greatest variations in relation to texture, differing significantly from the conventional sample with respect to the attributes of hardness, mouth melting and sandiness. Although some differences were detected among the samples in the descriptive test, in general, the acceptance test and the external map of preference demonstrated that all the samples showed good sensory acceptance, including those with a reduced fat content. Hence it is possible to develop bittersweet chocolates with no added sugar and with the addition of prebiotic fiber in order to substitute the sugar and fat.

*Key words:* Inulin, Quantitative Descriptive Analysis, Acceptance test

### *Introduction*

The worldwide consumption of chocolate increased 10% from 2002 to 2010 (Afoakwa 2014), which could be attributed to increasing knowledge concerning potential health benefits arising from the consumption of cocoa constituents. More than 200 compounds found in cocoa (Visioli et al. 2009) can provide protection against the development of a variety of pathologies, including cardiovascular diseases (Hooper et al. 2012). Despite these benefits, chocolate is a highly energy-rich product, containing elevated amounts of sugar and fat in its composition. Sugar may be present up to 60% of the composition, depending on the type of chocolate (Aidoo et al. 2013).

Currently the chocolate industries have been affected by increasing health concerns, and consumers are searching for less caloric foods without sugar. In this context the interest in natural sweeteners has greatly increased (Aidoo et al. 2015). Natural sweeteners extracted from the plant *Stevia rebaudiana* Bertoni have been considered as promising alternatives to substitute sugar, and the compounds responsible for the sweetness of this sweetener are known as steviol glycosides (Boileau et al. 2012). Rebaudioside A (Reb A) and stevioside are the main steviol glycosides found in the leaves of *S. rebaudiana* (Tavarini and Angelini 2013), but Reb A is more important due to its sensory characteristics as compared to stevioside, which is associated with the bitter aftertaste (Palmer et al. 2013). Hence the study of stevia varieties with greater Reb A contents is the target of many research groups (Ramesh et al. 2006; Rajasekaran et al. 2007; Yadav et al. 2014).

The substitution of sugar by sweeteners can cause structural alterations in the chocolate, thus one must combine the sweeteners with thickening agents, such as inulin. Inulin is a plant derived carbohydrate and is considered to be a prebiotic (Konar et al. 2014) and can be used in the production of chocolate with no added sugar and/or with fat reduced. Hence chocolate made with inulin has the advantage of possible reductions in the sugar and fat contents as well as showing a prebiotic characteristic, depending on the concentration in the formulation (Konar et al. 2016).

It is important that food products developed with no added sugar and with reduction in fat have sensory characteristics similar to the conventional product. In this way, sensory analysis represents the most effective tool to compare such products with the conventional one (Melo et al. 2009). Descriptive sensory analyses are the most sophisticated tools for use by sensory scientists, the Quantitative Descriptive Analysis (QDA) being the most used

(Lawless and Heymann 2010). This methodology provides a complete description of all the sensory properties of a product (Stone et al. 2012).

Sensory tests with consumers are carried out to investigate if the consumer prefers one product or another one, or find an acceptable product based on its sensory characteristics (Lawless and Heymann 2010). The external preference map, for its part, is a sensory tool used to correlate consumer reactions with descriptive data (Geel et al. 2005), allowing one to discover relationships between the attributes of a product and consumer acceptance (Meilgaard et al. 2007).

The objective of this study was to evaluate the influence of different rebaudioside A contents on the sensory profile of bittersweet prebiotic chocolates with no added sugar and with a reduction in the fat content, and correlate these descriptive data with the acceptance data.

### *Material and methods*

#### *Material*

The bittersweet chocolate samples were produced using the following ingredients: stevia with different rebaudioside A (Reb A) contents (60, 80 and 97%) (Steviafarma Industrial, Maringá, Brazil); Orafit GR<sup>®</sup> inulin (Beneo, Mannheim, Germany); maltitol (Sweet Pearl<sup>®</sup> P90 Roquette, Roquette Freres, France); icing sugar (Mais Doce, Limeira, Brazil); cocoa mass (Barry Callebaut, Extrema, Brazil); deodorized cocoa butter (Barry Callebaut, Extrema, Brazil); skimmed milk powder (Embaré, Lagoa da Prata, Brazil); Solec CH soy lecithin (Solae, Esteio, Brazil); Grinsted<sup>®</sup> - PGPR 90 polyglycerol polyricinoleate (Danisco, Cotia, Brazil); and artificial vanilla flavor (Synergy Aromas, Vinhedo, Brazil).

#### *Methods*

##### *Preparation of the bittersweet chocolates*

The bittersweet chocolates were produced in the Cereals and Chocolate Technology Center (CEREAL CHOCOTEC) of the Food Technology Institute (ITAL) located in Campinas, SP, Brazil.

The chocolates were conventionally produced. The ingredients were homogenized in a Kitchen Aid model K5SS planetary mixer (Kitchen-Aid, St. Joseph, MI, USA). So, the mixture was transferred to a Draiswerk GMBH three-roll refiner (Draiswerke GmbH,

Manheim Waldo, Mahweh, NJ, USA). The whole mass submitted to conching at a temperature of 65°C for 16 hours in an Inco longitudinal conch (Inco, Avaré, SP, Brazil). The chocolate mass was tempered on a marble table. The tempering index was measured using a Temperindex Sollich (Bad Salzflen, Germany) equipment to check if the tempering process was adequate. According to the equipment manual, correctly tempered chocolate should present a temperindex value between 4 to 6. The tempered chocolate was poured into polycarbonate molds and submitted to vibration on a Jafinox (BR) vibratory table to remove air bubbles for approximately 10 minutes. The molds were then passed twice through a SIAHT cooling tunnel (Siaht, Jundiaí, SP, Brazil), with entrance and exit temperatures of 18°C and central temperature at 12°C. The samples were removed from the molds, wrapped in aluminum foil and stored for fifteen days at 20°C in a BOD incubator (Novatecnica, BR, Brazil).

Table 1 presents the formulations used in this study. Seven bittersweet chocolate samples were produced, one being conventional (with added sugar), three samples with no added sugar, sweetened with stevia samples containing different amounts of rebaudioside A (60, 80 and 97%), and three samples produced using the same sweeteners but with a 50% reduction in the amount of cocoa butter. The concentrations of sweetener used were determined previously in a sweetness equivalence study using the time intensity analysis (Azevedo et al. 2016).

The inulin content used in the formulations was sufficient to guarantee the prebiotic activity of this fiber, since Brazilian legislation states that inulin can be considered prebiotic if a minimum amount of 3g is added to a portion of the solid food (25g in the case of chocolate) (Brasil 2008).

**Table 1** Formulation/Composition of the seven samples used in this study

Ingredients (%)	Chocolate samples						
	Sucrose	Stevia 60% Reb	Stevia 80% Reb	Stevia 97% Reb	Stevia 60% Reb	Stevia 80% Reb	Stevia 97% Reb
		A	A	A	A <sup>a</sup>	A <sup>a</sup>	A <sup>a</sup>
Icing sugar	47.5	0	0	0	0	0	0
Cocoa mass	30	30	30	30	30	30	30
Cocoa butter	15	15	15	15	7.5	7.5	7.5
Maltitol	0	32.34	32.34	32.34	32.34	32.34	32.34
Inulin	0	15	15	15	20	20	20
Skimmed milk powder	6.9	6.9	6.9	6.9	9.4	9.4	9.4
Soy lecithin	0.3	0.3	0.3	0.3	0.3	0.3	0.3
PGPR	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vanilla flavor	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Stevia	0	0.16	0.16	0.16	0.16	0.16	0.16

<sup>a</sup> Samples with reduced fat contents.

### *Proximate composition*

The proximate composition of all chocolate samples was determined at the Central Laboratory of the Department of Food and Nutrition (Unicamp / FEA), in three replicates.

The samples were characterized for moisture, ash, protein and fat content according AOAC methods (AOAC, 2006). Carbohydrates were determined by difference, thus they did not reflect solely the amount of sugar in the samples. The prebiotic fiber (inulin) and the polyol (maltitol) used in six chocolate formulations were also included in this group. The total caloric value was determined considering 4 Kcal per gram of protein and 9 Kcal per gram of fat. The caloric value of carbohydrates was determined considering 1.5 Kcal per gram of inulin (Roberfroid, 1999) and 2.1 Kcal per gram of maltitol (FDA, 2016). For the remaining carbohydrates, a value of 4 Kcal per gram was considered.

### *Sensory Analysis*

Sensory analyses were carried out in individual air-conditioned (22°C) booths in the Sensory Science and Consumer Studies Laboratory of the School of Food Engineering (FEA) of the University of Campinas (UNICAMP), Brazil.

The samples were presented to the judges on disposable napkins codified at random with three-digit algorithms (Meilgaard et al. 2007) and accompanied by taste free water and a



water cracker for palate cleansing. All the computers in the laboratory were equipped with FIZZ Network Sensory Software (Biosystemes, Couternon, France).

### *Quantitative Descriptive Analysis (QDA<sup>®</sup>)*

#### *Selection of tasters*

The initial step of the QDA<sup>®</sup> consisted of a pre-selection of tasters using Wald's sequential analysis (Meilgaard et al. 2007). Triangular difference tests were applied using commercial bittersweet chocolates containing different cocoa concentrations, which differed from one another at the 1% level of significance with respect to bitterness. A paired test was previously applied to 30 tasters to determine if the commercial samples used differed between each other at the 1% significance level.

The results obtained in the triangular test were analyzed by the sequential analysis [18] using the following values:  $r=0.45$  (maximum acceptable lack of ability) and  $r1=0.70$  (minimum acceptable ability), and for the risks  $a=0.05$  (probability to accept a candidate without sensory acuity) and  $b=0.05$  (probability to reject a candidate with sensory acuity).

#### *Generation of the descriptive terminology and training of the tasters*

Sixteen pre-selected individuals took part in generating the descriptive terms for the bittersweet chocolate samples using the network method cited by Moskowitz (1983). With the aid of a moderator and in consensus, the tasters generated the descriptive terms with their respective definitions and references for each extreme of the scale. The reference materials and the definition of each descriptive term were accessible to the tasters during the four training sessions, each lasting approximately one hour.

#### *Final selection of the sensory team*

In order to select the final QDA<sup>®</sup> team, all the descriptive terms were evaluated by the 16 pre-selected tasters using a linear, 9 cm non-structured scale, anchored at the extremes by none or weak (to the left) and strong (to the right). Each individual evaluated the seven bittersweet chocolate samples, presented in a monadic way with three repetitions, according to a complete balanced block design (Stone and Sidel 1993). Twelve assessors were selected according to their good discriminative power ( $pF_{\text{sample}} < 0.50$ ), good reproducibility in the judgements ( $pF_{\text{repetitions}} > 0.05$ ) and consensus within the team, according to the recommendations of

Damásio and Costell (1991). Graphs of the mean intensity of the attributes versus samples were drawn for each individual to evaluate consensus within the team.

#### *Sample evaluation*

In the QDA<sup>®</sup> tests, the samples of chocolate were also presented to the assessors in a monadic way (Stone and Sidel 2004) with three repetitions, using the same linear scale.

#### *Acceptance test*

Students and workers of the School of Food Engineering at University of Campinas, SP, Brazil, who liked bittersweet chocolate, were invited to take part in the acceptance test by way of posters and the sending of e-mails. One hundred and twenty consumers, aged between 18 to 60, of which 59% were female, evaluated the samples of bittersweet chocolates with respect to appearance, aroma, flavor, texture and global impression. The samples were presented to the consumers in a sequential monadic way in complete balanced blocks (Wakeling and MacFie 1995). Crackers and taste-free water were provided for palate cleansing.

The acceptance test was determined using a non-structured nine centimeter linear hedonic scale, anchored at the extreme left by disliked immensely, and at the extreme right by liked immensely. The evaluation of consumer perception is more sensitive when using a linear scale than when using a nine-point hedonic scale (Greene et al. 2006).

#### *Statistical analysis*

The results of the QDA<sup>®</sup> and the acceptance test were analyzed by ANOVA using two factors (taster and sample) and the interaction between them, followed by Tukey's test.

The Principal Component Analysis (PCA) with descriptive data and the internal preference map based on the global impression were also carried out.

The descriptive data obtained from the QDA<sup>®</sup> and the consumer preference data were correlated using the Partial Least Squares (PLS) regression. The statistical analyses were carried out using version 9.1.3 of SAS (SAS\_ Institute, Cary, NC) and the XLSTAT for Windows version 2012.5 (Addinsoft, Paris, France) at a 5% significance level.

### *Compliance with Ethics Requirements*

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national).

### *Results and Discussion*

#### *Proximate composition*

Table 2 shows the average values of the proximate composition of all bittersweet chocolates.

There was no significant difference in moisture and ash values. The low-fat samples had a significant difference ( $p > 0.05$ ) in protein, carbohydrate, and lipid values, which was expected since these formulations had half the amount of cocoa butter (7.5%) and higher amount of skimmed milk and inulin.

Regarding the caloric value of the products, the low-fat bittersweet chocolate presented, on average, a reduction of 27.62% of the calories, being considered light bittersweet chocolates, according to the Brazilian legislation, which recommends that a light product should have a minimum reduction of 25% in caloric value when compared to the conventional product (Brasil 1998).

**Table 2** - Chemical composition of the chocolate samples

Samples	Chemical composition (g/100 g)					Calorie (Kcal)
	Moisture	Ash	Protein	Lipid	Carbohydrate	
Sucrose	0.5±0.1 <sup>a</sup>	1.88±0.02 <sup>a</sup>	6.83±0.2 <sup>b</sup>	29.53±0.5 <sup>a</sup>	61.41 <sup>b</sup>	538.73 <sup>a</sup>
Stevia 60% Reb A	0.58±0.02 <sup>a</sup>	1.91±0.1 <sup>a</sup>	6.63±0.3 <sup>b</sup>	30.18±0.35 <sup>a</sup>	60.69 <sup>b</sup>	441.95 <sup>c</sup>
Stevia 80% Reb A	0.44±0.01 <sup>a</sup>	1.9±0.15 <sup>a</sup>	6.76±0.02 <sup>b</sup>	29.52±0.4 <sup>a</sup>	61.37 <sup>b</sup>	439.25 <sup>d</sup>
Stevia 97% Reb A	0.47±0.06 <sup>a</sup>	1.92±0.03 <sup>a</sup>	6.77±0.5 <sup>b</sup>	29.99±0.5 <sup>a</sup>	61.48 <sup>b</sup>	443.96 <sup>b</sup>
Stevia 60% Reb A <sup>a</sup>	0.52±0.15 <sup>a</sup>	2.03±0.05 <sup>a</sup>	7.86±0.1 <sup>a</sup>	22.23±0.6 <sup>b</sup>	67.34 <sup>a</sup>	389.32 <sup>g</sup>
Stevia 80% Reb A <sup>a</sup>	0.44±0.03 <sup>a</sup>	1.95±0.08 <sup>a</sup>	7.83±0.02 <sup>a</sup>	22.15±0.08 <sup>b</sup>	67.58 <sup>a</sup>	389.54 <sup>f</sup>
Stevia 97% Reb A <sup>a</sup>	0.49±0.01 <sup>a</sup>	2.05±0.04 <sup>a</sup>	7.9±0.05 <sup>a</sup>	22.47±0.2 <sup>b</sup>	67.1 <sup>a</sup>	390.78 <sup>e</sup>

<sup>a</sup> Samples with reduced fat contents

\* Means in the same column showing common letter are not significantly different ( $p = 0.05$ )

#### *Quantitative descriptive analysis (QDA<sup>®</sup>)*

Table 3 shows the list of the 20 descriptive terms generated, together with their respective definitions and references.

**Table 3** Descriptive terms used to describe the sensory profile of bittersweet chocolates

Descriptor	Definition	References
<i>Appearance</i>		
Brown color (Brc)	Medium brown to dark brown color (under white light) characteristic of bittersweet chocolates	<i>Weak:</i> Umbrella chocolate (Ki-Kakau <sup>®</sup> ) <i>Strong:</i> Extra dark chocolate 78% (Frey Swiss Premium <sup>®</sup> )
Brightness (Bri)	The degree to which the sample reflects light in one direction	<i>Weak:</i> Umbrella chocolate (Ki-Kakau <sup>®</sup> ) <i>Strong:</i> Chocolate Dairy Dessert (Batavo <sup>®</sup> )
<i>Aroma</i>		
Cocoa aroma (Coa)	Refer to cocoa mass used in sample production	<i>Weak:</i> Milk chocolate bar (Nestlé Classic <sup>®</sup> ) <i>Strong:</i> Cocoa mass (Barry Callebaut <sup>®</sup> )
Cocoa butter aroma (Cba)	Refer to cocoa butter used in sample production	<i>Weak:</i> Milk chocolate bar (Nestlé Classic <sup>®</sup> ) <i>Strong:</i> Deodorized cocoa butter (Barry Callebaut <sup>®</sup> )
Chocolate milk beverage aroma (Cma)	Characteristic aroma from Chocolate milk beverage	<i>Weak:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> ) - 1 part beverage dairy/1 part water <i>Strong:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> )
Sweet aroma (Swa)	Aroma due to the presence of sucrose and other substances	<i>Weak:</i> Extra dark chocolate 78% (Frey Swiss Premium <sup>®</sup> ) <i>Strong:</i> Milk chocolate bar (Nestlé Classic <sup>®</sup> )
Vanilla aroma (Vaa)	Characteristic aroma from vanilla used in sample production	<i>Weak:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> ) - 1 part beverage dairy/1 part water <i>Strong:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> ) with vanilla powder 0.2% (Synergy Aromas <sup>®</sup> )
Roasted aroma (Roa)	Characteristic aroma from roasted cocoa almonds	<i>Weak:</i> bittersweet chocolate 43% (Lacta Amaro <sup>®</sup> ) <i>Strong:</i> Extra dark chocolate 78% (Frey Swiss Premium <sup>®</sup> )
<i>Flavor</i>		
Cocoa flavor (Cof)	Refer to cocoa mass used in sample production	<i>Weak:</i> Milk chocolate bar (Nestlé Classic <sup>®</sup> ) <i>Strong:</i> Extra dark chocolate 78% (Frey Swiss Premium <sup>®</sup> )
Cocoa butter flavor (Cbf)	Refer to cocoa butter used in sample production	<i>Weak:</i> bittersweet chocolate 43% (Lacta Amaro <sup>®</sup> ) <i>Strong:</i> Aired Milk chocolate (Nestlé Suflair Duo <sup>®</sup> )
Sweetness (Swe)	Taste stimulated by the presence of sucrose and other substances, such as sweetener	<i>Weak:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> ) - 1 part beverage dairy/1 part water <i>Strong:</i> Baton milk chocolate (Garoto <sup>®</sup> )
Sweet aftertaste (Saf)	Sweet sensation perceived at the back of the throat after swallowing	<i>None:</i> Water <i>Strong:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> ) with aspartame (0.06%) (All

Chemistry do Brasil<sup>®</sup>)

Bitterness (Bit)	Characteristic taste from caffeine	<i>Weak:</i> Bittersweet chocolate 43% (Lacta Amaro <sup>®</sup> ) <i>Strong:</i> Extra dark chocolate 78% (Frey Swiss Premium <sup>®</sup> )
Bitter aftertaste (Baf)	Bitter sensation perceived at the back of the throat after swallowing	<i>Weak:</i> Bittersweet chocolate 43% (Lacta Amaro <sup>®</sup> ) <i>Strong:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> ) with stevia with 40% de rebaudioside A (0.07%) (Steviafarma Industrial <sup>®</sup> )
Vanilla flavor (Vaf)	Characteristic flavor from artificial vanilla flavor used in sample production	<i>Weak:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> ) - 1 part beverage dairy/5 part water <i>Strong:</i> Dairy beverage chocolate flavor (Shefa <sup>®</sup> ) with vanilla powder 0.1% (Synergy Aromas <sup>®</sup> )
Astringency (Ast)	Harsh sensation perceived in mouth and tongue, characteristic of polyphenols found in bittersweet chocolates	<i>Weak:</i> Milk chocolate bar (Nestlé Classic <sup>®</sup> ) <i>Strong:</i> Mixture of cocoa mass with cocoa butter (Barry Callebaut <sup>®</sup> ) (2:1) melted in the microwave
<i>Texture</i>		
Hardness (Har)	The force required to cut using central incisor teeth	<i>Weak:</i> Aired Milk chocolate (Nestlé Suflair Duo <sup>®</sup> ) <i>Strong:</i> Cocoa mass (Barry Callebaut <sup>®</sup> )
Melting rate (Mer)	Amount of time required for solid chocolate turn into liquid while moving the tongue	<i>Weak:</i> Umbrella chocolate (Ki-Kakau <sup>®</sup> ) <i>Strong:</i> Aired Milk chocolate (Nestlé Suflair Duo <sup>®</sup> )
Sandiness (San)	Amount of realizable particles while chewing	<i>Weak:</i> Milk chocolate bar (Nestlé Classic <sup>®</sup> ) <i>Strong:</i> Milk caramel bar (Embaré <sup>®</sup> )
Adherence (Adh)	The degree to which the sample sticks on molar teeth	<i>Weak:</i> Refrigerated Polenguinho UHT processed cheese (Pocket <sup>®</sup> ) <i>Strong:</i> Umbrella chocolate (Ki-Kakau <sup>®</sup> )

Table 4 shows the results obtained for each sample with respect to the terms generated by the assessors.

**Table 4** Attribute means for each sample of bittersweet chocolate

	Sucrose	Stevia 60% Reb A	Stevia 80% Reb A	Stevia 97% Reb A	Stevia 60% Reb A <sup>a</sup>	Stevia 80% Reb A <sup>a</sup>	Stevia 97% Reb A <sup>a</sup>
Brown color (Brc)	5.92 <sup>a</sup>	5.88 <sup>a</sup>	5.79 <sup>a</sup>	5.94 <sup>a</sup>	5.85 <sup>a</sup>	5.9 <sup>a</sup>	5.86 <sup>a</sup>
Brightness (Bri)	4.33 <sup>ab</sup>	4.43 <sup>ab</sup>	4.55 <sup>ab</sup>	4.14 <sup>b</sup>	4.29 <sup>ab</sup>	4.69 <sup>a</sup>	4.6 <sup>ab</sup>
Cocoa aroma (Coa)	6.13 <sup>a</sup>	5.86 <sup>a</sup>	5.73 <sup>a</sup>	5.96 <sup>a</sup>	6.19 <sup>a</sup>	6.31 <sup>a</sup>	6.18 <sup>a</sup>
Cocoa butter aroma (Cba)	4.32 <sup>ab</sup>	4.45 <sup>ab</sup>	4.55 <sup>a</sup>	4.39 <sup>ab</sup>	4.17 <sup>ab</sup>	4.24 <sup>ab</sup>	3.97 <sup>b</sup>
Sweet aroma (Swa)	3.6 <sup>ab</sup>	3.66 <sup>ab</sup>	4.04 <sup>a</sup>	3.81 <sup>ab</sup>	3.33 <sup>ab</sup>	3.22 <sup>b</sup>	3.28 <sup>ab</sup>
Roasted aroma (Roa)	4.59 <sup>a</sup>	4.44 <sup>a</sup>	4.09 <sup>a</sup>	4.69 <sup>a</sup>	4.71 <sup>a</sup>	4.71 <sup>a</sup>	4.51 <sup>a</sup>
Vanilla aroma (Vaa)	1.16 <sup>a</sup>	1.04 <sup>a</sup>	1.05 <sup>a</sup>	1.08 <sup>a</sup>	1.12 <sup>a</sup>	1.09 <sup>a</sup>	1.09 <sup>a</sup>
Cocoa flavor (Cof)	5.45 <sup>c</sup>	5.96 <sup>bc</sup>	6.07 <sup>ab</sup>	6.01 <sup>abc</sup>	6.5 <sup>a</sup>	6.42 <sup>ab</sup>	6.5 <sup>a</sup>
Cocoa butter flavor (Cbf)	3.67 <sup>a</sup>	3.53 <sup>a</sup>	3.2 <sup>ab</sup>	3.67 <sup>a</sup>	2.87 <sup>b</sup>	2.88 <sup>b</sup>	2.75 <sup>b</sup>
Sweetness (Swe)	4.89 <sup>a</sup>	4.84 <sup>a</sup>	4.87 <sup>a</sup>	4.73 <sup>a</sup>	3.86 <sup>b</sup>	4.25 <sup>ab</sup>	3.81 <sup>b</sup>
Sweet aftertaste (Saf)	3.27 <sup>b</sup>	4.69 <sup>a</sup>	4.08 <sup>ab</sup>	4.16 <sup>ab</sup>	3.68 <sup>ab</sup>	4.03 <sup>ab</sup>	3.74 <sup>ab</sup>
Bitterness (Bit)	3.66 <sup>c</sup>	4.07 <sup>bc</sup>	4.63 <sup>b</sup>	4.41 <sup>bc</sup>	5.77 <sup>a</sup>	5.51 <sup>a</sup>	5.69 <sup>a</sup>
Bitter aftertaste (Baf)	1.94 <sup>c</sup>	3.28 <sup>ab</sup>	2.71 <sup>bc</sup>	3.13 <sup>ab</sup>	3.6 <sup>ab</sup>	3.81 <sup>a</sup>	3.86 <sup>a</sup>
Vanilla flavor (Vaf)	1.31 <sup>a</sup>	1.1 <sup>abc</sup>	0.99 <sup>abc</sup>	1.15 <sup>ab</sup>	0.92 <sup>bc</sup>	0.87 <sup>bc</sup>	0.78 <sup>c</sup>
Astringency (Ast)	2.57 <sup>c</sup>	3.46 <sup>b</sup>	3.35 <sup>b</sup>	3.19 <sup>b</sup>	4.19 <sup>a</sup>	4.42 <sup>a</sup>	4.22 <sup>a</sup>
Hardness (Har)	4.65 <sup>b</sup>	4.65 <sup>b</sup>	4.7 <sup>b</sup>	4.75 <sup>b</sup>	6.01 <sup>a</sup>	5.94 <sup>a</sup>	5.84 <sup>a</sup>
Melting rate (Mer)	4.7 <sup>a</sup>	4.44 <sup>a</sup>	4.52 <sup>a</sup>	4.6 <sup>a</sup>	3.32 <sup>b</sup>	3.18 <sup>b</sup>	3.45 <sup>b</sup>
Sandiness (San)	1.3 <sup>b</sup>	1.43 <sup>b</sup>	1.38 <sup>b</sup>	1.47 <sup>b</sup>	3.92 <sup>a</sup>	4.06 <sup>a</sup>	3.89 <sup>a</sup>
Adherence (Adh)	3.38 <sup>ab</sup>	3.56 <sup>ab</sup>	3.77 <sup>a</sup>	3.3 <sup>ab</sup>	3.24 <sup>ab</sup>	3.17 <sup>ab</sup>	3.01 <sup>b</sup>

\* Means in the same line showing common letter are not significantly different (P = 0.05)

<sup>a</sup> Samples with reduced fat contents

With respect to brown color, there was no significant difference ( $p > 0.05$ ) between the samples, since all contained the same content of cocoa mass. In addition, the six samples with no added sugar did not differ significantly ( $p > 0.05$ ) from the sample with added sugar, with respect to brightness, and hence the reduction in fat content of three samples did not alter this

important attribute of the chocolate. Melo et al. (2009) also found no significant differences in the attribute of brightness between samples of milk chocolate which compared to samples with reduced fat contents. The fact that the appearances of the chocolates with no added sugar and with reduced fat contents were very similar to those with added sugar was very relevant for the development of the product, since the consumer's first contact with a food is by way of his/her vision, and the simple vision of a food can generate the desire to consume it (Marcelino et al. 2001).

None of the samples with no added sugar presented significant differences from the control sample added sugar with respect to the attribute of aroma. This was probably due to the fact that the predominant aroma of all the samples was that of the cocoa, and all the samples contained the same amount of cocoa. The accentuated aroma of cocoa can be masked by other aromas, making differentiation by the assessors difficult. The amount of vanilla aroma added to the samples was also the same. In addition, the presence of sucrose is not considered to be a factor that influences the formation of chocolate aroma. Beckett (2009) affirmed that the sugar is considered to be an inert chocolate ingredient, only contributing to the sweetness.

With respect to cocoa flavor and cocoa butter flavor, significant difference ( $p \leq 0.05$ ) was found among the samples with reduced fat content and the control sample. Of the three samples, the lowest mean awarded for the attribute of cocoa butter flavor was scored by the sample with the smallest content of this ingredient in the formulation.

On evaluating the means of the attributes of sweet taste, sweet aftertaste, bitter taste and bitter aftertaste of the three samples with no added sugar (Stevia 60% Reb A, Stevia 80% Reb A, Stevia 97% Reb A), it was found that the rebaudioside A content did not affect the perception of the assessors with respect to these characteristics. This also occurred with respect to the perception of these attributes in the three samples with reduced fat contents. Rebaudioside A is the glycoside which confers the sweet taste on stevia, whilst stevioside is responsible for the bitter taste (Wang et al. 2016). The greater the rebaudioside content the lesser the stevioside content, hence differences were expected in the means for the attributes of sweetness and bitterness among the bittersweet chocolate samples sweetened with stevia samples containing different rebaudioside contents. However, this behavior of the glycosides occurs for the pure stevia, but the food matrix can influence these characteristics. Probably the other ingredients in the sample formulations, for instance the liquor, had a predominant effect

on the flavor and masked possible differences among the different stevia samples. Melo et al. (2009) studied the sensory profiles of milk chocolates formulated with the addition of stevia and whey protein as a partial fat substitute. In this study they also found no differences between the samples for the attributes of sweet taste, sweet aftertaste, bitter taste and bitter aftertaste, demonstrating that it is possible to reduce the fat content of the chocolate and maintain the perception of these tastes. Although the rebaudioside A contents of the stevia samples did not interfere with the perception of these attributes, it could be seen that the means for bitter taste and bitter aftertaste of the three samples without sugar and with a reduction in fat, differed significantly in relation to the conventional sample. The bitterness of chocolate is caused mainly by the caffeine and theobromine, compounds present in the cocoa itself. Low sugar/ low fat samples are clearly perceived as bitter, since the sugar decreases the perception of bitterness and low fat samples tend to be bitterer since the fat has the ability to coat the bitter compounds (Guinard and Mazzucchelli 1999).

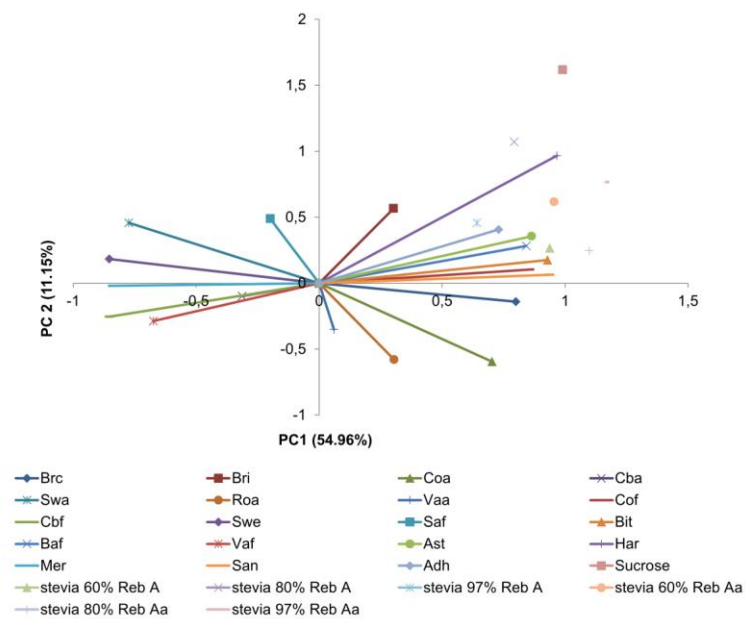
The astringency was affected in all samples with no added sugar, which differed significantly from the sample with added sugar. The vanilla flavor, for its part, was less intense in the low fat samples. Although the vanilla contents were the same in all seven formulations, the reduction in fat content could have prejudiced the palatability of these three samples.

It was also verified that the three samples with no added sugar and with reduced fat content showed the greatest variations in relation to texture, differing significantly from the conventional sample with respect to the attributes of hardness, mouth melting and sandiness. The cocoa butter is very important in the sensory profile of the chocolates, being the main component responsible for product texture (Efraim et al. 2013). On the other hand the three samples with no added sugar but without fat reduction (Stevia 60% Reb A; Stevia 80% Reb A; Stevia 97% Reb A) did not show significant differences from the conventional sample with respect to the attributes of hardness, mouth melting and sandiness, showing that the use of inulin as a thickening agent was efficient. The complete substitution of the sugar of chocolate represents a challenge, because it affects their rheological properties and texture. Inulin could be a good alternative to substitute the sugar of chocolate and maintain its sensory characteristics (Shourideh et al. 2012).

The PCA (Fig. 1) was carried out and the first and second principal components described 66.11% of the variation between the samples. The PCA suggested that the chocolate



samples produced without sugar were similar to each other, including those with a reduced fat content, since they were all located in the same quadrant and very close to each other, and were characterized by the same attributes adherence, astringency, bitter taste and bitter aftertaste. The sample with added sugar, although distant from the other samples, was located in the same quadrant and characterized by the same vectors, indicating that all the samples had similar sensory profiles.



**Fig. 1** Principal component analysis (PCA) loadings for descriptors and chocolate samples

### *Acceptance test*

Table 5 shows the results for the acceptance test, applied to 120 consumers.

**Table 5** Attribute means for each sample of bittersweet chocolate

	Sucrose	Stevia 60% Reb A	Stevia 80% Reb A	Stevia 97% Reb A	Stevia 60% Reb A <sup>a</sup>	Stevia 80% Reb A <sup>a</sup>	Stevia 97% Reb A <sup>a</sup>
Appearance	7.13 <sup>a</sup>	7.3 <sup>a</sup>	7.22 <sup>a</sup>	7.17 <sup>a</sup>	7.27 <sup>a</sup>	7.19 <sup>a</sup>	7.31 <sup>a</sup>
Aroma	6.92 <sup>ab</sup>	6.56 <sup>b</sup>	6.92 <sup>ab</sup>	6.74 <sup>ab</sup>	6.7 <sup>ab</sup>	7.1 <sup>a</sup>	7.01 <sup>ab</sup>
Flavor	6.72 <sup>a</sup>	5.53 <sup>b</sup>	5.87 <sup>b</sup>	6.71 <sup>a</sup>	6.60 <sup>a</sup>	7.12 <sup>a</sup>	6.81 <sup>a</sup>
Texture	6.58 <sup>a</sup>	4.51 <sup>b</sup>	7 <sup>a</sup>	4.52 <sup>b</sup>	5.04 <sup>b</sup>	6.96 <sup>a</sup>	6.77 <sup>a</sup>
Overall impression	6.79 <sup>a</sup>	5.44 <sup>b</sup>	5.56 <sup>b</sup>	6.99 <sup>a</sup>	6.67 <sup>a</sup>	7.29 <sup>a</sup>	6.95 <sup>a</sup>

\*Means in the same line showing the same common letter are not significantly different (P = 0.05)

<sup>a</sup>Samples with reduced fat contents

There was no significant difference ( $p > 0.05$ ) among the seven bittersweet chocolate samples with respect to appearance. Appearance is a very important attribute since it provides the consumer expectation with respect to the quality of the taste and palatability of the food, which can affect consumer acceptance of the product (Hurling and Shepherd 2003). In addition there was no significant difference ( $p > 0.05$ ) between the six diet samples and the conventional one with respect to aroma.

An analysis of the table also allows one to conclude that the most samples did not present significant differences ( $p > 0.05$ ) from the conventional sample with respect to taste.

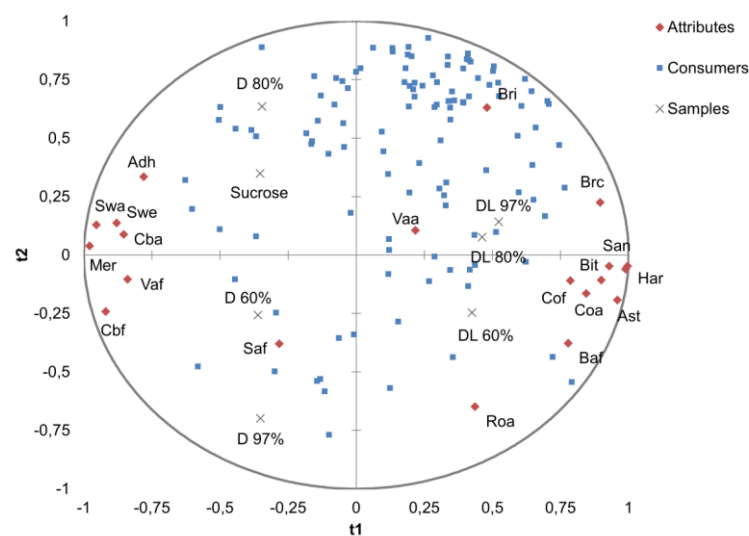
The results of the QDA<sup>®</sup> showed that the reduction in fat content caused a greater influence on the texture. This was not perceived by the consumers, who were untrained individuals, and in fact, two of the three samples with reduced fat content (Stevia with 80% Reb A<sup>a</sup>; Stevia with 97% Reb A<sup>a</sup>) did not present any significant difference from the conventional sample in relation to texture. Trained individuals may be capable of detecting even small differences between samples, whereas consumers normally cannot (Hough 1998).

With respect to global impression, it could be seen mainly that samples with no added sugar and with reduced fat were well accepted, being statistically the same as the conventional sample, showing that the removal of sugar from the formulation and reduction in the fat content did not prejudice consumer acceptance of these samples. According to the QDA<sup>®</sup> data, there was a difference in texture between the diet samples and the diet samples with fat reduction, but since this difference was not perceived by the consumers, it did not affect the global impression. Also according to the QDA<sup>®</sup>, the samples with fat reduction were bitterer, which could have prejudiced acceptance. However, since only individuals who liked

bittersweet chocolate were invited to take part in the acceptance test, the bitterness of these products was not an attribute that could prejudice the acceptance, and could even contribute to it. Rezende et al. (2015) evaluated the acceptance of diet chocolate with partial substitution of the cocoa butter by inulin and found acceptance similar to that of the standard chocolate (with added sucrose).

Figure 2 shows the relationship between the data for global impression and the QDA<sup>®</sup> data using PLS regression. PLS may be useful to guide in the selection of a subset of relevant attributes from the complete set of attributes, and the number of significant components to be evaluated is usually determined by a cross-validation procedure (Rossini et al. 2012). It can be seen that the majority of the consumers are located in the positive region of the y axis. The four samples closest to the consumers were the conventional sample, stevia with 80% Reb A and mainly stevia with 60, 80 and 97% Reb A<sup>a</sup>, indicating these as the most accepted samples.

This information allows one to conclude that the removal of sugar and reduction in fat from the formulation were well accepted by the consumers. It is interesting to note that the attribute of bitterness was close to the samples with reduced fat, which could have contributed to the good acceptance of these chocolates by pleasing the public which likes bittersweet chocolate. The attributes of brightness and vanilla aroma were positively correlated with the global impression.



**Fig. 2** Relation of acceptance (overall liking, OAL) to QDA data and the positions of chocolate samples, using PLS regression

### *Conclusions*

The results for the QDA<sup>®</sup> showed that the different contents of rebaudioside A tested did not interfere with the attributes of sweet taste, sweet aftertaste, bitter taste or bitter aftertaste. In addition the bitterness was more accentuated in the samples with reduced fat content.

With respect to texture, the samples with reduced fat content showed greater modifications when compared to the conventional sample, but these modifications were not detected by the consumers in the acceptance test.

Although some differences were detected among the samples in the descriptive test, in general, the acceptance test and the external map of preference demonstrated that all the samples showed good sensory acceptance, mainly those with a reduced fat content. Hence it is possible to develop bittersweet chocolates with no added sugar and with the addition of prebiotic fiber to substitute the sugar and fat.

The results of this study could be applied by the food industry in the development of a bittersweet chocolate sweetened with a natural sweetener, with inulin and with reduced calorie content (light). In addition the fact that the rebaudioside A did not affect the sensory characteristics of the samples, makes it possible for the food industry to use stevia with the smallest content of this glycoside, and therefore cheaper.

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### *Compliance with Ethics Requirements*

#### *Conflict of Interest*

The authors declare that they have no conflict of interest.

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**ARTIGO 3 – Artigo publicado no International Journal of Food Science and Technology.**

*“Bittersweet chocolates containing prebiotic and sweetened with stevia (Stevia rebaudiana Bertoni) with different Rebaudioside A contents: Multiple time-intensity analysis and physicochemical characteristics”*

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**Bittersweet chocolates containing prebiotic and sweetened with stevia (*Stevia rebaudiana* Bertoni) with different Rebaudioside A contents: Multiple time-intensity analysis and physicochemical characteristics**

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**Summary**

The objective of this study was to evaluate the time-intensity profile of the sensory attributes possibly affected in sugar-free and low-fat chocolates containing inulin and stevia with different Rebaudioside A contents, such as sweetness, bitterness, and melting rate. The bittersweet chocolates were analyzed by the multiple time-intensity analysis. The time intensity profile for the sweetness stimulus was similar for all chocolate samples. The differences between the contents of rebaudioside A were not perceived by the assessors. In relation to the bitterness stimulus, the low-fat samples had a more accentuated perception of this attribute by the assessors, with significant differences for I<sub>max</sub> and Area when compared to the sugar-free samples. The stimulus melting in the mouth was more affected in the low-fat samples. The sensory results obtained in this study are useful for food industry and researchers working with sweeteners and prebiotics in food, especially in chocolate.

**Keywords:** chocolate; prebiotics; sensory analysis; Multiple time-intensity analysis; glycosides.

## Introduction

In recent years, there is a growing demand for natural products and foods with nutritionally balanced profile and healthy ingredients. Health and physical fitness, along with cultural and ethical concerns, has led to new food choices (Falguera *et al.*, 2012). Therefore, researchers have been focused on reformulating food products traditionally rich in saturated fat and sugar, by the replacement with natural ingredients with positive physiological effects, including natural sweeteners and prebiotic compounds. Several studies have changed the traditional bar chocolate formulations into healthier alternatives, while maintaining the sensory characteristics (Morais-Ferreira *et al.*, 2016; Azevedo *et al.*, 2016; Saputro *et al.*, 2017).

*Stevia rebaudiana* is a sweetener that has gained a lot of attention, being the only natural non-nutritive sweetener approved in Europe (Philippe *et al.*, 2014). In addition, *Stevia rebaudiana* Bertoni leaves have high concentrations of steviol glycosides, including stevioside and rebaudioside A. Despite its commercial use, the stevioside has residual bitter taste, which limits its applications in the food industry. The number of carbohydrate units at positions C-13 and C-19 determines the degree of the component's sweetness. Rebaudioside A has an extra unit of glucose at position C-13 when compared to stevioside, presenting greater properties in terms of sweetness and flavor profile, thus becoming a potential component in stevia (Zhang & Li, 2013). The higher commercial demand has led to the need to increase the Rebaudioside A content of stevia leaves, which has been the target of many studies (Zhang *et al.*, 2015; Yadav *et al.*, 2014; Rajasekaran *et al.*, 2007; Ramesh *et al.*, 2006).

Sugar replacement for a high-intensity sweetener, such as stevia, can cause serious changes in chocolate manufacturing. Therefore, a technological alternative to solve this problem may be the addition of fibers, such as inulin. The incorporation of inulin into food products presents numerous technological advantages, including texturizing, thickening, gelling, and emulsifying properties, besides being sugar and fat substitutes (Shourideh *et al.*, 2012). In addition, the consumption of inulin is associated with health benefits, such as inhibition of colon cancer, immunomodulatory effects, reduction in the risk of cardiovascular diseases (Barclay *et al.*, 2010), relief in constipation (Shoaib *et al.*, 2016), and increase in mineral absorption (Al-Sheraji *et al.*, 2013), among others.

Sensory perception during food consumption is a dynamic phenomenon (Cliff & Heymann, 1993). The time-intensity (TI) analysis is a methodology widely used to obtain the

intensity variations of a specific attribute over time and has been successfully applied to several matrices, including chocolate (Morais-Ferreira *et al.*, 2016), French fries (Luckett *et al.*, 2016), beverages (Sokolowsky *et al.*, 2015), chocolate milk containing chia oil (Rodrigues *et al.*, 2015), and meat products (Lorido *et al.*, 2014). Multiple time-intensity analysis (MTIA) is a way to graphically represent simultaneously the dynamic profiles of two or more sensory attributes of a single sample (Morais *et al.*, 2014). The main objective of this study was to evaluate the time-intensity profile of the sensory attributes possibly affected in sugar-free and low-fat chocolates containing inulin and stevia with different Rebaudioside A contents.

## **Material and methods**

### **Material**

The following ingredients were used to prepare the bittersweet chocolate formulations: stevia extract with different rebaudioside A (Reb A) contents (60, 80, and 97%) (Steviafarma Industrial, Maringá, Brazil); Orafit GR<sup>®</sup> inulin (Beneo, Mannheim, Germany); maltitol (Sweet Pearl<sup>®</sup> P90 Roquette, Roquette Freres, France); icing sugar (Mais Doce, Limeira, Brazil); cocoa mass (Barry Callebaut, Extrema, Brazil); deodorized cocoa butter (Barry Callebaut, Extrema, Brazil); skimmed milk powder (Embaré, Lagoa da Prata, Brazil); Solec CH soy lecithin (Solae, Esteio, Brazil); Grinsted<sup>®</sup> - PGPR 90 polyglycerol polyricinoleate (Danisco, Cotia, Brazil); and artificial vanilla flavor (Synergy Aromas, Vinhedo, Brazil).

### **Methods**

#### *Bittersweet chocolate manufacturing*

All samples were produced in the Cereal and Chocolate Technology Center (CEREAL CHOCOTEC) of the Food Technology Institute (ITAL) located in Campinas, SP, Brazil.

The chocolates were conventionally produced. The ingredients were homogenized in a Kitchen Aid model K5SS planetary mixer (Kitchen-Aid, St. Joseph, MI, USA) and subsequently refined in a Draiswerk GMBH three-roll refiner (Draiswerke GmbH, Manheim Waldo, Mahweh, NJ, USA). After the conching step (65 °C/ 16h) in an Inco longitudinal conch (Inco, Avaré, SP, Brazil), the samples were tempered on a marble table. The chocolates were placed in polycarbonate molds with bar shape, and subjected to vibration on a Jafinox (BR) vibratory table. The molds were then passed twice through a cooling tunnel (Siaht,

Jundiaí, SP, Brazil). The samples were wrapped in aluminum foil and stored for fifteen days at 20°C in a BOD incubator (Novatecnica, BR, Brazil).

Seven formulations were prepared (Table 1), as follows: a conventional chocolate (containing sugar), 3 stevia-sweetened chocolates (using stevia with 60, 80, and 97% rebaudioside A), and 3 stevia-sweetened chocolates with a reduction of 50% cocoa butter. A previous study was carried out to determine the equivalent sweetness of each sweetener as compared with sucrose (Azevedo *et al.*, 2016).

According to the Brazilian legislation (Brasil, 2016), the amount of inulin in the chocolate samples of this study was sufficient to guarantee the prebiotic activity of this fiber.

**Table 1** Formulation of bittersweet chocolates

Ingredients (%)	Chocolate samples						
	Sucrose	Stevia 60% Reb A	Stevia 80% Reb A	Stevia 97% Reb A	Stevia 60% Reb A*	Stevia 80% Reb A*	Stevia 97% Reb A*
Icing sugar	47.5	0	0	0	0	0	0
Cocoa mass	30	30	30	30	30	30	30
Cocoa butter	15	15	15	15	7.5	7.5	7.5
Maltitol	0	32.34	32.34	32.34	32.34	32.34	32.34
Inulin	0	15	15	15	20	20	20
Skimmed milk powder	6.9	6.9	6.9	6.9	9.4	9.4	9.4
Soy lecithin	0.3	0.3	0.3	0.3	0.3	0.3	0.3
PGPR	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vanilla flavor	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Stevia	0	0.16	0.16	0.16	0.16	0.16	0.16

\*Low-fat samples.

### Sensory evaluation

Chocolate samples (4 g) were presented to the tasters in monadic form with 3 replicates in disposable plastic cups encoded with three digit numbers (Meilgaard *et al.*, 1999), using a complete balanced block design (Stone *et al.*, 2012). Crackers and taste-free water were provided for palate cleansing.

The sensory tests were performed in individually air-conditioned booths (22 °C) in the Sensory Science and Consumer Studies Laboratory of the Food Engineering Faculty (FEA) at University of Campinas (UNICAMP), Brazil. All computers were equipped with the software Time-Intensity Analysis of Food and Tastes (TIAFT) (Unicamp, 2012).

### *Time-Intensity analysis*

The time-intensity analysis was performed for the attributes sweetness, bitterness, and melting rate, which can be directly affected in sugar-free low-fat chocolates.

The individuals were recruited among undergraduate and graduate students and staff from the University of Campinas, bittersweet chocolate lovers interested in participating in the study. Only individuals with the ability to work with computers were selected at this stage.

Triangle tests were applied in the pre-selection of the tasters, using the Wald sequential analysis (Meilgaard *et al.*, 1999). Two commercial samples of bittersweet chocolate with different cocoa contents, with a significant difference of 0.1% previously determined in a paired test were used. The variable values used in Wald's sequential analysis were, as follows:  $r_0 = 0.45$ ,  $r_1 = 0.70$ ,  $a = 0.05$  and  $b = 0.05$ . Seventeen individuals were pre-selected in this step.

### *Training and selection of the time-intensity analysis team*

The seventeen pre-selected individuals were trained for the attributes sweetness, bitterness, and melting rate, and the references are shown in Table 2. The training aimed to form the sensory memory on the maximum perception for each stimulus and consisted in the direct contact of the individuals with the reference of maximum intensity occurring for each stimulus, during three sessions of one hour each.

The conditions for the time-intensity analysis were standardized as follows: (1) judge's wait time of 10 s; (2) time with the sample in the mouth of 15 s; (3) time after swallowing of 90 s; and (4) 9 intensity scales. The scale used for performing the tests was linear and horizontal from 0 to 9, in which 0 corresponded to none (far left), 4.5 corresponded to moderate (middle), and 9 corresponded to strong (far right) (Palazzo & Bolini, 2014).

To perform the analysis, after hearing the signal given by the computer (10 s), the tasters were instructed to place the entire sample (4 g) in the mouth and indicate the intensity of perception according to time, by sliding the mouse over the scale.

At the end of the test, the following parameters were collected and analyzed statistically: maximum intensity (**I<sub>max</sub>**, the maximum sweetness intensity of each sample), time for maximum intensity (**T<sub>I<sub>max</sub></sub>**, the maximum intensity time), Area under the curve

(**Area**, total area under the time-intensity curve), total time (**T<sub>tot</sub>**, time for the perception of sweetness from the first to the last perception).

For the selection of the final team, each taster evaluated the stimulus separately, and twelve assessors of the seventeen pre-selected individuals were chosen to participate according to their discriminative ability ( $P < 0.30$ ), repeatability ( $P > 0.05$ ), and group consensus (Damasio & Costell, 1991).

**Table 2** Definition and references of the stimuli for bittersweet chocolates evaluated by the assessors

<b>Attributes</b>	<b>Definition</b>	<b>Reference</b>
Sweetness	Taste stimulated by the presence of sucrose and other compounds, such as sweetener	Bittersweet chocolate containing 49% sucrose (produced in CEREAL CHOCOTEC of ITAL)
Bitterness	Characteristic taste of caffeine	Bittersweet chocolate 43% (Lacta Amaro <sup>®</sup> )
Melting rate	Time required for solid chocolate turn into liquid while moving the tongue	Aired Milk chocolate (Nestlé Suflair Duo <sup>®</sup> )

#### *Multiple time-intensity analysis*

Twelve selected assessors (7 female, 5 male) evaluated all attributes (sweetness, bitterness, and melting in the mouth) of the monadic samples, according to a complete block design (Wakeling & MacFie, 1995), with three repetitions. The attributes were evaluated separately.

#### *Physicochemical characterization*

##### *Texture profile*

The texture profile of all chocolate samples was performed at the Central Laboratory of the Department of Food and Nutrition (Unicamp / FEA). Samples were evaluated in five replicates. A Texture Analyzer TA-XT2, Stable Micro Systems, with the software Texture Expert for Windows was used, which measured the compression force of all samples. The probe comprised two metallic, parallel bars and a third bar coupled to the machine arm, which descended vertically in predetermined speeds, causing sample fracture. The test conditions were: pre-test speed: 3.0 mm/s; test speed: 1.7 mm/s; post-test speed: 10.0 mm/s; rupture test distance: 5.0 mm; distance: 20.0 mm; force: 0.981 N; and time: 5.0 seconds.

The mean hardness values were expressed as compressive force (g), and the standard deviations were calculated.

#### *Color measurements*

The color of the bittersweet chocolates was determined according to the CIELab system. The parameters L \* (brightness), a \* (redness) and b \* (yellowness) were measured in a spectrophotometer (Color Quest II model, Hunter Associates Laboratory, Reston, VA, USA), adjusted with the following parameters: 10° angle, illuminant D65 and calibration mode RSIN (Minolta, 1994).

#### *Statistical analysis*

The parameters collected by the time-intensity curves for each attribute were evaluated by ANOVA and Tukey's test ( $P > 0.05$ ). Time-intensity curves were constructed in Microsoft Excel 2010 using the parameters collected on the computer.

The results of the physicochemical analyses were analyzed by univariate statistical analysis (ANOVA), and the means were compared by Tukey's test (at 5% significance). A completely randomized experimental design was used.

The software Statistical Analysis System was used (SAS, 2012).

## **Results and Discussion**

#### *Time-intensity analysis for sweetness stimulus*

Sweetness was the first attribute evaluated by the 12 assessors previously selected and trained. Table 3 shows the mean values of the four parameters evaluated in relation to sweetness, using the time-intensity analysis. No significant differences ( $P > 0.05$ ) were observed in the sweetness time profile of the sugar-free bittersweet chocolates when compared to the sugar-sweetened chocolate. Palazzo and Bolini (2014) evaluated sweetness of milk chocolate formulated with different sweeteners, using the time-intensity analysis, and found significant differences between the samples.

Rebaudioside A is responsible for the sweet taste of stevia (Wang *et al.*, 2016). Therefore, a higher sweetness intensity and longer duration of the stimulus was expected for the samples sweetened with stevia containing 97% rebaudioside A, which was not observed in



this study. This finding may be due to the differences between the contents of this glycoside were not perceived by the assessors, once the food matrix has a strong and characteristic flavor. Bittersweet chocolate has a predominant flavor of cocoa liquor, with hints of nuts and caramel (Liu *et al.*, 2015). Dutra and Bolini (2013) have also found no differences in the sweetness profile of acerola juice sweetened with stevia containing different rebaudioside A levels, probably due to the high acidity of that juice, which diminished the perception of sweetness.

The results in Table 3 demonstrate that the fat reduction in the samples (Stevia 60% Reb A\*, Stevia 80% Reb A\*, Stevia 97% Reb A\*) did not affect the perception of sweetness by the assessors, which was also observed in a study with low-fat ice cream (Cadena *et al.*, 2012). According to Wiet *et al.* (1993), fat reduction may cause a slight increase in sweetening power of the sweeteners used to replace sucrose. However, according to those authors, small differences in the perception of sweetness as a function of fat content are dependent on the food matrix, thus it cannot be generalized.

**Table 3** Time-intensity analysis for sweetness stimulus

<b>Samples</b>	<b>TImax</b>	<b>Imax</b>	<b>Area</b>	<b>Ttot</b>
Sucrose	22.1 <sup>a</sup> ±1.23	6.61 <sup>a</sup> ±0.89	206.01 <sup>ab</sup> ±8.21	56.28 <sup>a</sup> ±3.13
Stevia 60% Reb A	21.8 <sup>a</sup> ±2.17	6.26 <sup>ab</sup> ±0.95	212.87 <sup>a</sup> ±11.12	62.6 <sup>a</sup> ±3.54
Stevia 80% Reb A	22.49 <sup>a</sup> ±2.82	5.86 <sup>ab</sup> ±1.11	176.47 <sup>ab</sup> ±7.53	60.59 <sup>a</sup> ±4.17
Stevia 97% Reb A	20.96 <sup>a</sup> ±1.86	6.49 <sup>a</sup> ±1.01	202.3 <sup>ab</sup> ±10.67	60.09 <sup>a</sup> ±3.22
Stevia 60% Reb A*	21.45 <sup>a</sup> ±1.55	5.81 <sup>ab</sup> ±0.79	171.05 <sup>b</sup> ±7.22	57.2 <sup>a</sup> ±2.96
Stevia 80% Reb A*	21.58 <sup>a</sup> ±2.34	6.28 <sup>ab</sup> ±1.22	197.41 <sup>ab</sup> ±8.19	58.67 <sup>a</sup> ±3.1
Stevia 97% Reb A*	21.96 <sup>a</sup> ±2.41	5.87 <sup>ab</sup> ±1.08	193.33 <sup>ab</sup> ±9.25	59.64 <sup>a</sup> ±2.79

Means with the same superscript letter are not significantly different at a 5% level.

\* Low-fat samples.

#### *Time-intensity analysis for bitterness stimulus*

Bitterness was the second attribute evaluated by the assessors. The polyphenolic compounds from cocoa confer bitter and astringent characteristics on bittersweet chocolates (Harwood *et al.*, 2013). Therefore, bitterness was expected in all samples due to the presence of cocoa liquor to the formulations, with a higher intensity in the samples sweetened with stevia containing lower rebaudioside A levels which contain higher stevioside levels, responsible for the bitter taste (Torri *et al.*, 2016).

As shown in Table 4, there was no significant difference ( $P > 0.05$ ) among the seven samples for the TImax parameter. In contrast, a significant difference was observed in the Imax and Area among the sugar-free samples (Stevia 60, 80, and 97% Reb A) and the sugar-free low-fat samples (Stevia 60, 80, and 97% Reb A\*). According to Guinard and Mazzucchelli (1999), low-fat chocolates tend to be more bitter, since fat has the ability to mask the bitter compounds. In the present study, bitterness increased with fat reduction.

A significant difference in the total duration of the stimulus was observed for all stevia-sweetened samples when compared to the sugar-sweetened sample, due to the residual bitterness characteristic of stevia (Chranioti *et al.*, 2016). Morais *et al.* (2014) studied analyze the TI profile in chocolate dairy dessert in the traditional version and with prebiotic and light version (sweetened with different sweeteners) and didn't observe changes in the total duration of the bitterness for stevia-sweetened sample when compared to the sugar-sweetened sample.

Again, there was no significant difference ( $P > 0.05$ ) in the temporal perception of bitterness between stevia with different levels of rebaudioside A. Bittersweet chocolate is a product with characteristic bitterness, which may have affected the perception by the assessors.

**Table 4** Time-intensity analysis for bitterness stimulus

Samples	TImax	Imax	Area	Ttot
Sucrose	17.14 <sup>a</sup> ±1.09	4.47 <sup>c</sup> ±0.83	111.33 <sup>c</sup> ±10.1	46.98 <sup>b</sup> ±4.12
Stevia 60% Reb A	19.24 <sup>a</sup> ±1.17	5.89 <sup>b</sup> ±0.67	174.92 <sup>b</sup> ±11.87	54.12 <sup>a</sup> ±3.89
Stevia 80% Reb A	19.3 <sup>a</sup> ±2.06	5.78 <sup>b</sup> ±0.75	176.57 <sup>b</sup> ±9.93	56.54 <sup>a</sup> ±3.33
Stevia 97% Reb A	18.97 <sup>a</sup> ±1.36	5.81 <sup>b</sup> ±0.73	175.04 <sup>b</sup> ±12.34	57.74 <sup>a</sup> ±4.01
Stevia 60% Reb A*	19.29 <sup>a</sup> ±2.01	6.77 <sup>a</sup> ±1.01	216.93 <sup>a</sup> ±10.73	58.62 <sup>a</sup> ±3.76
Stevia 80% Reb A*	18.55 <sup>a</sup> ±2.22	7.01 <sup>a</sup> ±0.98	217.51 <sup>a</sup> ±11.21	56.34 <sup>a</sup> ±3.91
Stevia 97% Reb A*	18.42 <sup>a</sup> ±2.31	6.78 <sup>a</sup> ±0.76	220.08 <sup>a</sup> ±10.11	57.32 <sup>a</sup> ±2.98

Means with the same superscript letter are not significantly different at a 5% level.

\* Low-fat samples.

#### *Time-intensity analysis for melting rate stimulus*

Melting rate was the last attribute evaluated by the assessors. This evaluation aimed to verify possible changes in the melting rate due to the reduction of cocoa butter, and the effectiveness of inulin as a fat substitute in this product. Melting of chocolates in the mouth is defined by

the characteristics of the fat phase and affects the perception of chocolate taste and texture, thus it is considered a quality attribute (Afoakwa *et al.*, 2007).

As shown in Table 5, no significant differences ( $P > 0.05$ ) were observed among the samples for the parameter TImax. However, the low-fat chocolates (Stevia 60, 80, and 97% Reb A\*) were significantly different from all other samples for the parameters Imax, Area, and Ttot, demonstrating that there was a change in the temporal perception of the melting rate of the low-fat samples, even those containing a higher amount of inulin, which is a functional ingredient widely used to replace sugar and fat (Aidoo *et al.*, 2017).

Aidoo *et al.* (2015) studied sugar-free chocolates with prebiotic fibers and also observed changes in the melting behavior.

**Table 5** Time-intensity analysis for melting rate stimulus

Samples	TImax	Imax	Area	Ttot
Sucrose	45.85 <sup>a</sup> ±3.45	7.3 <sup>a</sup> ±1.22	297.34 <sup>a</sup> ±14.65	78.83 <sup>a</sup> ±4.11
Stevia 60% Reb A	46.2 <sup>a</sup> ±3.76	7.26 <sup>a</sup> ±2.02	272.26 <sup>ab</sup> ±13.98	78.07 <sup>a</sup> ±4.51
Stevia 80% Reb A	45.29 <sup>a</sup> ±3.92	7.19 <sup>a</sup> ±1.45	266.5 <sup>b</sup> ±14.14	77.71 <sup>ab</sup> ±5.34
Stevia 97% Reb A	47.37 <sup>a</sup> ±2.74	7.21 <sup>a</sup> ±1.12	287.93 <sup>ab</sup> ±14.54	79.35 <sup>a</sup> ±5.02
Stevia 60% Reb A*	45.12 <sup>a</sup> ±2.98	5.68 <sup>b</sup> ±1.31	209.38 <sup>c</sup> ±15.1	73.31 <sup>bc</sup> ±4.97
Stevia 80% Reb A*	45.02 <sup>a</sup> ±3.1	5.52 <sup>b</sup> ±1.21	212.44 <sup>c</sup> ±13.79	72.75 <sup>c</sup> ±4.31
Stevia 97% Reb A*	45.1 <sup>a</sup> ±2.45	5.61 <sup>b</sup> ±0.99	208.7 <sup>c</sup> ±14.23	73.36 <sup>bc</sup> ±6.02

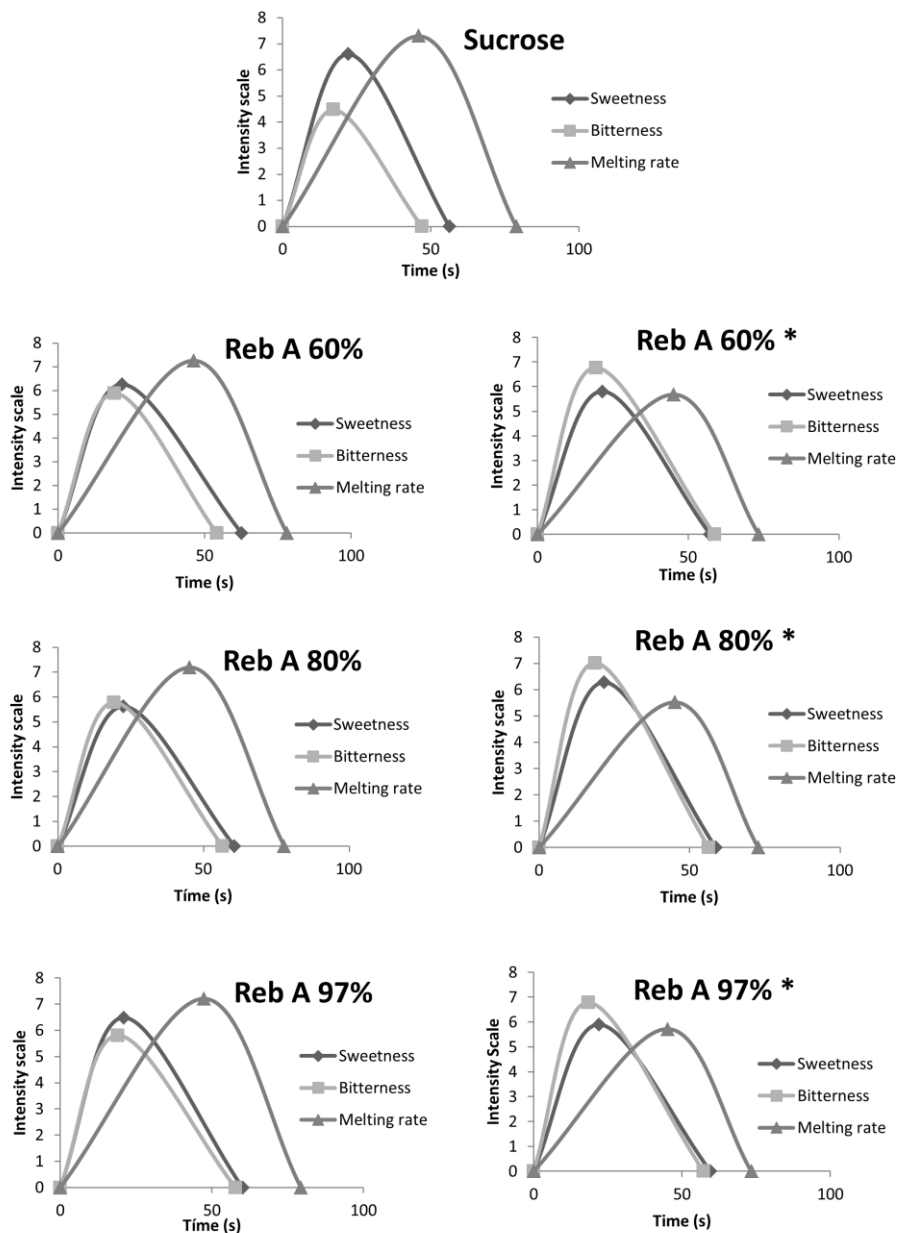
Means with the same superscript letter are not significantly different at a 5% level.

\* Low-fat samples.

#### *Multiple time-intensity analysis (MTIA)*

MTIA should be applied in studies on the development of new formulations, especially those using sugar and fat substitutes, since it demonstrates that the use of different ingredients can lead to changes in the time-intensity profile during consumption for each attribute and product, which is not assessed by the Classical Descriptive Sensory Analysis (Palazzo & Bolini, 2014).

The time-intensity curves for the three stimuli (sweetness, bitterness, and melting rate) of each sample (multiple time-intensity representations) are shown in Figure 1.



**Figure 1** Multiple time-intensity of bittersweet chocolates samples sweetened with sucrose, with stevia 60, 80 and 97% rebaudioside A, and with stevia 60, 80 and 97% rebaudioside A\*. \* Samples with reduced fat contents.

Figure 1 is a graphical form of representing the mean values of the four parameters evaluated in relation to the three stimuli. The samples without fat reduction exhibited a temporal profile more similar to the sugar-sweetened sample, mainly concerning the sweetness and melting in the mouth. The low-fat samples were more bitter and presented lower melting rate in the mouth. These results are essential for the development of products

with sensory characteristics similar to the conventional product, thus affecting the consumer' purchase.

### *Physicochemical characterization*

#### *Texture and color measurements*

The hardness values and color parameters of the samples are shown in Table 6.

**Table 6** Hardness and color (L\*, a\*, b\*) of bittersweet chocolate samples

<b>Samples</b>	<b>Hardness (g)</b>	<b>L*</b>	<b>a*</b>	<b>b*</b>
Sucrose	7180.94±125.3 <sup>b</sup>	28.74±0.17 <sup>b</sup>	6.96±0.13 <sup>b</sup>	6.35±0.18 <sup>b</sup>
Stevia 60% Reb A	6246.01±137.09 <sup>c</sup>	28.22±0.15 <sup>c</sup>	6.59±0.22 <sup>c</sup>	6.03±0.22 <sup>bc</sup>
Stevia 80% Reb A	6340.95±104.04 <sup>c</sup>	28.2±0.12 <sup>c</sup>	6.56±0.15 <sup>c</sup>	6.07±0.08 <sup>bc</sup>
Stevia 97% Reb A	6377.7±116.64 <sup>c</sup>	28.1±0.05 <sup>c</sup>	6.57±0.08 <sup>c</sup>	6.03±0.19 <sup>c</sup>
Stevia 60% Reb A*	7801.03±98.08 <sup>a</sup>	30.08±0.2 <sup>a</sup>	7.46±0.16 <sup>a</sup>	7.1±0.06 <sup>a</sup>
Stevia 80% Reb A*	7776.14±121.49 <sup>a</sup>	30.07±0.05 <sup>a</sup>	7.49±0.18 <sup>a</sup>	7.11±0.1 <sup>a</sup>
Stevia 97% Reb A*	7644.69±120.38 <sup>a</sup>	30.05±0.03 <sup>a</sup>	7.48±0.08 <sup>a</sup>	7.07±0.13 <sup>a</sup>

\* Low-fat samples.

Changes in composition, processing, and storage, among others, can affect chocolate texture, impairing its quality. Low hardness leads to the development of a sticky texture, while high hardness makes it difficult to chew (Alvis *et al.*, 2011).

The low-fat chocolates presented higher hardness values, and were significantly different from the other samples. Different behavior was observed by Rezende *et al.* (2015), who found an increase in hardness of chocolates with the increase in cocoa butter contents, as the solid particles became more dispersed with the increase of the lipid phase, causing less interference in the crystallization process, which resulted in hard chocolates (Beckett, 2009). In contrast, Aidoo *et al.* (2017) reported that the increase in fat content also decreased the hardness of bittersweet chocolates.

In the present study, the low-fat samples had higher inulin levels, which may also have influenced the present result. Furlán *et al.* (2017) also found higher hardness in white chocolate samples containing higher inulin levels.

Regarding the color parameters, the L\* values ranged from 27.5 to 29.6, characterizing the samples as bittersweet chocolates. Low-fat samples with higher inulin content had higher L\*, a\*, and b\* values. Shourideh *et al.* (2012) have shown that the increase in inulin

concentration leads to an increase in  $L^*$  value. *Morais-Ferreira et al.* (2016) found higher  $a^*$  and  $b^*$  values in low-fat food products.

## **Conclusion**

According to the time-intensity analysis, no significant differences were observed in the sweetness temporal profile of the six sugar-free chocolates when compared to the sugar-sweetened sample, demonstrating that stevia and inulin have proven to be excellent sucrose substitutes.

In relation to the bitterness stimulus, the low-fat samples had a more accentuated perception of this attribute by the assessors, with significant differences for  $I_{max}$  and Area when compared to the sugar-free samples.

The stimulus melting in the mouth was more affected in the low-fat samples.

Data have shown no significant differences between stevia with different rebaudioside A levels. Therefore, stevia with 60% rebaudioside A is the most indicated due to its lower cost and similar sensory profile, as demonstrated by the time-intensity analysis of sweetness, bitterness, and melting rate, which is the most relevant attributes in a sugar-free low-fat chocolate.

Although the samples with lower cocoa butter content presented changes in bitterness and melting rate, the product may appeal to an audience that enjoys bitter chocolate and searches for light and healthier products. The sugar-free sample sweetened with stevia containing 60% rebaudioside A may be an excellent alternative healthy product similar to the conventional, once it is less caloric and sweetened with a cheap natural sweetener, besides containing prebiotic fiber with functional properties and presenting a profile similar to the sugar-sweetened sample.

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## 4 DISCUSSÃO GERAL

Para o desenvolvimento adequado de chocolates meio amargos sem açúcar e com reduzido teor de gordura, é fundamental a aplicação da ciência da Análise Sensorial, uma vez que a retirada ou diminuição destes ingredientes pode afetar as características sensoriais do produto, em termos de sabor e textura principalmente, acarretando em prejuízo na aceitação do consumidor.

A primeira etapa realizada neste estudo foi o teste de Doçura Ideal, que possibilitou a determinação da concentração ideal de sacarose a ser adicionada no chocolate meio amargo. A mesma foi calculada usando a equação de regressão e o valor obtido foi 47,5% (m/m), com um valor de  $R^2$  de 99,64% (CALLEGARI-JACQUES, 2003).

Resultado similar foi encontrado por Morais-Ferreira e colaboradores (2016) encontraram doçura ideal de 40,46% em chocolate branco. Estudo com chocolate ao leite em barra determinou que a doçura ideal deste produto é de 43% (MELO; BOLINI; EFRAIM et al., 2007), já com o chocolate amargo foi encontrado uma doçura maior que 50% (KOMES et al., 2013). A doçura encontrada no chocolate amargo (50%) nesse estudo foi superior aos valores encontrados no chocolate branco e ao leite, provavelmente, porque os consumidores tentaram compensar a amargura deste produto aumentando a sua doçura. Ao misturar diferentes estímulos gustatórios, a intensidade de um frequentemente diminui a percepção do outro (KEAST, 2008), sendo a supressão do amargor pelo aumento da doçura um exemplo (ARES et al., 2009).

As pesquisas com diferentes resultados enfatizam a importância da realização deste teste em diferentes matrizes alimentares, e, até mesmo, em diferentes variedades do mesmo produto, como é o caso dos chocolates ao leite e meio amargo (MELO; BOLINI; EFRAIM, 2007; MORAIS-FERREIRA et al., 2016).

Após a determinação da Doçura Ideal do chocolate meio amargo, foi possível obter as concentrações equivalentes de cada edulcorante utilizado, com o objetivo de atingir doçuras semelhantes à encontrada na amostra de chocolate meio amargo com sacarose.

A Análise Tempo-Intensidade tem se tornado uma ferramenta extremamente útil para a indústria de alimentos, por possibilitar a comparação temporal da percepção de doçura do alimento com sacarose em relação aos alimentos adicionados de edulcorantes. Morais-Ferreira e colaboradores (2016) aplicaram a análise tempo-intensidade para determinar as

concentrações equivalentes de doçura dos edulcorantes sucralose e estévia no chocolate branco, adicionado ou não de *goji berry*.

No presente estudo, determinaram-se as concentrações equivalentes de estévias contendo diferentes teores de Rebaudiosídeo A (60, 80 e 97%) no chocolate meio amargo, sendo que cada amostra foi produzida em 3 concentrações (0,16%, 0,22% e 0,27%) de cada edulcorante.

Ao comparar os parâmetros das curvas tempo-intensidade do chocolate meio amargo adoçado com sacarose com as seis amostras adoçadas com diferentes estévias, verificou-se que o percentual de rebaudiosídeo A não interferiu na intensidade de doçura do chocolate meio amargo. Os resultados encontrados não tiveram diferença significativa ( $p < 0,05$ ) independente do tipo de estévia usado. Era esperado que a amostra com estévia contendo 97% de Rebaudiosídeo A apresentasse maior intensidade de doçura que a amostra com estévia contendo 60% rebaudiosídeo A na mesma concentração, uma vez que este glicosídeo é responsável por conferir doçura à estévia (WANG et al., 2016). Provavelmente, isto ocorreu devido ao efeito sinérgico dos edulcorantes com outros ingredientes utilizados, como o poliol (maltitol). Os poliois são sinérgicos com edulcorantes de alta intensidade, tais como a estévia (NABORS, 2011). Dutra & Bolini (2014) avaliaram as doçuras equivalentes de estévias com diferentes teores de rebaudiosídeo A adicionadas em suco de acerola e também não encontraram diferenças significativas nos resultados. Os autores relataram que a acidez do suco pode ter prejudicado a percepção dos provadores. No caso do chocolate meio amargo, possivelmente, o gosto amargo intenso das amostras pode ter dificultado esta percepção.

Outro ponto a ser observado nos resultados da análise de equivalência de doçura foi que a redução de 7,5% no teor de gordura de algumas amostras não alterou a percepção da intensidade de doçura pelos provadores. Paixão e colaboradores (2014) encontraram diferenças na percepção de doçura em leites achocolatados (integral e desnatado) adoçados com o edulcorante neotame, mas também não encontraram diferenças na percepção de doçura nas amostras adoçadas com estévia contendo 97% rebaudiosídeo A. Melo e colaboradores (2009) também não encontraram diferença na percepção da intensidade de doçura de chocolates ao leite *diet* e com calorias reduzidas, adoçados com sucralose e estévia.

Além disso, não houve diferença significativa nas concentrações testadas (0,16%; 0,22%; 0,27%) para cada estévia (60%, 80% e 97% de rebaudiosídeo A), em relação aos parâmetros avaliados ( $I_{max}$ ,  $T_{I_{max}}$ ,  $T_{tot}$  e  $Area$ ). Palazzo e colaboradores (2011) não

encontraram diferença significativa nas quatro diferentes concentrações testadas de estévia (0,269%; 0,215%; 0,161%; 0,107%) no chocolate ao leite, em relação aos mesmos parâmetros. Uma possível explicação para isto seria que a doçura das amostras de chocolate meio amargo com menor concentração de estévia já teria atingido o limiar de detecção (*threshold*) em termos de intensidade de doçura (KEAST; ROPER, 2007). Segundo Keast e Breslin (2003), ao atingir o limiar de detecção, mesmo que a concentração do edulcorante aumente, a percepção da intensidade de doçura não aumentará.

Após a realização do teste de equivalência de doçura, foi possível determinar a melhor e mais conveniente concentração de estévia que deveria ser adicionada no chocolate meio amargo para as etapas seguintes do estudo. Uma vez que não houve diferença significativa nas diferentes concentrações testadas para cada estévia, a concentração de 0,16% foi considerada a mais indicada para ser utilizada por ser essa quantidade suficiente para atingir a doçura ideal do produto. Vale ressaltar que a utilização da menor concentração de estévia estudada diminuiria os custos da fabricação do produto e, provavelmente, diminuiria, também, o amargor das amostras, atributo comum encontrado no edulcorante estévia.

Após a determinação da doçura ideal e da equivalência de doçura das amostras de chocolate meio amargo, realizou-se o perfil sensorial das amostras por meio de testes descritivos, como a Análise Descritiva Quantitativa (ADQ<sup>®</sup>) e Análise Tempo-Intensidade Múltipla, e testes afetivos, como o Teste de Aceitação. Além disso, foi realizada a caracterização instrumental (análises de textura e cor) das amostras, e a composição centesimal aproximada.

Para a realização da Análise Descritiva Quantitativa (ADQ<sup>®</sup>), 16 indivíduos pré-selecionados geraram, de forma consensual, 20 termos descritores das amostras, com suas respectivas referências e definições. Para a definição da equipe final da ADQ<sup>®</sup>, todos os termos descritores foram avaliados pelos 16 indivíduos pré-selecionados, usando uma escala linear, não estruturada, de nove centímetros, ancorada nos extremos por nenhum ou fraco (à esquerda) e forte (à direita). Doze assessores foram selecionados de acordo com o bom poder discriminativo ( $pF$  amostra  $< 0,50$ ), boa reprodutibilidade nos julgamentos ( $pF$  repetição  $> 0,05$ ) e consenso com o grupo, de acordo com as recomendações de Damásio & Costell (1991).

Em relação ao perfil sensorial das amostras obtido por ADQ<sup>®</sup>, observou-se que dentre os 20 termos descritores analisados, as principais diferenças observadas foram em relação ao

sabor e à textura entre as amostras sem açúcar e as amostras sem açúcar com redução no teor de gordura.

Não houve diferença significativa ( $p > 0,05$ ) entre as amostras em relação à cor marrom, uma vez que todas as amostras apresentavam o mesmo teor de liquor de cacau, responsável por este atributo.

Além disso, as seis amostras sem açúcar não diferiram significativamente ( $p > 0,05$ ) da amostra com adição de açúcar com relação ao atributo brilho, portanto, a redução no teor de gordura não alterou esse relevante atributo do chocolate. A manteiga de cacau é um dos ingredientes mais importantes na formulação do chocolate e é responsável por diversas características de qualidade no chocolate, entre elas o brilho (AFOAKWA, 2016). Provavelmente, a redução do teor de manteiga de cacau nas amostras não foi suficiente para causar diferença na percepção visual deste atributo. Melo e colaboradores (2009) também não encontraram diferença significativa para o atributo brilho em amostras de chocolate ao leite com redução no teor de gordura.

Para o desenvolvimento de um novo produto com características nutricionais mais saudáveis, é fundamental que a aparência seja semelhante à amostra convencional. Isto porque a visão é o primeiro contato do consumidor com o alimento, e já pode gerar o desejo de consumi-lo (MARCELINO et al., 2001).

Nenhuma das amostras sem açúcar apresentou diferença significativa da amostra com açúcar em relação aos atributos de aroma. Segundo a ADQ, o aroma predominante de todas as amostras foi o aroma de cacau. Este aroma por estar acentuado pode ter mascarado os outros aromas, dificultando a diferenciação dos mesmos pelos assessores. Vale ressaltar que o aroma de baunilha pode não ter apresentado diferença significativa entre as amostras porque a quantidade adicionada na formulação foi a mesma em todas as amostras. Além disso, a presença de sacarose não é considerada um fator que influencia a formação de aroma de chocolate (Beckett, 2009). Portanto, não eram esperadas diferenças significativas em relação ao aroma entre a amostra convencional e as amostras adoçadas com rebaudiosídeo A.

Com relação aos sabores de cacau e manteiga de cacau, foram encontradas diferenças significativas ( $p \leq 0,05$ ) entre as três amostras com redução no teor de gordura e a amostra convencional. A menor média do atributo sabor de manteiga de cacau nessas três amostras adoçadas com rebaudiosídeo A e com redução de gordura ocorreu pelo menor teor deste ingrediente nestas formulações.



Ao avaliar as médias dos atributos gosto doce, gosto residual doce, gosto amargo e gosto residual amargo das três amostras sem açúcar (Estévia com 60, 80 e 97% de Rebaudiosídeo A), verificou-se que a diferença no teor de Rebaudiosídeo A entre elas não afetou a percepção dos assessores em relação a essas características. Isso também ocorreu com a percepção destes mesmos atributos nas três amostras com redução de gordura. Rebaudiosídeo A é o glicosídeo que confere o gosto doce na estévia, enquanto o esteviosídeo é responsável pelo gosto amargo (WANG et al., 2016). Quanto maior o teor de Rebaudiosídeo A na estévia, menor será o teor de esteviosídeo. Portanto, eram esperadas diferenças nas médias dos atributos de doçura e amargor nas amostras de chocolates meio amargos adoçadas com estévias contendo diferentes teores de rebaudiosídeo A. No entanto, este comportamento dos glicosídeos ocorre na estévia ainda não adicionada ao alimento, considerando que a matriz alimentar pode influenciar nestas características. Provavelmente, os outros ingredientes presentes nas amostras tiveram predominância no sabor e mascararam as possíveis diferenças entre as estévias. Melo e colaboradores (2009) estudaram o perfil sensorial de chocolates ao leite formulados com adição de estévia e de proteína do soro do leite como substituta parcial de gordura. Neste estudo, também não foram encontradas diferenças nos atributos gosto doce, gosto residual doce, gosto amargo e gosto residual amargo, demonstrando que é possível reduzir o teor de gordura do chocolate e manter a percepção de alguns gostos.

Embora os teores de rebaudiosídeo A das estévias não tenham interferido na percepção destes atributos, foi possível verificar que as três amostras sem açúcar e com redução de gordura tiveram maiores médias para os atributos gosto amargo e gosto amargo residual e diferiram significativamente em relação à amostra com açúcar. Amostras com redução no teor de gordura tendem a ser mais amargas, uma vez que a gordura tem a capacidade de revestir os compostos amargos (GUINARD; MAZZUCHELLI, 1999).

Foi verificado ainda que as três amostras sem açúcar e com teor de gordura reduzido apresentaram as maiores variações em relação à textura, diferindo de forma significativa da amostra convencional nos atributos de dureza, derretimento na boca e arenosidade. A manteiga de cacau tem grande importância no perfil sensorial de chocolates, sendo responsável principalmente pela textura deste produto (EFRAIM et al., 2013). Por sua vez, as três amostras sem açúcar, que não tiveram redução no teor de gordura, não tiveram diferença significativa da amostra convencional nos atributos de dureza, derretimento na boca e arenosidade, demonstrando que a utilização da inulina como agente de corpo foi eficiente. A

substituição completa do açúcar no chocolate representa um desafio, porque pode afetar as propriedades reológicas e a textura do mesmo. A inulina pode ser uma boa alternativa para substituir o açúcar do chocolate e manter suas características sensoriais (SHOURIDEH et al., 2012).

Os atributos gosto doce, gosto amargo e derretimento na boca foram avaliados por meio da Análise Tempo-Intensidade, sendo que cada atributo foi avaliado em um período diferente.

A doçura foi o primeiro atributo avaliado pelos assessores. Não foram observadas diferenças significativas ( $p > 0,05$ ) no perfil temporal de doçura das amostras sem açúcar em comparação com a amostra convencional.

Rebaudiosídeo A é o glicosídeo responsável pelo gosto doce da estévia (WANG et al., 2016). Portanto, uma alta intensidade de doçura e longa duração do estímulo eram esperadas para as amostras adicionadas de estévia contendo 97% rebaudiosídeo A, o que não ocorreu neste estudo. Provavelmente, por se tratar de uma matriz alimentar de sabor forte e característico, as diferenças entre os conteúdos deste glicosídeo não foram percebidas pelos assessores. O chocolate meio amargo tem sabor predominante do liquor de cacau, com notas de nozes e caramelo (LIU et al., 2015).

A redução no teor de gordura das três amostras não afetou a percepção temporal de doçura pelos assessores. Em um estudo com sorvete de creme, a redução no teor de gordura também não afetou a doçura das amostras (CADENA et al., 2012). Segundo Wiet e colaboradores (1993), a redução do teor de gordura pode causar um ligeiro aumento na potência de edulcorantes usados para substituir a sacarose. No entanto, segundo estes autores, pequenas diferenças na percepção da doçura em função do teor de gordura são dependentes da matriz alimentar estudada e não podem ser generalizadas.

O amargor foi o segundo atributo avaliado pelos assessores. Os compostos polifenólicos do cacau conferem características amargas e adstringentes ao chocolate meio amargo (HARWOOD et al., 2013). Portanto, o atributo amargor era esperado em todas as amostras por causa do uso do liquor de cacau, mas com maior intensidade nas amostras adoçadas com estévia com menor teor de rebaudiosídeo A e, conseqüentemente, maior quantidade de esteviosídeo, responsável pelo gosto amargo (TORRI et al., 2016). A percepção de amargor aumentou com a redução de gordura. Segundo Guinard & Mazzucchelli (1999), o

chocolate com redução no teor de gordura tende a ser mais amargo, uma vez que a gordura tem a capacidade de revestir os compostos amargos.

Todas as amostras adoçadas com estévia apresentaram diferença significativa no tempo total de estímulo em relação à amostra com sacarose, devido ao gosto residual amargo característico deste edulcorante (CHRANIOTI et al., 2016).

O derretimento na boca foi o último atributo avaliado. O objetivo de avaliar este atributo foi verificar se a redução da manteiga de cacau de algumas amostras de chocolate meio amargo alterou o derretimento na boca, além de avaliar se a inulina foi uma boa substituta de gordura neste produto.

As amostras com redução no teor de gordura diferiram de forma significativa de todas as outras amostras nos parâmetros I<sub>max</sub>, Area e T<sub>tot</sub>, demonstrando que houve alteração na percepção temporal do derretimento na boca nas amostras com menor teor de gordura, mesmo com a presença de uma maior quantidade de inulina, que é um ingrediente funcional muito utilizado para substituir açúcar e gordura (AIDOO et al., 2017).

A aceitação das amostras desenvolvidas foi avaliada por 120 consumidores com idade entre 18 e 60 anos, sendo 59% do sexo feminino. Alunos e funcionários da Faculdade de Engenharia de Alimentos (Unicamp, SP), que apreciavam chocolate meio amargo, foram convidados a avaliar as sete amostras de chocolates meio amargos em relação à aparência, aroma, sabor, textura e impressão global.

Em relação à aparência, não houve diferença significativa ( $p > 0,05$ ) entre as 7 amostras de chocolate meio amargos produzidas. A aparência é um atributo muito importante, uma vez que fornece ao consumidor expectativas sobre a qualidade do sabor e palatabilidade do alimento, que pode afetar a aceitação e o consumo do produto (HURLING; SHEPHERD, 2003). Além disso, não houve diferença significativa ( $p > 0,05$ ) em relação ao aroma entre as seis amostras sem açúcar com a amostra convencional.

A maioria das amostras com diferentes teores de Rebaudiosídeo A não apresentou diferença significativa ( $p > 0,05$ ) da amostra com sacarose em relação ao sabor.

Os resultados da ADQ demonstraram que as amostras com redução no teor de gordura sofreram maior influência na textura. Isto não foi percebido sensorialmente pelos consumidores, que são indivíduos não treinados, já a maioria das amostras com redução no teor de gordura não apresentaram diferença significativa da amostra convencional em relação

à textura. Indivíduos treinados podem ser capazes de detectar pequenas diferenças entre as amostras, enquanto os consumidores normalmente não (HOUGH, 1998).

Em relação à impressão global, foi possível observar que principalmente as amostras sem açúcar e com redução no teor de gordura tiveram boa aceitação, sendo estatisticamente iguais à amostra com sacarose, demonstrando que a retirada do açúcar e a redução no teor de gordura não prejudicaram a aceitação destas amostras pelo consumidor. Segundo os dados da ADQ, houve diferença na percepção pelos assessores na textura entre as amostras sem sacarose e as amostras sem sacarose e com redução no teor de gordura, mas como esta diferença não foi percebida pelos consumidores, isso não afetou a aceitação global. Ainda segundo a ADQ, as amostras com redução no teor de gordura eram mais amargas, o que poderia ter prejudicado a aceitação. No entanto, uma vez que o teste de aceitação convidou indivíduos que apreciavam chocolate meio amargo, o amargor deste produto não era um atributo que poderia prejudicar a aceitação, podendo até contribuir para a mesma, o que foi verificado no mapa de preferência externo.

A correlação dos dados descritivos obtidos por ADQ com os dados de aceitação foi representada pelo Mapa Externo de Preferência, usando a Regressão dos Mínimos Quadrados Parciais (PLS). As quatro amostras localizadas mais próximas aos consumidores no gráfico (Figura 2 – artigo 2), e, portanto, as mais aceitas, foram as amostras com sacarose, com estévia contendo 80% Rebaudiosídeo A e com estévia contendo 80 e 97% Rebaudiosídeo A (com redução no teor de gordura).

Esta informação permite concluir que a retirada da sacarose e a redução do teor de gordura foram bem aceitas pelos consumidores. É interessante observar que o atributo amargor ficou próximo das amostras com redução de gordura, o que contribuiu para a boa aceitação destes chocolates, ao agradar um público que aprecia chocolate meio amargo.

As análises químicas e instrumentais fornecem informações importantes para o desenvolvimento adequado de novos produtos e devem ser correlacionadas com os parâmetros sensoriais.

Em relação à composição centesimal, não houve diferença significativa nos valores de umidade e cinzas. As amostras com redução de gordura tiveram diferença significativa ( $p > 0,05$ ) nos valores de proteína, carboidratos e lipídeos, uma vez que as formulações destas três amostras apresentavam metade da quantidade de manteiga de cacau (7,5%), maior quantidade de leite em pó desnatado e maior quantidade de inulina.

Foi verificado ainda que as amostras de chocolate meio amargo com redução de gordura apresentaram, em média, uma redução de 27,62% das calorias, podendo ser considerados chocolates meio amargos *light*, segundo a legislação brasileira, que preconiza que um produto *light* deve ter uma redução mínima de 25% no valor energético em comparação ao produto tradicional (BRASIL, 1998).

Em relação à análise de textura, as amostras de chocolate com redução no teor de gordura apresentaram maiores médias de dureza e diferiram significativamente das outras amostras. Um resultado diferente foi encontrado no estudo de Rezende e colaboradores (2015), no qual houve um aumento da dureza dos chocolates com o aumento no teor de manteiga de cacau, pois com o aumento da fase lipídica, as partículas sólidas tornaram-se mais dispersas, causando uma menor interferência no processo de cristalização, o que resultou em chocolates mais duros (BECKETT, 2009). No entanto, no estudo de Aidoo e colaboradores (2017), o aumento no teor de gordura também diminuiu a dureza de chocolates amargos.

No presente estudo, a redução no teor de gordura e adição de maiores teores de inulina pode ter influenciado a dureza das amostras. Furlán e colaboradores (2017) também encontraram maiores valores de dureza para amostras de chocolate branco com teores mais altos de inulina.

Em relação à cor, a análise instrumental mostrou que o parâmetro  $L^*$  ficou entre 27.5 e 29.6, caracterizando as amostras como escuras. As amostras com redução no teor de gordura e maior teor de inulina apresentaram maiores médias de  $L^*$ ,  $a^*$  e  $b^*$ . Shourideh e colaboradores (2012) demonstraram que o aumento na concentração de inulina causa um aumento no valor de  $L^*$ . Em outro estudo, os parâmetros  $a^*$  e  $b^*$  avaliados em produtos com menor teor de gordura foram maiores (MORAIS et al., 2016).

## 5 CONCLUSÃO GERAL

Este estudo apresentou todas as etapas fundamentais, em relação à análise sensorial, para o desenvolvimento de chocolates meio amargos mais saudáveis, adicionados de substitutos de sacarose e ingredientes com alegações funcionais.

A partir da análise da determinação de Doçura Ideal, estabeleceu-se que a concentração ideal de sacarose para o chocolate meio amargo é de 47,5%.

Com relação à equivalência de doçura, uma vez que não houve diferença significativa nas três concentrações testadas para cada estévia, concluiu-se que a concentração de 0,16% é a mais indicada para ser utilizada. Isso porque essa quantidade já foi suficiente para atingir a doçura equivalente do produto, não existindo a necessidade de acrescentar mais edulcorante. Além disso, a utilização da menor concentração de estévia estudada diminuiu os custos da fabricação deste produto para a indústria de alimentos.

Os resultados dos testes descritivos (ADQ e Análise Tempo-intensidade) demonstraram que os diferentes teores de Rebaudiosídeo A testados não interferiram nos atributos de gosto doce, gosto doce residual, gosto amargo e gosto amargo residual. Estes são os principais atributos afetados pela estévia, segundo a literatura.

Em relação ao amargor, a ADQ e a Análise Tempo-intensidade demonstraram que as amostras com redução de gordura tiveram percepção deste atributo de forma mais acentuada pelos assessores. O derretimento na boca também foi mais afetado nestas amostras.

Uma vez que os diferentes teores de Rebaudiosídeo A estudados apresentaram perfil sensorial muito semelhante no chocolate meio amargo, a utilização da estévia com 60% de rebaudiosídeo A foi a mais indicada, por ter o menor custo, uma vez que quanto maior o teor de rebaudiosídeo A, maior é o seu custo. Além disso, esta estévia apresentou perfil sensorial muito semelhante às outras amostras, segundo a análise tempo-intensidade de doçura, amargor e derretimento, os três atributos mais relevantes em um chocolate sem açúcar e com redução de gordura.

Em relação à textura, as amostras com redução no teor de gordura apresentaram maiores modificações quando comparadas à amostra convencional. No entanto, apesar de algumas diferenças entre as amostras terem sido detectadas nos testes descritivos, o teste de aceitação e o mapa de preferência externo demonstraram que, de forma geral, todas as amostras tiveram boa aceitação sensorial, mas principalmente as amostras com redução no

teor de gordura. Portanto, o desenvolvimento de chocolates meio amargos sem açúcar, com redução no teor de gordura e com adição de fibra prebiótica como substituta de açúcar e gordura é viável.

Os resultados deste estudo poderiam ser aplicados pela indústria de alimentos para o desenvolvimento de um produto voltado para indivíduos que realmente apreciam chocolates com gosto amargo acentuado e que buscam por opções mais saudáveis. O produto desenvolvido seria adoçado com edulcorante natural de menor custo, adicionado de prebiótico e com teor calórico reduzido, sendo considerado *light* pela legislação brasileira.

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## APÊNCICE A

### **TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**

Você está sendo convidado (a) para participar, como voluntário (a), da pesquisa: **DESENVOLVIMENTO E PERFIL SENSORIAL DE CHOCOLATES MEIO AMARGOS COM PROPRIEDADES FUNCIONAIS E ADOÇADOS COM REBAUDIOSÍDEO A COMO SUBSTITUTO DA SACAROSE.**

O principal objetivo deste trabalho será avaliar o efeito do uso de diferentes estévias, como substitutas da sacarose, e da inulina, como substituta de gordura, em chocolate meio amargo, através de técnicas sensoriais adequadas.

É muito improvável qualquer desconforto ou risco para você que participar da pesquisa, uma vez que todos os edulcorantes estudados são permitidos para uso no Brasil.

Os pesquisadores irão tratar a sua identidade com padrões profissionais de sigilo. A participação no estudo não acarretará custos para você e não será disponível nenhuma compensação financeira adicional.

Sua colaboração se fará de forma anônima e o acesso e a análise dos dados coletados se farão apenas pelo (a) pesquisador (a) e/ou seu(s) orientador(es).

### **CONSENTIMENTO DA PARTICIPAÇÃO DA PESSOA COMO SUJEITO**

Declaro, por meio deste termo, que concordei em participar do projeto de pesquisa intitulado “Desenvolvimento e perfil sensorial de chocolates meio amargos com propriedades funcionais e adoçados com rebaudiosídeo a como substituto da sacarose”, desenvolvido por Bruna Marcacini Azevedo, que poderá ser contatada pelo endereço de e-mail: bma1703@fea.unicamp.br ou celular: (035) 8804-1581, que trabalha no endereço: Cidade Universitária “Zeferino Vaz”, s/n - Departamento de Alimentos e Nutrição da Faculdade de Engenharia de Alimentos (FEA) da Unicamp de Campinas – SP, CEP 13083-862 C.P.: 6121.

Fui informado (a), ainda, de que a pesquisa é orientada pela Profa. Dra. Helena Maria André Bolini, a quem poderei contatar / consultar a qualquer momento que julgar necessário, através do endereço de e-mail: hellini@fea.unicamp.br. Fui informado (a), também, que em caso de denúncias ou reclamações sobre minha participação e sobre questões éticas do estudo,

eu poderei entrar em contato com a secretaria do Comitê de Ética em Pesquisa (CEP) da UNICAMP: Rua: Tessália Vieira de Camargo, 126; CEP 13083-887 Campinas – SP; telefone (19) 3521-8936; fax (19) 3521-7187; e-mail: cep@fcm.unicamp.br

Afirmo que aceitei participar por vontade própria, sem receber qualquer incentivo financeiro ou ter qualquer ônus e com a finalidade exclusiva de colaborar para o sucesso da pesquisa. Fui informado (a) dos objetivos estritamente acadêmicos do estudo.

Fui também esclarecido (a) de que os usos das informações por mim oferecidas estão submetidos às normas éticas destinadas à pesquisa envolvendo seres humanos, da Comissão Nacional de Ética em Pesquisa (CONEP) do Conselho Nacional de Saúde, do Ministério da Saúde.

Fui ainda informado (a) de que posso me retirar dessa pesquisa a qualquer momento, sem sofrer quaisquer sanções ou constrangimentos.

Atesto recebimento de uma cópia assinada deste Termo de Consentimento Livre e Esclarecido, conforme recomendações da Comissão Nacional de Ética em Pesquisa (CONEP).

Campinas, \_\_\_\_ de \_\_\_\_\_ de \_\_\_\_\_.

Assinatura do (a) participante: \_\_\_\_\_

Assinatura da pesquisadora: \_\_\_\_\_

Assinatura do (a) testemunha: \_\_\_\_\_

## ANEXO A – Comitê de Ética



### COMPROVANTE DE ENVIO DO PROJETO

#### DADOS DO PROJETO DE PESQUISA

**Título da Pesquisa:** Desenvolvimento e Perfil Sensorial de chocolates meio amargos Diet e Diet/Light com propriedades funcionais.

**Pesquisador:** Bruna Marcacini Azevedo

**Versão:** 2

**CAAE:** 41358214.0.0000.5404

**Instituição Proponente:** Faculdade de Ciências Médicas - UNICAMP

#### DADOS DO COMPROVANTE

**Número do Comprovante:** 006419/2015

**Patrocinador Principal:** MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

Informamos que o projeto Desenvolvimento e Perfil Sensorial de chocolates meio amargos Diet e Diet/Light com propriedades funcionais, que tem como pesquisador responsável Bruna Marcacini Azevedo, foi recebido para análise ética no CEP UNICAMP - Campus Campinas em 04/02/2015 às 13:18.

## ANEXO B - Permissão da editora para inclusão do artigo 1 na tese

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### JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Apr 12, 2017

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This Agreement between Bruna Azevedo ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4086631123395
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Licensed Content Title	The Influence of the Rebaudioside A Content of Stevia (Stevia rebaudiana Bertoni) on the Determination of Sweetness Equivalence in Bittersweet Chocolates, Using the Time-Intensity Analysis
Licensed Content Author	Bruna M. Azevedo, Janaína M. M. Ferreira, Valdecir Luccas, Helena M. A. Bolini
Licensed Content Date	Nov 3, 2016
Licensed Content Pages	1
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	DESENVOLVIMENTO E PERFIL SENSORIAL DE CHOCOLATES MEIO AMARGOS COM PROPRIEDADES FUNCIONAIS E ADOÇADOS COM REBAUDIOSÍDEO A COMO SUBSTITUTO DA SACAROSE
Expected completion date	Jun 2017
Expected size (number of pages)	133
Requestor Location	Bruna Azevedo Rua Monteiro Lobato, 80, Campinas, SP

**ANEXO C – Comprovante de submissão do artigo 2**

The image is a screenshot of a Gmail inbox. At the top, the Google search bar contains the text "journal of food science and technology". Below it, the Gmail header shows navigation icons and the text "Mover para a Caixa de Entrada" and "Mais". The main content area displays an email titled "JFST-D-16-02506 - Submission Confirmation" from "Journal of Food Science and Technology <em@editorialmanager.com>". The email is dated "23/11/2016" and is addressed to "para mim". The body of the email contains the following text:

Dear Ms Azevedo,

Your submission entitled "The importance of the Rebaudioside A content of Stevia (Stevia rebaudiana Bertoni) in the sensory profile of semi-bitter chocolates with no added sugar and with prebiotic and relationship with consumer expectations" has been received by Journal of Food Science and Technology

The submission id is: JFST-D-16-02506  
Please refer to this number in any future correspondence.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is <http://jfst.edmgr.com/>.

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to our journal.

Kind regards,  
Editorial Office

The left sidebar of the Gmail interface shows various folders and options, including "Entrada (1)", "Com estrela", "Importante", "Enviados", "Rascunhos", "Círculos", "Facebook", and a contact list with "Bruna". At the bottom of the sidebar, there is a message: "Nenhum contato do Hangouts Encontre alguém".

**ANEXO D - Aceite de publicação do artigo 3**

The screenshot shows a Gmail interface with a search bar at the top. The left sidebar contains navigation options like 'ESCREVER', 'Entrada', 'Com estrela', 'Importante', 'Enviados', 'Rascunhos', 'Círculos', and 'Facebook (1)'. The main content area displays an email from Wiley Author Services, dated 25-Mar-2017, addressed to Ms. Azevedo. The email text discusses the acceptance of a manuscript titled '# IJFST-2017-22067.R1' and provides instructions regarding the publication process, including the need for a signed license agreement and the availability of OnlineOpen for open access.

Google

Gmail

ESCREVER

Entrada  
Com estrela  
Importante  
Enviados  
Rascunhos  
Círculos  
Facebook (1)  
Bruna

25-Mar-2017

Dear Ms. Azevedo,

It is a pleasure to accept your manuscript entitled "# IJFST-2017-22067.R1 entitled "Bittersweet chocolates containing prebiotic and sweetened with stevia (Stevia rebaudiana Bertoni) with different Reubaudioside A contents: Multiple time-intensity analysis and physicochemical characteristics" which you submitted to the International Journal of Food Science and Technology" in its current form for publication in the International Journal of Food Science and Technology.

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Thank you for your fine contribution. On behalf of the Editors of the International Journal of Food Science and Technology, we look forward to your continued contributions to the Journal.

Sincerely,  
Prof. Charles Brennan  
Editor-in-Chief, International Journal of Food Science and Technology  
[charles.brennan@lincoln.ac.nz](mailto:charles.brennan@lincoln.ac.nz)



**ANEXO E – Permissão da editora para inclusão do artigo 3 na tese**

The screenshot shows a Gmail interface with the following elements:

- Header:** Google logo, search bar, and user profile 'B'.
- Navigation:** 'Gmail' label, navigation icons (back, forward, search, etc.), and 'Mais' dropdown.
- Left Sidebar:** 'ESCREVER' button, 'Entrada' (Inbox) selected, and other folders like 'Com estrela', 'Importante', 'Enviados', 'Rascunhos', 'Círculos', and 'Facebook (1)'. A 'Bruna' contact card is visible.
- Message 1:**
  - From:** Bruna Marcacini Azevedo <bma.nutricao@gmail.com>
  - To:** para Charles
  - Time:** 11:56 (Há 21 horas)
  - Body:**

Dear Prof. Charles Brennan,

I would like permission to include the manuscript entitled "# IJFST-2017-22067.R1 entitled "Bittersweet chocolates containing prebiotic and sweetened with stevia (Stevia rebaudiana Bertoni) with different Rebaudioside A contents: Multiple time-intensity analysis and physicochemical characteristics" which you submitted to the International Journal of Food Science and Technology" in my PhD thesis.

Sincerely,

Bruna Marcacini Azevedo.
- Message 2:**
  - From:** Brennan, Charles
  - To:** para mim
  - Time:** 17:07 (Há 16 horas)
  - Body:**

Thank you for your email and from my perspective I have no problem with this request

Charles

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