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RAFAEL SOUSA LIMA

PERFIL SENSORIAL DINÂMICO E DIRECIONADORES DE PREFERÊNCIA EM BEBIDA FUNCIONAL DE BAIXA CALORIA À BASE DE TAMARINDO – *Tamarindus indica* L.

DYNAMIC SENSORY PROFILE AND DRIVERS OF LIKING ON LOW-CALORIE FUNCTIONAL TAMARIND BEVERAGE – *Tamarindus indica* L.

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Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Alimentos e Nutrição

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Orientadora: Profa. Dra. Helena Maria André Bolini

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RESUMO

O objetivo desta pesquisa foi elaborar um perfil sensorial dinâmico e direcionador de preferências, que possibilite o melhor comportamento sensorial da bebida funcional de tamarindo – *Tamarindus indica* L. de baixa caloria. As amostras de bebida funcional foram feitas com polpa comercial de tamarindo congelada com adição de frutooligossacarídeos (FOS). A razão de diluição foi de 100 g de polpa por 200 mL de água. A quantidade de FOS utilizada foi de 1,5 g por 100 mL, de acordo com as recomendações da legislação brasileira para alimentos líquidos. Ao todo, foram elaboradas sete amostras, uma padrão com sacarose e seis com diferentes edulcorantes não nutritivos em substituição à sacarose. Os edulcorantes utilizados nas formulações foram: estévia com 97% de rebaudiosídeo A, sucralose, acessulfame-K, sacarina, neohesperidina e neotame. Inicialmente, a intensidade ideal de sacarose na bebida funcional de tamarindo foi determinada pela escala do ideal (*Just-About-Right scale*), seguida pela determinação da equivalência de doçura dos seis diferentes edulcorantes em relação à concentração ideal de sacarose. O perfil sensorial das amostras foi determinado por Análise Descritiva Quantitativa (ADQ) e questionário *Check-All-That-Apply* (CATA). A avaliação da aceitação em relação à aparência, aroma, sabor, textura e impressão global das sete amostras de bebida funcional de tamarindo foi realizada utilizando teste de aceitação com escala hedônica de 9 cm. Após a caracterização do perfil sensorial, foi realizada a avaliação da intensidade dos estímulos percebidos durante o consumo da bebida com a aplicação do teste Tempo-Intensidade (TI). A pesquisa também avaliou o impacto de diferentes temperaturas ambiente durante o consumo da bebida funcional por meio da aplicação de três métodos de *Temporal Dominance of Sensations* (TDS), um para cada variação de temperatura (20, 24 e 26 ºC). Todos os dados foram analisados por ANOVA e *Tukey's honestly significant difference procedure* para comparação das médias das amostras e a Análise de Componentes Principais (ACP). As informações descritivas obtidas foram relacionadas com a aceitação do consumidor usando o método *Partial Least Squares* (PLS) e Mapa Externo de Preferência. A amostra adoçada com sucralose mostrou ser a melhor opção de substituto de sacarose na bebida funcional de tamarindo, apresentando um perfil sensorial mais próximo à amostra com sacarose, sem apresentar diferença significativa em relação à aparência, aroma, sabor, textura e impressão global. As amostras adoçadas com edulcorantes naturais apresentaram menor impressão global e menor aceitação por percentual de intenção de compra. A aplicação do teste TI foi importante para avaliar a duração e intensidade de estímulo de duas características naturais da fruta (refrescância e sabor de tamarindo), identificadas pela ADQ e CATA. A temperatura do laboratório, como fator extrínseco, também influenciou na percepção dos atributos sensoriais da bebida, com destaque para o ambiente frio, que acentuou a sensação de gosto amargo nas amostras adoçadas com estévia e neohesperidina. Sugerem-se novos estudos sobre a abrangência da análise sensorial em relação ao impacto dos fatores extrínsecos na aceitação e no perfil sensorial de bebidas à base de frutas.

Palavras-chave: Análise Sensorial; Bebidas; Frutas; Alimentos Funcionais; Adoçantes não Calóricos.

ABSTRACT

The purpose of this work consisted in elaborating a dynamic sensory and drivers of liking on low-calorie functional tamarind beverage. The samples of the functional beverage were made with commercial frozen tamarind pulp with the addition of frutctooligosaccharides (FOS). The dilution ratio was 100 g of pulp per 200 mL of water. The quantity utilized of FOS was 1,5 g per 100 mL, according to the recommendations of the Brazilian legislation for liquid foods. Altogether, seven samples were elaborated, one standard with sucrose and six with different non-nutritive sweeteners to replace sucrose. The utilized sweeteners in the formulations were: stevia with 97% rebaudioside A, sucralose, acesulfame-K, saccharin, neohesperidin, and neotame. Initially, the sucrose ideal intensity in the functional tamarind beverage was determined by the Just-About-Right scale, followed by the determination of sweetness equivalence of the six different sweeteners concerning the ideal sucrose concentration. The sensory profile of the samples was determined by the Quantitative Descriptive Analysis (QDA) and Check-All-That-Apply (CATA) questionnaire. The evaluation of the acceptance of the appearance, aroma, flavor, texture, and global impression of the seven tamarind functional drink samples was performed by the acceptance test with 9 cm hedonic scale. After the characterizing the sensory profile, the evaluation of the intensity of the stimuli perceived during the consumption of the beverage was performed by the application of the Time-Intensity (TI) test. This research also evaluated the impact of different ambient temperatures during the consumption of the functional beverage by application of three Temporal Dominance of Sensations (TDS) tests, one for each temperature variation (20, 24, and 26 ºC). All data were analyzed by ANOVA and Turkey´s honestly significant difference procedure for comparison of the sample averages and the Principal Components Analysis (PCA). The descriptive information obtained was related to consumer acceptance using the Partial Least Squares (PLS) method and the External Preference Mapping. The sample sweetened with sucralose showed to be the best option of substitute for sucrose in the functional tamarind beverage, presenting a sensory profile closer to the sample with sucrose, without showing significant differences concerning the appearance, aroma, flavor, texture, and global impression. Samples sweetened with natural sweeteners presented lower global impression and less acceptance by percentage or purchase intention. The application of the TI test was important to assess the duration and intensity of the stimulus of two natural characteristics of the fruit (refreshing and tamarind flavor), identified by QDA and CATA. The laboratory temperature, as an extrinsic factor, also influenced the perception of the sensory attributes of the beverage, having prominence the cold ambient, which increased the bitter taste sensation in the samples sweetened with stevia and neohesperidin. Further studies are suggested on the scope of sensory analysis concerning the impact of extrinsic factors on the acceptance and sensory profile on fruit-based drinks.

Keywords: Sensory Analysis; Beverages; Fruits; Functional Food; Non-nutritive Sweeteners.

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LISTA DE ABREVIATURAS E SIGLAS

ABNT	Associação Brasileira de Normas Técnicas
ADA	American Dietetic Association
ADQ	Análise Descritiva Quantitativa
AOAC	Association of Official Analytical Chemists
AGCC	Ácidos Graxos de Cadeia Curta
ANVISA	Agência Nacional de Vigilância Sanitária
BIT	Brazil Ingredients Trends
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
DCNT	Doenças Crônicas Não Transmissíveis
DHA	Ácido docosahexaenoico
DPPH	2,2-Difenil-1-Picril-Hidrozil
DTS	Dominância Temporal das Sensações
EPA	Ácido Eicosapentaenoico
FAO	Food and Agriculture Organization of The United Nations
FDA	Food and Drug Administration
FOS	Fruto-oligossacarídeos
FOSHU	Food for Specified Health Use
HDL	High Density Lipoprotein
IDA	Ingestão Diária Aceitável
INC	Informação Nutricional Complementar
ITAL	Instituto de Tecnologia de Alimentos
LDL	Low Density Lipoprotein
NEPA	Núcleo de Pesquisa em Alimentos
PCA	Principal Component Analysis
PLS	Partial Least Squares
QDA	Quantitative Descriptive Analysis
SAS	Statistical Analysis System
SD	Standard Deviation
TDS	Temporal Dominance Sensation
TI	Time Intensity
TRPM5	Transient receptor potential
WHO	World Health Organization

SUMÁRIO

1. INTRODUÇÃO

As doenças crônicas não transmissíveis (DCNT), consideradas um dos grandes desafios do século XXI, são as principais causa de morte em todo o mundo e estão causalmente ligadas a fatores de risco comportamentais. O consumo de tabaco, uso nocivo de álcool, inatividade física e dieta inadequada são os principais comportamentos evitáveis que levam a alterações metabólicas/fisiológicas desencadeadoras de doenças crônicas (WORLD HEALTH ORGANIZATION, 2016).

Desses fatores de risco, a dieta é o fator modificável com maior impacto na saúde pública, pois tem o potencial de reduzir o alto nível de obesidade, doenças cardiovasculares, diabetes mellitus e câncer (NEUHOUSER, 2019). Por esse motivo, tem-se observado o crescente interesse da população por hábitos alimentares mais equilibrados em relação aos nutrientes, caracterizado por redução do consumo de alimentos ricos em calorias (como o excesso de gorduras saturadas ou trans e também de sacarose) e por preferência por gorduras insaturadas, além de alimentos de origem vegetal e marinha (WORLD HEALTH ORGANIZATION, 2016).

O interesse do consumidor em adicionar alimentos mais nutritivos na dieta é evidenciado pelos estudos sobre perspectivas de desenvolvimento de novos produtos da indústria de bebidas, observando-se a inclusão de diversos ingredientes que agregam valor nutricional, como a utilização de frutas e verduras. Além disso, notou-se um aumento na quantidade de pedidos por produtos premium, com qualidade aprimorada, tais como, smothies, água de coco, sucos de frutas exóticas e alimentos funcionais (DEL BUONO, 2017; REGO; VIALTA; MADI, 2016).

Deste modo as frutas exóticas, em especial algumas frutas tropicais, se destacam no setor de ingredientes por apresentarem compostos fenólicos com propriedade funcional, além de acrescentar características sensoriais atraentes, como aroma e o sabor de fruta (VIALTA; AMARAL REGO, 2014). Assim, um fruto em destaque é o tamarindo – *Tamarindus indica L.*, que é caracterizado pelo sabor agridoce e presença de compostos fenólicos como catenina, procianidina, epicatequina, ácido tartárico, triterpenoide que comprovadamente, por meio de estudos *in vitro* e em *in vivo*, desempenham atividade antioxidante (BHADORIYA et al., 2012).

O tamarindo é utilizado de diversas formas culinárias, tais como doces, bebidas e temperos. Porém quando utilizado em bebidas, por conta da sua acidez característica, é comumente ingerido com adição de sacarose, que em excesso pode intereferir negativamente na resposta glicêmica do indivíduo, especialmente em diabéticos. Desta forma, torna-se interessante reduzir e/ou substituir a sacarose por outros agentes adoçantes que não interfiram de forma negativa na resposta glicêmica ou no nível calórico da bebida.

A utilização de agentes adoçantes em bebidas, como substituto de sacarose, é um grande desafio para a indústria de alimentos, pois cada agente vai apresentar uma característica diferenciada de acordo com o tipo e/ou meio de aplicação. Além disso, esses agentes adoçantes precisam superar suas limitações quanto ao sabor residual desagradável (VIALTA; AMARAL REGO, 2014). Entre os agentes adoçantes mais utilizados pode-se destacar a sucralose, acessulfame de K, sacarina, neotame e estévia.

Assim, com a utilização de agentes adoçantes pode-se desevolver uma bebida à base de tamarindo de baixa caloria com a doçura desejável pelo consumidor. Entretando, a substituição da sacarose pode tornar a bebida menos "encorpada" e desagradar uma parte dos consumidores. Por isso, é interessante também adicionar agentes de corpo em bebidas sem adição de sacarose, como exemplo os fruto-oligossacarídeos (FOS), que são fibras solúveis com propiedades funcionais (BRASIL, 2016).

Desse modo, observa-se que utilização de diversos ingredientes pode oferecer ao consumidor uma bebida nutritiva, com baixa caloria e com características sensoriais agradáveis. Para isso a indústria de alimentos utiliza as técnicas de mudanças e adição de ingredientes para atender às exigências do mercado consumidor cada vez mais preocupado com a saúde, o que evidencia a importância da análise sensorial por exercer uma grande influência na melhoria das características sensoriais e propriedades do produto, incluindo aparência, aroma, sabor e textura em um nível que é particularmente aceitável para os consumidores (IANNARIO et al., 2012).

1.1 OBJETIVOS

O objetivo geral desta pesquisa foi desenvolver uma bebida funcional de baixa caloria à base de tamarindo e elaborar o perfil sensorial dinâmico e direcionadores de preferências que possibilitem uma melhor caracterização sensorial do produto. Para tal, foram estabelecidos sete objetivos específicos para a melhor condução da pesquisa:

- Identificar a concentração ideal de sacarose e dos edulcorantes para formulação das amostras de bebida funcional de tamarindo;
- Definir os dados físico-químicos e de composição centesimal das amostras;

- Definir o perfil sensorial descritivo das amostras por meio de Análise Descritiva Quantitativa (ADQ) e *Check-All-That-Apply* (CATA);
- Determinar a aceitação e intenção de compra do produto;
- Definir o mapa externo de preferência;
- Estabelecer o perfil tempo-intensidade e as curvas de dominância das sensações em função de tempo dos gostos básicos e atributos característicos das diferentes amostras;
- Avaliar o impacto da temperatura ambiente na dominância de sensações durante o consumo das amostras.

2. REFERENCIAL TEÓRICO

2.1 O MERCADO DE BEBIDAS NÃO ALCOÓLICAS E OS DESAFIOS DAS MUDANÇAS DE INGREDIENTES

A indústria de alimentos é um dos setores mais dinâmicos da economia brasileira, pois dá resposta à demanda alimentar doméstica do país além de atender ao comércio exterior. Uma das categorias de maior destaque da indústria alimentar no Brasil é a relacionada à produção de bebidas, sendo o país o terceiro maior produtor e consumidor de cervejas e refrigerantes do mundo, essa categoria é caracterizada pelo forte crescimento neste início de século pelas oportunidades oriundas do crescimento econômico e da emergência de uma nova classe de consumo (LUCENA; SIBIN; SILVA, 2017).

A indústria de bebidas é complexa e possui duas categorias de grande impacto no mercado, a categoria de bebidas alcoólicas e de bebidas não alcoólicas. Nesta revisão, será abordada a categoria de bebidas não alcoólicas que, de acordo com o estudo Brasil Beverage Trends 2020 (REGO; VIALTA; MADI, 2016), pode apresentar diferentes classificações de acordo com cada país. No Brasil, há uma classificação, convencionada pelo Instituto de Tecnologia de Alimentos - ITAL, que utiliza seis grandes categorias, que podem ser observadas na Tabela 1.

Tabela 1. Definição das categorias de bebidas não alcoólicas no Brasil.

(continua)

CATEGORIA	DEFINIÇÃO
Refrigerantes e outras bebidas carbonatadas	Bebida gaseificada, obtida pela dissolução, em água potável, de suco ou extrato vegetal de sua origem, adicionada de açúcar.
Águas	Bebidas minerais, gaseificadas ou não, não adoçadas, sem adição de sabor; embalagens diversas, inclusive garrafões de 20 litros.
Sucos, néctares e refrescos	Suco: bebida não fermentada, não concentrada, não diluída, destinada ao consumo, obtida da fruta madura e sã, ou parte do vegetal de origem, por processamento tecnológico adequado, submetida a tratamento que assegure a sua apresentação e conservação até o momento do consumo. Néctares: bebida não fermentada, obtida da diluição em água potável da parte comestível do vegetal ou de seu extrato, adicionado de açúcares, destinada ao consumo direto. Refresco: bebida não fermentada, obtida pela diluição, em água potável, do suco de fruta, polpa ou extrato vegetal de sua origem, com ou sem adição de açúcares

Tabela 1. Definição das categorias de bebidas não alcoólicas no Brasil.

(conclusão)

CATEGORIA	DEFINIÇÃO
Energéticos, isotônicos e funcionais	Energéticos: Bebidas gaseificadas, obtidas pela mistura de cafeína e/ou outros ingredientes “estimulantes” e açúcar e/ou edulcorantes. Isotônicos: Bebida obtida pela mistura de ingredientes com o objetivo de repor os sais minerais perdidos pelo corpo humano durante a prática de atividades esportivas. Funcionais: Bebidas com ingredientes ou composição associados a funções específicas para a saúde e o bem-estar (melhora do desempenho cognitivo; controle do peso; saciedade; probiótica e prébiótica; saúde da pele etc.)
Chás e cafés	Chá: bebida obtida pela maceração, infusão ou percolação de folhas e brotos de várias espécies de chá do gênero Thea (*Thea sinensis* e outras), de folhas, hastes, pecíolos e pedúnculos de erva-mate da espécie *llex paraguariensis* ou de outros vegetais, podendo ser adicionado de outras substâncias de origem vegetal e de açúcares. Café: Bebida à base de café pronta para beber, gaseificada ou não.
Lácteas e substitutas	São considerados os laticínios líquidos que compreendem o leite, em suas diferentes apresentações (integral, desnatado, sem lactose, vitaminado, aromatizado, em pó etc.), e seus derivados em estado líquido prontos para beber (leites fermentados e iogurte líquido), além das bebidas lácteas que são produtos formulados com leite, soro de leite e outros ingredientes.

Fonte: BRASIL (2009); ASSOCIAÇÃO DA INDÚSTRIA DE REFRIGERANTES E DE BEBIDAS NÃO ALCOÓLICAS (2016).

Com base nessas categorias, os estudos de tendências para o mercado de bebidas não alcoólicas mostram uma mudança estrutural no mix de bebidas produzidas, que em grande parte é movida pelas novas tendências de mercado relacionadas à saúde. Da mesma forma que vem ocorrendo em outros países, no mercado brasileiro houve uma redução do volume de produção de refrigerantes, acompanhada de um aumento expressivo na produção de águas engarrafadas, isotônicos e energéticos, chás prontos para beber, sucos, néctares e refrescos (REGO; VIALTA; MADI, 2016).

Os sucos de frutas e vegetais, por exemplo, tem experimentado um crescimento global, e estimou-se que em 2019 o faturamento tenha sido em torno de US$ 198 bilhões (TELFORD, 2015). Esse crescimento tem sido alavancado pelos países emergentes, cujo consumo passou de 30 para 50 bilhões de litros / ano. O Brasil apresentou crescimento significativo nos últimos anos, com movimentação de mais de 1,5 bilhões de reais (ESPERANCINI, 2014). O mercado brasileiro de sucos é bastante diversificado e pode ser classificado em cinco categorias: sucos naturais, sucos em pó, sucos concentrados, sucos prontos para beber e sucos de polpa. Porém, os consumidores brasileiros têm preferência por sucos naturais, feitos na hora, e são ainda resistentes a consumir sucos industrializados (ESPERANCINI, 2014).

Há atualmente uma grande preocupação, em todo o mundo, com a qualidade de vida e a saúde. Com isso, a indústria de alimentos tem-se motivado a criar produtos mais saudáveis, por meio da substituição de ingredientes (SEYHAN; YAMAN; ÖZER, 2016). Porém, é importante ressaltar que a substituição total ou parcial de ingredientes representa um enorme desafio para a indústria, devido à complexidade tecnológica envolvida em desenvolver um produto mais similar possível ao tradicional (VIALTA; AMARAL REGO, 2014).

De acordo com o estudo do Brazil Ingredients Trends 2020 (BIT2020), os ingredientes de maior destaque na indústria de alimentos que são utilizados na categoria de bebidas não alcoólicas são os aromatizantes, adoçantes, corantes, agentes de textura, nutrientes, frutas e vegetais. Esses ingredientes são utilizados com o intuito de se desenvolverem novos produtos com maior valor agregado, além da adequação dos produtos existentes às novas demandas do mercado (VIALTA; AMARAL REGO, 2014).

Assim, em resposta à essa demanda por alimentos com ingredientes nutritivos e com funções benéficas ao organismo (alimentos funcionais) e de calorias controladas, desde os anos 80, tem surgido um grande número de edulcorantes alternativos. Os edulcorantes podem ser classificados por propriedades intrínsecas ou origem, mas também podem apresentar outras classificações sendo as mais comuns as que são relacionadas aos termos de valor nutritivo, poder adoçante e procedência (CAROCHO; MORALES; FERREIRA, 2017). Deste modo, eles podem ser divididos em edulcorantes nutritivos e intensivos, como também entre origem sintética e natural (Tabela 2).

Tabela 2. Classificação dos edulcorantes.

NUTRITIVOS	INTENSIVOS	NATURAIS	SINTÉTICOS
Glicose, isoglicose Açúcar invertido, sorbitol, manitol, xilitol e fruto-oligossacarídeo	Aspartame, acessulfame-K, sacarina, dulcina, taumatina, estévia, monellina, neohesperidina e glicerina	Glicosídeos: monossacarídeos, dissacarídeos, sorbitol, xilitol e manitol Não glicosídeos: maltitol, isomaltitol, taumatina e neohesperidina	Sacarina, ciclamato, aspartame e sucralose

Fonte: CAROCHO; MORALES; FERREIRA (2017)

A principal estratégia utilizada para reduzir total ou parcialmente o açúcar é a introdução de um ou mais edulcorantes de alta intensidade para recuperar o dulçor, e de hidrocolóides para resgatar o “corpo” do produto (VIALTA; AMARAL REGO, 2014). Portanto, na Tabela 3, observam-se os principais edulcorantes e agentes de corpo utilizados para redução da densidade calórica nas indústrias de alimentos.

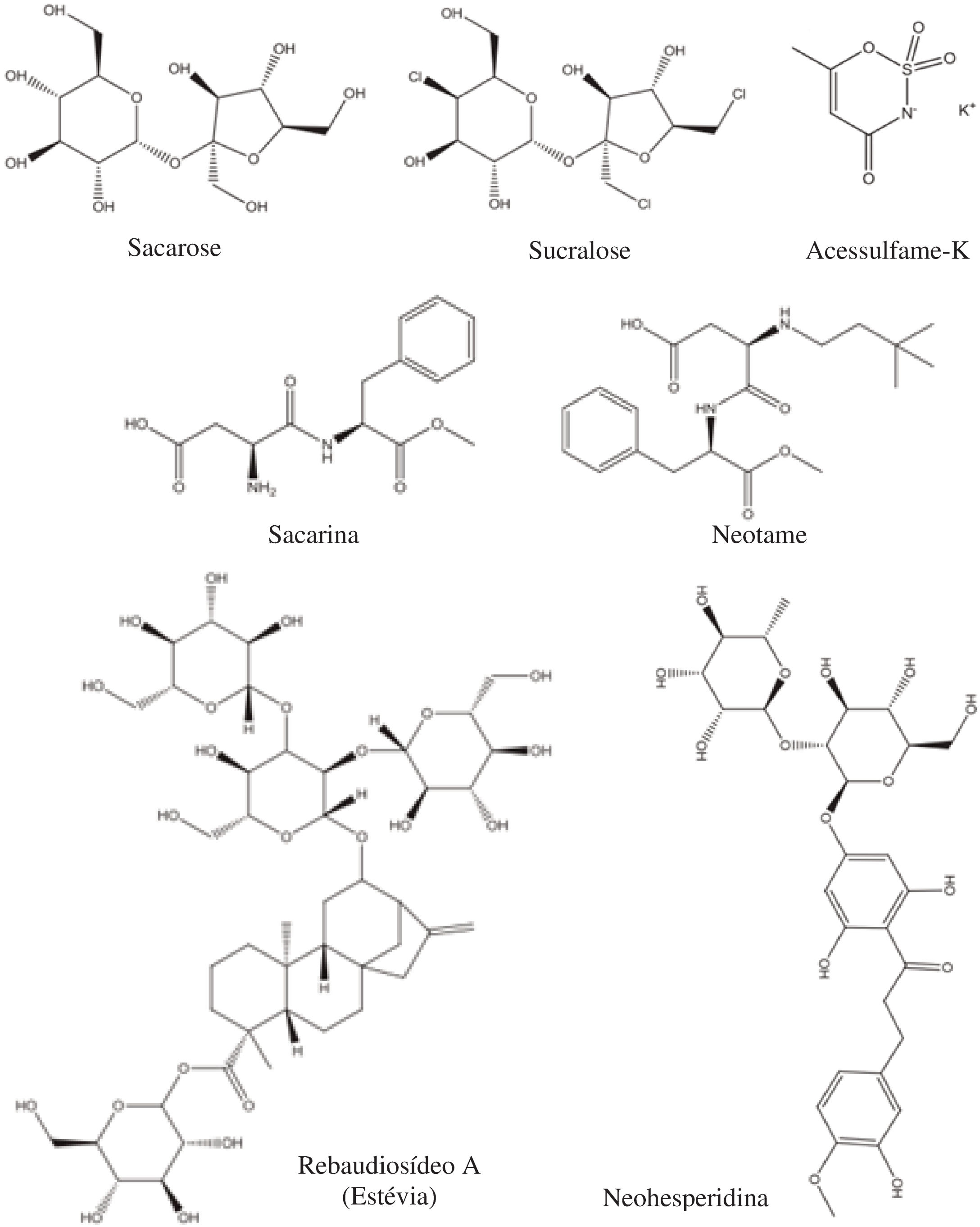
Tabela 3. Principais edulcorantes e agentes de corpo utilizados para redução da densidade calórica.

FUNÇÃO	ADOÇANTES
Edulcorantes artificiais de alta intensidade	Acessulfame-K, aspartame, ciclamato, sacarina, sucralose
Edulcorantes naturais de alta intensidade	Esteviosídeos, fruta do monge (*Monk fruit*), adoçantes proteicos
Edulcorantes e agentes de corpo	Polióis (eritrol, isomalte, lactiol, maltitol, manitol, sorbitol, xilitol)
Agentes de corpo	Gomas (xantana, gelana, carragena, guar, etc.), maltodextrinas, amidos, polidextrose, pectina e celuloses

Fonte: VIALTA; AMARAL REGO (2014)

A classificação dos edulcorantes em intensivos sintéticos e intensivos naturais é bastante utilizada e facilita a sua compreensão, sendo também importante conhecer a estrutura química de cada um. Na Figura 1 observa-se a estrutura molecular de cada edulcorante, tendo em vista que, biologicamente, a percepção de doçura acontece pela interação entre o alimento e os receptores das papilas gustativas por meio de um sistema próton doador / receptor que estabelece uma ligação covalente. A sensação de doçura também depende da configuração da molécula, que nos açúcares provém das conformações dextrorotárias, e não levorotárias (CAROCHO; MORALES; FERREIRA, 2017).

Figura 1. Estrutura química da sacarose e demais edulcorantes.



Fonte: Adaptada de CAROCHO, MORALES E FERREIRA (2017)

Os edulcorantes intensivos sintéticos apresentam alta potência adoçante, superior à sacarose, sendo necessárias apenas doses muito baixas para se obter doçura intensa. Sua contribuição calórica também é muito baixa ou praticamente nula, eles também não apresentam perigo em termos de cariogênese ou em reação à insulina, e não têm outra função nos alimentos além de adoçar (CAROCHO; MORALES; FERREIRA, 2017). Os mais conhecidos são: sucralose, sacarina, acessulfame-K e neotame, que seguem caracterizados na Tabela 4.

Tabela 4. Características, ingestão diária recomendada, metabolismo e toxicidade dos edulcorantes intensivos sintéticos.

(continua)

NOME	DOÇURA	CARACTERÍSTICA	IDA*	METABOLISMO	TOXICIDADE
Sucralose	750x	Estabilidade no calor e em pH baixo. Altamente solúvel em água.	15 mg/kg de peso corporal	Não é metabolizado pelas enzimas hepáticas (BRUSICK et al., 2010).	Segurança no consumo (BRUSICK et al., 2010). Descartada possível conexão do consumo de sucralose com câncer Berry et al. (2016). Em 2016, uma pesquisa evidenciou que o consumo de sucralose resultaria no aumento da ingestão de alimentos por meio de uma resposta do jejum neuronal (WANG et al., 2016). Recentemente, uma nova pesquisa implicou o contrário: a sucralose suprime a ingestão de alimentos, usando os mesmos critérios de teste. Em 2017, um estudo mostrou que, devido ao menor peso e volume sanguíneo que possuem, as crianças têm maior quantidade de sucralose em circulação, evidenciando que medidas devem ser tomadas a fim de determinar a segurança dessa ocorrência (SYLVETSKY et al., 2017; PARK et al., 2017).
Sacarina	300x	Estável a pH baixo e resiste a altas temperaturas.	5mg/kg*/ 15mg/kg** de peso corporal	Excretado pela urina. Pode atravessar a placenta. Transferida pelo leite-materno.	Numerosos estudos consideram a sacarina segura e seu consumo é hoje considerado seguro.
Acessulfame-K	200x	Ausência de sabor residual. Pode ser usado em sinergias com outros adoçantes, como aspartame, ciclamatos e sucralose, para melhorar o poder de doçura e o sabor.	15mg/kg de peso corporal	Excretado pela urina.	Muitos estudos descreveram sua segurança em consumo. Embora outros estudos até 2000 apontassem a presença de algum tipo de toxicidade, foram refutados.

Tabela 4. Características, ingestão diária recomendada, metabolismo e toxicidade dos edulcorantes intensivos sintéticos.

(conclusão)

NOME	DOÇURA	CARACTERÍSTICA	IDA*	METABOLISMO	TOXICIDADE
Neotame	7000 – 13000x	Intensificador de sabores naturais, especialmente gosto ácido e sabores de frutas.	2mg/Kg de peso corporal	Ausência de fenilalanina. Metade do neotame ingerido não é absorvido, sendo excretado pelas fezes, enquanto a outra metade é excretada na urina como neotame desesterificado.	Não foi detectada toxicidade.

* Ingestão Diária Aceitável (IDA) pelo Comitê Conjunto de Especialistas em Aditivos Alimentares da FAO / OMS (JECFA), 2018. ** U.S. FDA (U.S. *Food and Drug Administration*).

Fonte: CAROCHO; MORALES; FERREIRA (2017)

Já os edulcorantes intensivos naturais incluem açúcares naturais, álcoois de açúcar, glicosídeos terpenóides, além de alguns aminoácidos e polifenóis. Atualmente, dentro dessa classe, a estévia e a neohesperidina vêm ganhando destaque, devido a problemas de saúde decorrentes do alto consumo de sacarose e outros açúcares naturais. Na Tabela 5 a seguir foram descritas as principais informações sobre esses dois edulcorantes naturais.

Tabela 5. Características, ingestão diária recomendada, metabolismo e toxicidade dos edulcorantes intensivos naturais.

NOME	DOÇURA	CARACTERÍSTICA	IDA*	METABOLISMO	TOXICIDADE
Estévia	Rebaudiosídeo A: 250-450x Rebaudiosídeo B: 300-350x Rebaudiosídeo C: 50-120x Rebaudiosídeo D: 250-400x Rebaudiosídeo E: 150-300x	O Rebaudiosídeo A gera um sabor doce mais limpo, semelhante ao da sacarose, em comparação com o esteviosídeo, que proporciona um sabor amargo mais forte (CHÉRON; MARCHAL; FIORUCCI, 2019)	4mg/kg de peso corporal*	---	Preocupação com a toxicidade e genotoxicidade, embora muitos autores afirmem que o banco de dados de estudos *in vitro* e *in vivo* é robusto e não há indicação da toxicidade do esteviosídeo e do Rebaudiosídeo (CAROCHO; MORALES; FERREIRA, 2017)
Neohesperidina	1500x	Estável em altas temperaturas, principalmente durante a pasteurização. Disfarça gostos indesejáveis.	Não apresenta*. 35mg/kg de peso corporal**.	Não se acumula nos tecidos devido à sua rápida metabolização e excreção.	---

* Ingestão Diária Aceitável (IDA) pelo Comitê Conjunto de Especialistas em Aditivos Alimentares da FAO / OMS (JECFA), 2018. ** (CAROCHO; MORALES; FERREIRA, 2017).

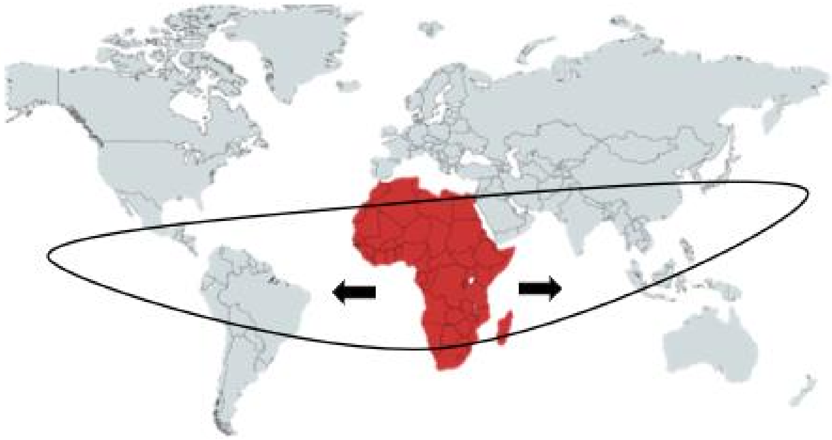
Em geral, há preocupação sobre o consumo seguro dos edulcorantes presente nos produtos alimentares. Os estudos já realizados para determinar as exposições ao consumo dessas substâncias mostraram que os consumidores estão apresentando um consumo geralmente dentro dos limites da IDA para cada edulcorante (MARTYN et al., 2018). O IDA foi selecionado como um parâmetro apropriado para investigar possíveis preocupações de segurança quanto à ingestão de edulcorantes de baixa / sem caloria, pois esse é o principal valor de orientação baseado em saúde usado pelo autoridades competentes em todo o mundo, e o valor usado nos estudos de ingestão disponíveis identificados na pesquisa bibliográfica (MARTYN et al., 2018). Esta declaração de posição, apoiada por evidências de pesquisa, afirma a inclusão de adoçantes nutritivos e não nutritivos no contexto das recomendações atuais de dieta para o público (AMERICAN DIETETIC ASSOCIATION, 2004b).

À medida em que os consumidores procuram assumir o controle de sua saúde e bem-estar, outros segmentos de ingredientes também podem se beneficiar, por exemplo o mercado de frutas exóticas. Acerola, camu camu, fruta dragão e tamarindo emergem como opções tropicais para formulações de bebidas, pois além de seus benefícios para a saúde e relevante valor nutricional, esses ingredientes podem compor uma bebida diferente e atrativa (JACOBSEN, 2015)

O Tamarindo (*Tamarindus indica* L.), por exemplo, é uma árvore que pertence à família *Fabaceae*, subfamília *Caesalpinioidae*. É originária da África tropical, mas se adaptou nas Américas do Norte e do Sul, e é também cultivada na China subtropical, Índia, Paquistão, Filipinas, Java e Espanha (JIMOH; ONABANJO, 2012). As plantações comerciais de tamarindo são relatadas nos países da América Central e no Norte do Brasil. Além disso, o *T. indica* é comumente utilizado na medicina popular na Índia, Sudão, Nigéria, Bangladesh e na maioria dos países tropicais. Quase todas as partes da árvore encontram algum uso nas indústrias alimentar, química e farmacêutica (BHADORIYA et al., 2011, 2012). A Figura 2 ilustra o mapa de origem e distribuição do fruto Tamarindo pelo mundo.

O fruto é uma vagem, indeiscente, alongada, reta ou curvada, aveludada, de cor marrom. A casca do fruto é quebradiça e as sementes são incorporadas em uma polpa comestível pegajosa. As sementes são encontradas na quantidade de 3-10, apresentam aproximadamente 1,6 cm de comprimento, forma irregular, dura, brilhante e lisa (BHADORIYA et al., 2011). A polpa do tamarindo, que apresenta sabor agridoce, é utilizada em uma grande variedade de finalidades domésticas e industriais, como o preparo de doces, bolos, sorvetes, xaropes, bebidas, licores, refrescos, sucos concentrados e, ainda, tempero para arroz, carne, peixe e outros alimentos (JIMOH; ONABANJO, 2012).

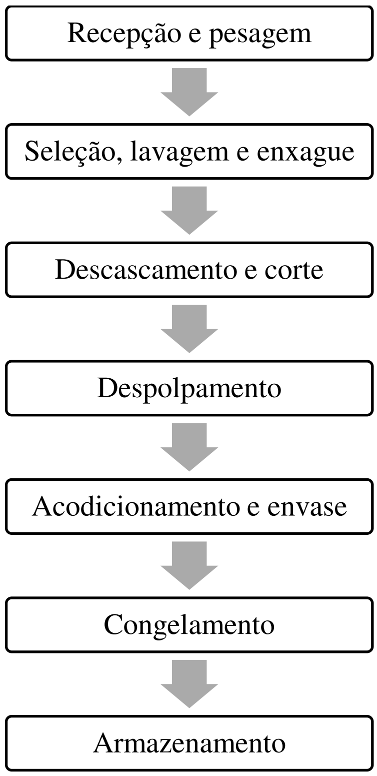
Figura 2. Mapa de origem e distribuição do Tamarindo no mundo.



Fonte: Adaptado de iTi TROPICALS (2017)

A polpa de fruta é o produto não-fermentado, não-concentrado e não-diluído, com teor mínimo de sólidos totais, provenientes da parte comestível da fruta, obtido de frutas polposas, por processo tecnológico adequado (DA MATTA et al., 2005). O fluxograma que detalha o processo de produção de polpa congelada está ilustrado na Figura 3. Na Tabela 6 está descrita a composição nutricional da polpa de tamarindo.

Figura 3. Fluxograma do processo de produção de polpa de fruta congelada.



Fonte: DA MATTA et al. (2005)

O tamarindo é rico em nutrientes e desempenha um papel importante na nutrição humana, principalmente nos países em desenvolvimento. O fruto contém níveis elevados de proteínas (aminoácidos essenciais) e carboidratos, e é rico em minerais como potássio, fósforo, cálcio e magnésio, além de fornecer quantidades menores de ferro e vitamina A (BHADORIYA et al., 2012; ISHOLA; AGBAJI; AGBAJI, 1990). A literatura tem evidenciado a presença de diversos compostos fenólicos com atividade oxidante em praticamente todas as partes que compõem o tamarindo, seja folhas, flores, casca, semente ou polpa (JAIN et al., 2011).

Tabela 6. Composição centesimal, vitaminas e minerais por 100 gramas da parte comestível de tamarindo.

Umidade (%)	22	**Cálcio (mg)**	37	**Cobre (mg)**	**0,29**
Energia (kcal)	276	**Magnésio (mg)**	59	**Zinco (mg)**	**0,7**
Proteína (g)	3,2	**Manganês (mg)**	0,34	**Tiamina (mg)**	**0,31**
Lipídeos (g)	0,5	**Fósforo (mg)**	55	**Riboflavina (mg)**	**Tr**
Carboidrato (g)	72,5	**Ferro (mg)**	0,6	**Piridoxina (mg)**	**0,1**
Fibra alimentar (g)	6,4	**Sódio (mg)**	0	**Niacina (mg)**	**Tr**
Cinzas (g)	1,9	**Potássio (mg)**	723	**Vit. C (mg)**	**7,2**

Fonte: NÚCLEO DE ESTUDOS E PESQUISAS EM ALIMENTAÇÃO NEPA - UNICAMP (2011)

Nos estudos clínicos, em relação à polpa, foi verificado que seu extrato propiciou a diminuição nos níveis de colesterol total sérico, LDL (do inglês, Low Density Lipoprotein) e triglicerídeo, e aumento dos níveis de colesterol HDL (do inglês, High Density Lipoprotein) em ratos hipercolesterolêmicos. Em estudo in vitro, o extrato apresentou capacidade de eliminação de radicais livres, avaliada pelos ensaios dos radicais 2,2-difenil-1-picril-hidrazil (DPPH) e superóxido, enquanto em ensaios in vivo, o extrato melhorou a eficiência do sistema de defesa antioxidante, avaliado pelas atividades das enzimas superóxido dismutase, catalase e glutationa peroxidase (BHADORIYA et al., 2011).

Em relação à indústria de alimentos, especialmente no segmento de bebidas, o tamarindo tem se destacado por seus atributos sensoriais caraterísticos, que o tornam atraente, e por suas características de alimento nutritivo e funcional. Desse modo, é importante compreender as resoluções e normas que qualificam um alimento ou um produto com alegação de nutrição e funcionalidade.

2.2 COMPONENTES BIOATIVOS DOS ALIMENTOS FUNCIONAIS

O conceito inicial de alimentos funcionais foi introduzido no Japão em referência aos alimentos de uma dieta normal, que além de suas funções básicas de nutrição, demonstravam benefícios fisiológicos ou reduziam o risco de doenças crônicas. Tais alimentos foram definidos como de uso específico de saúde (FOSHU, do inglês Food for Specified Health Use) e foram rapidamente adotados em todo o mundo. No entanto, os critérios de alegações e seus critérios para aprovação dependem da regulamentação local ou regional (COSTA; ROSA, 2016).

Em diversos países, como os Estados Unidos, Canadá, países da União Europeia e Austrália, foram adotados diferentes conceitos para os alimentos funcionais, porém, apenas no Japão a expressão “alimento funcional” é definida por lei. A American Dietetic Association (ADA) define alimentos funcionais como “alimento integral e fortificado, enriquecido ou aprimorado, que apresenta um efeito potencialmente benéfico à saúde quando consumido de forma regular, em níveis efetivos, dentro de uma dieta variada” (AMERICAN DIETETIC ASSOCIATION, 2004a).

No Brasil, não há uma definição de alimentos funcionais, mas há resoluções específicas da Agência Nacional de Vigilância Sanitária (ANVISA), descritas na Tabela 7, que avaliam e aprovam a alegação de propriedade funcional, de saúde e estabelecem as diretrizes para sua utilização, bem como as condições de registro de alimentos. Na Tabela 8 estão listados os nutrientes e não nutrientes com as alegações padronizadas e os respectivos requisitos específicos, segundo a legislação brasileira (BRASIL, 2016).

Tabela 7. Resoluções aprovadas pela ANVISA sobre alimentos com alegação de propriedades funcionais.

RESOLUÇÃO	REGULAMENTO TÉCNICO	FONTE
nº 2, de 07/01/2002	Substâncias bioativas e probióticos isolados, com alegação de propriedades funcional e/ou de saúde	(BRASIL, 2002)
nº 19, de 30/04/1999	Procedimento de registro de alimentos com alegações de propriedades funcionais e/ou de saúde no rótulo	(BRASIL, 1999a)
nº 18, de 30/04/1999	Estabelece as diretrizes básicas para análise e comprovação de propriedades funcionais e/ou de saúde alegadas no rótulo de alimentos	(BRASIL, 1999b)
nº 17, de 30/04/1999	Estabelece as diretrizes básicas para a avaliação de risco e a segurança dos alimentos	(BRASIL, 1999c)
nº 16, de 30/04/1999	Registro de Alimentos e ou Novos Ingredientes	(BRASIL, 1999d)

Fonte: Elaborada pelo autor

Tabela 8. Alimentos, nutrientes e bioativos com alegações de propriedades funcionais padronizadas.

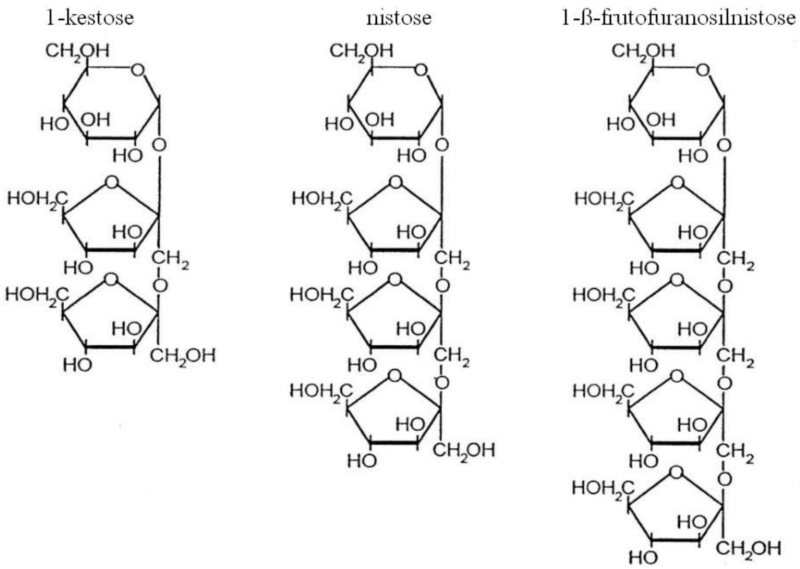
ALIMENTO / NUTRIENTE	ALEGAÇÃO	REQUISITOS
Ácidos graxos EPA e DHA	"Auxilia na manutenção de níveis saudáveis de triglicerídeos, desde que associado a uma alimentação equilibrada e hábitos de vida saudáveis"	As quantidades mínimas anteriormente exigidas de 100 mg de EPA e DHA não são suficientes para produção dos efeitos benéficos relacionados aos níveis de triglicerídeos. Portanto, os pedidos de avaliação de eficácia de alimentos e suplementos com alegações para os ácidos graxos EPA e DHA serão avaliados caso a caso.
Carotenoides: Licopeno Luteína Zeaxantina	"Têm ação antioxidante que protege as células contra os radicais livres. Seu consumo deve estar associado a uma alimentação equilibrada e hábitos de vida saudáveis"	Somente para uso em suplementos contendo licopeno extraído do tomate ou licopeno sintético, fontes já aprovadas pela A quanto à segurança de uso.
Fibras alimentares	"Auxiliam o funcionamento do intestino. Seu consumo deve estar associado a uma alimentação equilibrada e hábitos de vida saudáveis".	O relatório técnico-científico deve conter informações detalhadas sobre o processo de fabricação desses ingredientes e as especificações e laudos analíticos do fabricante, a fim de demonstrar o atendimento aos requisitos de qualidade estabelecidos em referências oficiais reconhecidas: Farmacopeia Brasileira e outras farmacopeias oficiais reconhecidas
Beta glucano	"Pode auxiliar na redução do colesterol. Seu consumo deve estar associado à uma alimentação equilibrada e baixa em gorduras saturadas e a hábitos de vida saudáveis."	Esta alegação pode ser aprovada para aveia em flocos, farelo e farinha de aveia. A utilização da alegação em outros produtos/alimentos está condicionada à comprovação científica de eficácia
Frutooligossacarideo (FOS)	"FOS (prebiótico) contribuem 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 recomendação de consumo diário do produto pronto para consumo forneça no mínimo 5 g de FOS. A porção deve fornecer no mínimo 2,5 g de FOS.
Probióticos	A alegação de propriedade funcional ou de saúde deve ser proposta pela empresa e será avaliada, caso a caso, com base nas definições e princípios estabelecidos na Resolução n. 18/1999.	Deve ser apresentado laudo de análise que comprove a quantidade mínima viável do microrganismo para exercer a propriedade funcional no final do prazo de validade do produto e nas condições de uso, armazenamento e distribuição
Proteína de soja	"O consumo diário de no mínimo 25 g pode ajudar a reduzir o colesterol. Seu consumo deve estar associado a uma alimentação equilibrada e hábitos de vida saudáveis".	Para uso desta alegação, o produto deve atender, no mínimo, aos requisitos estabelecidos para o atributo "fonte" definidos na Resolução sobre Informação Nutricional Complementar (INC)

Fonte: BRASIL (2016)

Para a presente pesquisa, que desenvolverá uma bebida funcional à base de tamarindo, é importante destacar as fibras alimentares, especialmente os FOS. As fibras, como visto na Tabela 8, contribuem para o funcionamento intestinal, sendo classificadas em: polissacarídeos não amido, oligossacarídeos (frutooligossacarideos - FOS), carboidratos análogos, lignina, substâncias associadas aos polissacarídeos não amido, e fibra de origem não vegetal (BERNAUD; RODRIGUES, 2013).

Atualmente, os oligossacarídeos são exemplos de fibras facilmente fermentáveis no intestino humano. FOS é um nome comum dado apenas a oligômeros de frutose que são compostos de 1-kestose, nistose e frutofuranosil nistose (Figura 4), em que as unidades de frutosil (F) são ligadas na posição beta-2,1 da sacarose, o que os distingue de outros oligômeros (PASSOS; PARK, 2003).

Figura 4. Estrutura química dos principais frutooligossacrídeos (kestose; nistose; frutofuranosilnistose).



Fonte: Adaptado de PASSOS; PARK (2003).

As aplicações dos FOS na indústria de alimentos são variadas, especialmente em formulações que levem no rótulo alegação de açúcar reduzido, calorias reduzidas e/ou produto sem açúcar. Também são utilizados em produtos funcionais que promovam efeito nutricional adicional nas áreas de prebióticos, simbióticos, fibras dietéticas e em bebidas (sucos, néctares e refrescos), substituindo a sacarose e gerando produtos com teor reduzido de açúcar (PASSOS; PARK, 2003).

Na saúde humana, os FOS destacam-se pelo efeito positivo de sua fermentação pela microbiota intestinal formando ácido lático e ácidos graxos de cadeia curta (AGCC). Essas substâncias atuam no equilíbrio da flora gastrointestinal e estimulam outros benefícios no metabolismo humano, como a regulação da pressão sanguínea, alteração do metabolismo de ácidos gástricos, e redução da absorção de carboidratos e lipídeos (DAVANI-DAVARI et al., 2019).

Além do apelo saudável, os FOS possuem características que permitem sua aplicação em vários produtos, uma vez que não cristalizam, não precipitam, possuem maior solubilidade em relação à sacarose, e não são calóricos (PASSOS; PARK, 2003). A utilização desse nutriente na indústria alimentar configura a importância da análise sensorial como um sistema otimizado para elaboração de novos produtos com características semelhantes aos tradicionais.

2.3 ANÁLISE SENSORIAL

A definição consensual de análise sensorial é descrita como um método científico usado para evocar, medir, analisar e interpretar as respostas aos produtos (STONE; SIDEL, 2004). Também pode ser definida como um conjunto de técnicas para medir com precisão as respostas humanas para alimentos, minimizando as possíveis influências da identidade de marca e outras variáveis de influência (LAWLESS; HEYMANN, 2010).

Do ponto de vista estatístico, a avaliação sensorial consiste em um método científico em que os resultados experimentais são coletados a partir de um conjunto de consumidores que expressam preferências e reações às características de alimentos e bebidas. Essas preferências são produto de uma decisão humana mediada por interações complexas condicionadas pela história pessoal, variáveis ambientais, subjetivas e características dos objetos (IANNARIO et al., 2012). A análise sensorial é importante no cenário das indústrias de alimentos, pois mediante os testes sensoriais, são desenvolvidas decisões estratégias para responder às principais exigências do mercado, evitando riscos e incertezas na elaboração de um produto.

Essas decisões são tomadas pela correlação dos resultados dos testes sensoriais com medidas instrumentais, variáveis de ingredientes, fatores de armazenamento, tempo de vida útil, ou outras condições conhecidas por afetar as propriedades sensoriais de um produto. Na maioria das aplicações, os testes sensoriais funcionam como redução de risco e incertezas para pesquisadores das empresas e gerentes de marketing. Além da utilização já consolidada no desenvolvimento de produtos, a avaliação sensorial pode fornecer informações para outros departamentos na indústria de alimentos, como setores de desenvolvimento de embalagens e outros ligados à publicidade (LAWLESS; HEYMANN, 2010).

Com a ênfase atual em gestão da qualidade, tem sido notável a preocupação da indústria alimentícia em maximizar seus lucros fornecendo ao consumidor produtos de qualidade comprovada. Nesse contexto, a análise sensorial destaca-se por exercer uma grande influência na melhoria das características sensoriais e propriedades de um produto, incluindo aparência, aroma, sabor e textura em um nível que é particularmente aceitável para os consumidores (IANNARIO et al., 2012).

Assim, pode-se observar na Tabela 9 algumas das principais aplicações para análise sensorial na indústria de alimentos. Entre elas estão inclusas a preferência dos consumidores e comportamento de compra, análise de produtos concorrentes, teste de protótipo, desenvolvimento de produtos, ensaios de prateleira e controle de qualidade e garantia (LAWLESS; HEYMANN, 2010).

Tabela 9. Principais aplicações da análise sensorial na indústria de alimentos.

APLICAÇÕES	BENEFÍCIO
Preferência dos consumidores e comportamentos de compra	Avalia as características sensoriais que indicam qualidade.
Análise de produtos concorrentes	Útil para monitorar a aceitação de produtos concorrentes.
Teste de protótipo	Dar indicação de aceitabilidade do produto e direção de desenvolvimento.
Desenvolvimento de produtos	Ajuda a finalizar as decisões nas características e propriedades dos produtos.
Ensaio de prateleira	Avalia as mudanças organolépticas na vida de prateleira de um produto.
Controle de qualidade e garantia	Verifica o processo/lote e qualidade das matérias-primas até o produto.

Fonte: LAWLESS; HEYMANN (2010).

A principal preocupação de qualquer especialista em avaliação sensorial é garantir que o método escolhido seja apropriado para responder às perguntas feitas sobre o produto no teste. Por esse motivo, os testes geralmente são classificados de acordo com seu objetivo principal e o uso mais válido. Há três tipos de testes sensoriais comumente utilizados: teste discriminativo, descritivo e o teste afetivo (LAWLESS; HEYMANN, 2010).

O objetivo dos testes discriminativos, segundo ABNT NBR ISO 6658 (2019), é determinar se existe uma diferença perceptível entre dois produtos (se há preferência por algum), ou se há semelhança entre eles. A análise é baseada nos resultados dos testes dos avaliadores em cada categoria específica. Os testes mais conhecidos são: teste comparação pareada, teste triangular, e teste duo-trio.

Em relação aos testes descritivos, é observado na literatura que são aplicados para uma ou mais amostras, com o objetivo de caracterizar, qualitativa ou quantitativamente, um ou mais atributos sensoriais. Os testes mais comumente usados são: perfil sensorial qualitativo, perfil sensorial quantitativo, perfil por consenso, perfil livre, perfil flash, desvio do perfil de referência, e dominância temporal das sensações (DTS) (ABNT NBR ISO 6658, 2019).

Por fim, os testes afetivos são definidos como testes sensoriais que atuam na tentativa de quantificar o grau de gostar ou não gostar de um produto, sendo chamados também de métodos de testes hedônicos. A abordagem mais direta dos testes afetivos é de oferecer às pessoas uma escolha entre produtos alternativos, e se há uma preferência clara da maioria dos respondentes (LAWLESS; HEYMANN, 2010).

3. ARTIGO 1 - IMPACT OF NON-NUTRITIVE SWEETENERS ON THE SENSORY PROFILE AND ACCEPTANCE OF A FUNCTIONAL TAMARIND BEVERAGE

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Impact of non-nutritive sweeteners on the sensory profile and acceptance of a functional tamarind beverage

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ABSTRACT

The current consumer aversion for sucrose excess and the use of artificial ingredients has encouraged the production of beverages that preserve the fruit's wholeness and aggregate nutritional values, such as the availability of dietary fibers. Thus, the aim of this study was to evaluate the impact of non-nutritive sweeteners on the sensory profile and acceptance of a functional tamarind beverage. The tests were applied in individual cabins, with 30 mL samples, served in 3 random digits coded plastic cups, presented in monadic balanced complete blocks. Ideal sweetness, equi-sweetness, quantitative descriptive analysis (QDA) and consumer affective tests were performed, and results were analyzed through Analysis of Variance (ANOVA) and Tukey's means test ($p < 0.05$). The ideal sweetness of the reference sample was 10.70% of sucrose. For the quantitative descriptive analysis, 17 descriptive terms for the observed perceptions of the product were used. Most of the used sweeteners, except for stevia, showed no significant difference for perceptions tamarind aroma and flavor. The neohesperidin sample showed a greater intensity of refreshment sensation, while the sucrose and sucralose samples presented lower perception of bitter taste. The stevia sample had the least preference and greater perception of bitter taste, sweet and bitter aftertaste. Stevia and neohesperidin samples obtained lower acceptance by consumers regarding the overall impression, while sucralose had the best sweetness acceptance. Also, the astringency perception of the product interfered negatively in the acceptance of the samples.

Keywords: Sensory analysis, Quantitative descriptive analysis, Sweeteners, Functional food, Tropical food.

3.1 INTRODUCTION

The present scenario of increased prevalence of chronic noncommunicable diseases in the world observed by the World Health Organization [1] has led consumers to seek a better quality of life, which is usually linked to changes in the dietary pattern. This is evidenced by studies on the Beverage Industry's New Product Development Outlook, where consumers consider the "natural quality" as the most important attribute in a product [2].

This concern impacts on the beverage market, which tends to meet demand through products with different claims for health benefits. In the United States, for example, there was a reduction in the traditional beverages market due to consumer aversion to excess sucrose and artificial ingredients, and an increase in consumer requests for products with improved quality. Euromonitor shows that smoothies, coconut water and exotic fruit juices have gained strength due to a more premium positioning [3,4].

In this context, non-alcoholic beverage market invests in products that preserve the fruit and other vegetables integrity, as practical and easy alternatives for healthy eating. Fruit and vegetable juices are at the top of current global market trends, with an expected revenue of USD 198 billion in 2019 [5]. Developing exotic fruit beverages with sensory attributes, nutritional and functional claims is in the best interest of current consumer demands.

Following this scenario, a functional beverage with tamarind (*Tamarindus indica L*), besides its striking flavor, has a low glycemic index [6]. The fruit presents phenolic compounds such as catenin, procyanidin, epicatechin, tartaric acid, triterpenoid and other substances with important nutritional properties for a functional claim product [7,8].

Beyond the benefits of phenolic compounds [9], the addition of soluble dietary fibers, including fructooligosaccharides (FOS), has also been associated with health benefits. Adding FOS has positive effects on glycemic control, being associated with increased viscosity and gel formation of the intestinal contents, reduction in glucose diffusion through the water layer in the intestine and increased sensitivity to insulin [10].

Thus, based on the associated risk factors of diabetes on the population, and considering new consumer’s sucrose aversion and demands for natural and healthy products, the purpose of this study was to evaluate the impact of non-nutritive sweeteners on the sensory profile and acceptance of a functional tamarind beverage.

3.2 MATERIALS AND METHODS

3.2.1 Tamarind beverage sample

The beverage samples were made using a commercial frozen tamarind pulp (Ricaeli®, Cabreúva, Brazil) with addition of fructooligosaccharides (FOS) from SweetMix® (Sorocaba, Brazil). Seven different samples were made, the standard sample with added sucrose, and six subsequent samples with different sweeteners. União® sucrose (São Paulo, Brazil) was used to formulate the reference sample. The non-caloric sweeteners used for the subsequent samples were stevia with 97% of rebaudioside A (Clariant®, São Paulo, Brazil), sucralose, acesulfame-K, saccharin, neohesperidin and neotame (Nutramax®, São Paulo, Brazil).

The pulps were purchased and stored at -20 °C, as recommended by the manufacturer. The dilution ratio was 100 g of pulp per 200 ml of water. The amount of FOS was 1.5 g per 100 ml, according to the recommendations of the Brazilian legislation for liquid foods [11].

3.2.2 Proximate composition

The proximate composition of the samples base formulation was performed in quadruplicate, according to the Association of Official Analytical Chemists (AOAC) methodology for moisture, protein, carbohydrates and ashes [12]. Lipid determination was made by cold extraction [13]. Values were expressed in mean and standard deviation.

3.2.3 Sensory analysis

For this study, ideal sweetness, equi-sweetness, quantitative descriptive analysis and consumer affective tests were performed. All tests were carried out at the Laboratory of Sensory Analysis and Consumer Studies at the Department of Food and Nutrition the School of Food Engineering, University of Campinas. The research was approved by the Ethics and Research Committee of the University of Campinas (CAAE #84575518.0.0000.5404).

The participants of the research were invited through institutional e-mail and posters, available in different buildings at the University of Campinas. Before starting the tests, the individuals were informed about the criteria for participating in this research.

The tests were applied in individual cabins, at conditioned temperature of 22 ºC. Each sample consisted of 30 ml, served in 50 ml plastic cups coded with 3 random digits, presented in monadic balanced complete blocks, always with water availability. Data collection was performed by Fizz Sensory Software model 2.47b [14].

3.2.4 Ideal sweetness and equi-sweetness determination

Initially 122 consumers performed the ideal sucrose test [15] to define the ideal sweetness of the samples using Just about Right (JAR) scale. Five samples of the functional tamarind beverage (pulp + FOS) with different concentrations of sucrose were elaborated: 5.0g, 7.5g, 10.0g, 12.5g, and 15.0g / 100ml. Results were analyzed through distribution histograms of the sensory responses as a function of the sucrose concentration, and by simple linear regression analysis between the hedonic values and the sucrose concentration. For determining the sweetness equivalence by the magnitude estimation method, the tasters were pre-selected through WALD's sequential analysis [15], using triangular difference tests to select candidates with the ability to differentiate the samples. For triangular difference tests, three samples of a tamarind functional beverage (3.5%, 3.5% and 5% / 100mL) were prepared.

The sweeteners relative sweetness was defined by 12 selected tasters through the Magnitude Estimation [16] test. Five samples of the tamarind beverage with different concentrations of each sweetener were elaborated based on the ideal sucrose value, using a multiplying factor of 1.6 [17,18]. The selected and trained tasters received a 50 ml reference sample, with an arbitrary value of 100 for sweetness intensity, followed by several samples (30 ml each) in encoded, randomized cups, always with water to clean the palate.

Each sweetener was evaluated on different days, and the tasters estimated the sweetness intensities of the coded samples relative to the reference, e.g., a sample that presented twice the sweetness from the reference received the value 200, the one that presented half of the sweetness received the value 50, and so on [19]. The obtained values from the magnitude scale were converted to logarithmic values expressed in the geometric means. The relation between the concentration curves and the sensory response for each sweetener corresponded to a potency function [20].

3.2.5 Quantitative descriptive analysis (QDA®)

A total of 17 undergraduate, graduate students and employees from the University of Campinas were selected as panelists through WALD's sequential analysis. The panelists, all between 20 and 40 years old, male and female were then invited to evaluate seven samples of the tamarind beverage, one being the reference sample with sucrose, and six subsequent samples (with stevia with 97% of rebaudioside A, sucralose, acesulfame-K, saccharin, neohesperidin and neotame).

In the second stage, the repertory grid [19] test was applied to define the samples descriptive terms. The panelists received a paired combination of the beverage and individually described their similarities and differences regarding appearance, aroma, flavor and texture. Then they discussed together the chosen terms by each individual, and with the panel leader supervision they defined the descriptors that adequately described the assessed attributes among the samples, noting their definitions and suggesting references for training purposes [21]. Table 1 shows the seventeen consensually generated sensory descriptors, their written definitions and suggested references.

Table 1. Descriptive terms definitions and reference standards chosen by the QDA group

Attributes	Definitions	References
Appearance		
Particle presence	Particle/residue presence in the glass wall	Little: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Much: unfrozen tamarind pulp (Ricaeli®)
Turbidity	Reduced transparency due to the presence of suspended materials that interfere with the passage of light through the fluid	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: unfrozen tamarind pulp (Ricaeli®)
Brown color	Brown color characteristic of a tamarind drink	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: unfrozen tamarind pulp (Ricaeli®)
Aroma		
Acid	Acid aroma characteristic of the oxidation of tamarind juice	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: unfrozen tamarind pulp (Ricaeli®)
Tamarind	Characteristic aroma from natural tamarind juice	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: unfrozen tamarind pulp (Ricaeli®)
Sweet	Aroma related to aromatic compounds that provide the sweet sensation	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 30% sucrose (União®) heated to 70° C
Taste		
Acid taste	Acid taste characteristic of the oxidation of tamarind juice	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: unfrozen tamarind pulp (Ricaeli®)
Sweet taste	Sweet taste characteristic of added sucrose or sweetener	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 30% sucrose (União®)
Tamarind	Characteristic flavor of natural tamarind	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: unfrozen tamarind pulp (Ricaeli®)
Bitter taste	Bitter taste characteristic of caffeic acid	None: filtered water Strong: tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 0.208% stevia (Clariant®)
Bitter aftertaste	Bitter taste that lingers in the mouth after swallowing	None: filtered water Strong: tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 0.208% stevia (Clariant®)
Sweet aftertaste	Sweet taste that lingers in the mouth after swallowing	None: filtered water Strong: tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 0.011% neohesperidin (Nutramax®)

Attributes	Definitions	References
Astringency	Resulting sensation from the contraction of the mouth muscles caused by substances such as tannins	Weak: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Strong: unfrozen tamarind pulp (Ricaeli®)
Refreshment sensation	Sensation of vigor, freshness	None: filtered water Strong: tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 0.011% neohesperidin (Nutramax®)
Texture		
Viscosity	Perceived flow time during swallowing	Little: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Much: unfrozen tamarind pulp (Ricaeli®)
Body	Capacity of the mouth filling, consistency of a beverage	Little: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Much: unfrozen tamarind pulp (Ricaeli®)
Particle presence	Particle/residue presence in the mouth after swallowing	Little: tamarind pulp (Ricaeli®) 1-part pulp / 6-parts water Much: unfrozen tamarind pulp (Ricaeli®)

In the third stage, a round table was conducted, where the samples' attributes for "little", "much", "weak", "strong", and "none" were displayed during a week, being constantly replaced to maintain temperature and quality for training. The panelists carried out the training for at least four times so that they could retain the memory to recognize the characteristics evaluated in the products.

In the fourth stage, the panelists performed the intensity determination tests for each of the descriptive terms. In individual booths, a 9 cm linear intensity scale (non-structured), with sequential monadic presentation of the seven samples, evaluated in quadruplicate was used [22]. The results were analyzed by the Statistical Analysis System (SAS) [23], expressed by Analysis of Variance (ANOVA) with two sources of variation between sample and reference, for each attribute and each panelist, where the p-values of the F-test were obtained both for sample and repetition. With this analysis the panelists were selected according to their discriminating ability ($p\text{-value} \leq 0.30$), replication ($p\text{-value} > 0.05$), and agreement with the rest of the panel.

After the previous tests, 12 panelists were selected to perform a new evaluation of the seven samples, during 4 sessions, using a 9-point scale for each attribute descriptive term. The QDA results were obtained by analyzing the attributes from the sensory profile through Analysis of Variance (ANOVA), followed by a Tukey's means test and Principal Component Analysis (PCA).

3.2.6 Consumer study

For this study, 113 untrained consumers aged from 18 to 60 years who presented habitual consumption of tropical fruit evaluated the seven tamarind functional beverage samples, analyzing appearance, aroma, taste, texture and overall impression of the samples. The test was applied using a 9 cm non-structured linear hedonic scale anchored with "dislike extremely" on the left and with "like extremely" on the right [16].

The acceptance results were analyzed by ANOVA, using two factors (consumer and sample), and Tukey's means test ($p < 0.05$). The QDA data were compared with the consumer preference data using partial least squares (PLS) regression [24,25]. For statistical analysis, XLSTAT for Windows [26] with a 5% level of significance was used.

3.3 RESULTS AND DISCUSSION

3.3.1 Proximate composition and sweetness ideal

The results of the proximate composition of the base formula beverage (without sucrose) are shown in Table 2.

Table 2. Proximate composition of the base sample tamarind functional beverage (Pulp + FOS)

Proximate composition	Sucrose-free beverage Means ± SD[1] (%)
Moisture	96.30 ± 0.08
Protein	0.22 ± 0.09
Lipids	0.19 ± 0.04
Ashes	0.18 ± 0.00
Carbohydrates	3.16 ± 0.16

[1]Mean values ± standard deviation (SD)

n=4 (quadruple analysis)

According to Brazilian resolution [27], the functional beverage can be classified as a low energy food (< 20 kcal / 100 mL), with a non-significant amount of lipids (5 g / 100 mL). The proximate composition was not performed for the other samples, since the sweeteners had a very low (close to zero) caloric contribution [28].

These results indicate that the tamarind beverage has a low-calorie claim. Currently there is a growing market for low-calorie and low-sugar products, especially beverages with low-calorie sweeteners [29]. In the United States, for example, there is a trend for these products market as national policies and industry efforts encourage manufacturers to reformulate and reduce the energy density of food products [30].

The samples' ideal sweetness was obtained through the ideal sucrose test by simple linear regression analysis between the hedonic values and the sucrose concentration. The result value was 10.70%, higher than other studies with different fruits, such as mango [17], passion fruit [18], peach [29], and pitanga [31], possibly due to the tamarind acidic property by the presence of tartaric acid [32]. However, the high value of sucrose ideal is not only explained by the acidic characteristic of the product, but also by factors like the food pattern of a population.

In Brazil, according to a food consumption survey that analyzed the registration of foods, beverages and preparations consumed inside and outside the household, there was an excessive consumption of sugar by more than 60% of the population [33]. Soft drinks and juices are directly related to this high sucrose consumption, indicating the preference of Brazilians for sweeter foods [33], and revealing a concern to replace or reduce the sucrose of these products.

With the ideal sucrose value, the concentration of each sweetener was defined by the magnitude estimation method, where the tasters analyzed coded samples compared to the reference sample with 10.70% sucrose. The logarithmic scale of each sweetener concentration and their sweetness perception as a reference to the sucrose scale, represented by the standard sample is shown in Figure 1. The samples with neotame and neohesperidin required smaller amounts of the product, while a greater amount of stevia was used to obtain a better sweetness perception. The samples with acesulfame-K and saccharin presented intermediate values.

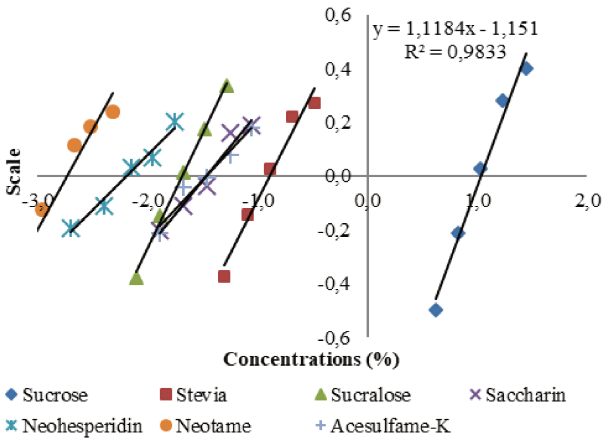

Figure 1. Relation between perceived sweetness and sweetener concentrations of the tamarind functional beverage by logarithmic scale

The equivalent concentration and sweetness potency of each sweetener compared to the reference sample with 10.70% sucrose are shown in Table 3. The stevia sample presented the highest equivalent concentration, but with lower sweetness potency (86x). On the other hand, a small amount of neotame was needed to obtain a 5,944 times greater sweetness potency. Neohesperidin also presented a high sweetness potency, inversely proportional to the used concentration. The same concentration and sweetness potency (315x) for acesulfame and saccharin were observed.

Table 3. Equivalent concentration and sweetness potency of the tamarind functional beverage compared to the reference sample with 10.70% of sucrose

Sweetener	Equivalent concentration (%)	Sweetness potency (times)
Sucralose	0.0200	535
Acesulfame-K	0.0340	315
Stevia	0.1295	86
Saccharin	0.0340	315
Neohesperidin	0.0064	1,672
Neotame	0.0018	5,944

The sweetness potency found in the samples with acesulfame-K differed for more, and stevia differed for less from the values found in the literature [17,28]. The substance must first be dissolved into the saliva and then contact the tongue receptors so the sweet taste intensity can be perceived [28]. Thus, the high percentage of rebaudioside in the stevia sample and the presence of other substances may interfere with the sweet taste perception mechanism of the samples [34].

3.3.2 Quantitative Descriptive Analysis (QDA®) and Consumer Study

After obtaining the sweetness ideal and sweetness equivalence of the sweeteners, the seven samples for the QDA were defined. 12 tasters evaluated the attributes appearance, aroma, flavor and texture considering the 17 descriptors. The results of Tukey's means test are shown in Table 4, where samples with equal caption letters do not differ significantly from each other ($p \leq 0.05$). According to the table, all sweeteners (except stevia), did not present significant difference for important perceptions like tamarind aroma and flavor. Neohesperidin and acesulfame-K presented the lowest intensity for sweet taste, while stevia presented the highest perception of sweet taste and bitter taste. Sucrose and sucralose were the sweeteners with the lowest perception of bitter taste, while neohesperidin showed the highest intensity refreshment.

These results are supported by the literature. Sucralose, for example, is known to have less bitter taste compared to other sweeteners [35], while stevia has a bitter component and neohesperidin has a very slow sweetening speed and residual taste of menthol, which is responsible for the refreshment sensation [28]. The lower bitter perception of sucralose may be a determining factor in its choice, since it is reported as the most used sweetener by consumers [29]. The bi-dimensional representation of the Principal Component Analysis (PCA) of the samples' descriptive terms are shown in Figure 2A. The red vectors represent the QDA defined descriptors, while the blue circles are the descriptors PCA. Principal components 1 and 2 together explain 69.24% of the variations between the samples. The stevia sample is isolated from the other ones, indicating a difference regarding the sensory attributes. The proximity of saccharin and neohesperidin indicates a possible similarity, especially as to appearance by the presence of particles.

According to the external preference map (Figure 2B) the consumers (represented by the blue circles) were concentrated next to the samples (green squares) with greater acceptance. There is a higher concentration of consumers close to the sucrose and sucralose samples, characterized by acid aroma and viscosity. On the opposite side, stevia had the lower concentration of consumers. The sweetener is characterized by bitter taste, residual sweet and residual bitter, which possibly were considered as negative characteristics for the sample.

Table 4. Tukey's means of Quantitative Descriptive Analysis (QDA) of the functional tamarind beverage for the terms sensorial descriptors evaluated. Means in the same line showing common letter are not significantly different (*p* ≥ 0.05)

Attributes	Sucrose	Sucralose	Acesulfame-K	Stevia	Saccharin	Neohesperidin	Neotame	MSD[1]
Particle presence	4.1^{a}	4.31^{a}	4.15^{a}	4.02^{a}	4.37^{a}	4.4^{a}	4.2^{a}	0.47
Turbidity	5.02^{bc}	5.03^{bc}	4.87^{c}	5.29^{ab}	5.31^{ab}	5.41^{a}	5.19^{abc}	0.35
Brown color	3.95^{ab}	4.36^{a}	4.25^{a}	3.6^{b}	4.22^{a}	4.25^{a}	4.18^{a}	0.52
Acid aroma	5.19^{a}	5.15^{a}	4.65^{a}	4.49^{a}	5.01^{a}	5.2^{a}	5.04^{a}	0.82
Tamarind aroma	5.7^{ab}	5.86^{a}	5.76^{a}	5.23^{b}	5.61^{ab}	5.87^{a}	5.47^{ab}	0.51
Sweet aroma	3.52^{a}	3.93^{a}	3.46^{a}	4.1^{a}	4.26^{a}	3.43^{a}	3.77^{a}	0.90
Acid taste	5.46^{bc}	5.38^{c}	5.89^{abc}	5.31^{c}	6.05^{ab}	6.29^{a}	5.78^{abc}	0.59
Sweet taste	5.14^{bc}	5.75^{ab}	3.19^{e}	6.57^{a}	4.38^{dc}	3.95^{de}	5.71^{ba}	1.02
Tamarind flavor	5.7^{a}	5.86^{a}	5.76^{a}	5.23^{b}	5.61^{ab}	5.87^{a}	5.47^{ab}	0.51
Bitter taste	1.54^{de}	1.28^{e}	3.54^{ba}	4.58^{a}	3.07^{bc}	2.38^{dc}	2.41^{dc}	1.07
Bitter aftertaste	1.22^{c}	1.2^{c}	2.59^{b}	3.82^{a}	2.11^{cb}	1.58^{cb}	1.56^{c}	1.02
Sweet aftertaste	1.67^{cb}	2.16^{cb}	1.24^{c}	4.67^{a}	2.14^{cb}	2.41^{b}	4.43^{a}	1.02
Astringency	4.08^{bc}	4.05^{c}	4.76^{a}	4.54^{bac}	4.49^{bac}	4.72^{ba}	4.43^{bac}	0.65
Refreshment sensation	1.67^{cb}	2.09^{cb}	1.52^{c}	2.25^{b}	1.77^{cb}	3.15^{a}	2.32^{b}	0.67
Viscosity	4.46^{ba}	4.78^{a}	4.36^{ba}	4.25^{b}	4.82^{a}	4.66^{ba}	4.52^{ba}	0.49
Body	3.95^{ba}	4.36^{a}	4.25^{a}	3.6^{b}	4.22^{a}	4.25^{a}	4.18^{a}	0.52
Particle presence (texture)	4.1^{a}	4.31^{a}	4.15^{a}	4.02^{a}	4.37^{a}	4.4^{a}	4.2^{a}	0.47

[1] Minimum significant difference

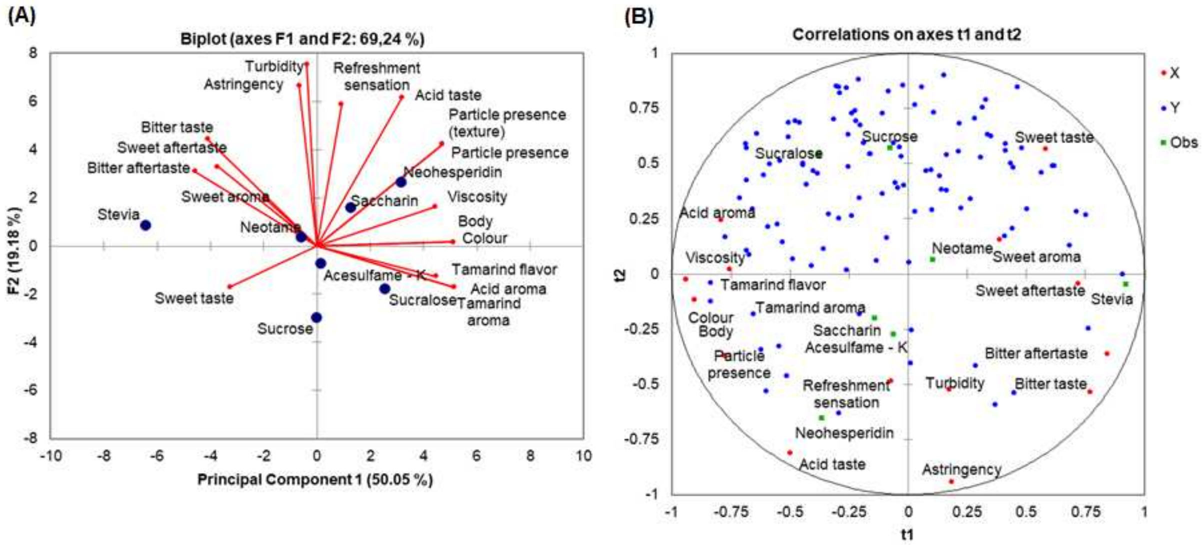

Figure 2. (A) Principal Component Analysis (PCA) generated with the sensory data for appearance, aroma, flavor and texture of the tamarind functional beverage. (B) External preference map. Axes t1 and t2 were obtained by partial least squares regression of descriptive data and respondent's overall liking scores for the sensory attributes of the tamarind functional beverage (square = samples; blue circle = consumers; red circle = QDA attributes)

The correlation between the overall impression and sensory descriptors is shown in Figure 3. The columns on the positive side of the Y axis are positively correlated with the acceptance of the functional tamarind beverage samples, while the columns on the negative side represent the attributes that were negatively correlated with the samples' acceptance. The vertical lines represent a 95% confidence interval. The astringency was the only attribute that affected negatively sample's choice, i.e., the higher the astringency the smaller the acceptance of the sample. The astringency perception in the results can be originated by the interaction of the tannins present in the tamarind pulp with the salivary proteins of the buccal mucosa. This perception is considered as an unpleasant sensory attribute, characterized as a dry and frowned mucosa sensation, observed mainly with the consumption of foods of plant origin due to the amount of proanthocyanins [36,37].

Due to this negative association of astringency sensation with the product acceptance, it is necessary to identify means that allow a lesser perception of this stimulus. Some environmental factors associated with tamarind production, such as soil characteristics of each production site, season of the year, stage of development and mineral availability affect the level of tannins in the fruits and, therefore, in the astringency perception. There are also several secondary treatments available in the food industries that can decrease the astringency level in fruits, so it is necessary to identify the best process that can be used in the preparation of beverages.

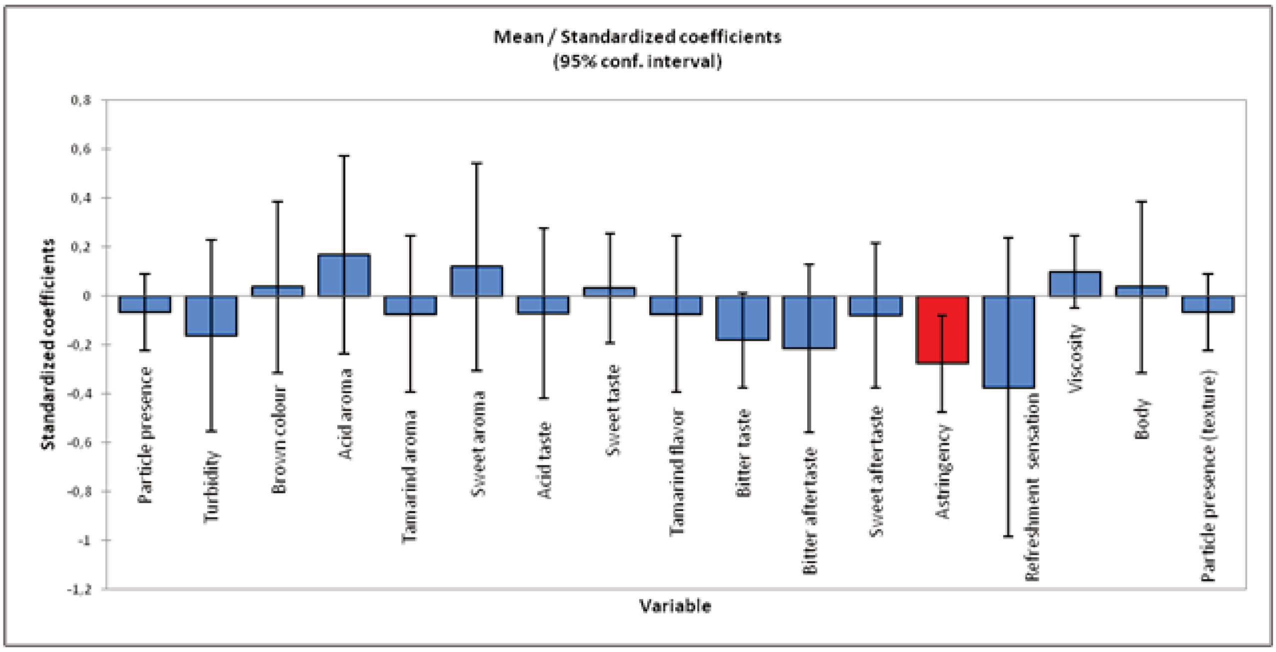

Figure 3. Partial least squares standardized coefficients of the tamarind functional beverage (red = descriptor term with negative contribution to consumer acceptance)

3.4 CONCLUSION

There was an evident impact of the different sweetening agents used in the sensory profile and acceptance in the studied beverage. With the QDA®, neohesperidin and acesulfame-K had the lowest intensity for sweet taste, while stevia presented the highest perception of sweet taste and bitter taste. Sucrose and sucralose presented the lowest perception of bitter taste, while neohesperidin showed the highest intensity of refreshment sensation. Consumers found stevia as the sweetener with the highest bitter taste perception, sweet and bitter aftertaste. The consumer study also showed that the beverage astringency attribute negatively affected the preference. Other studies are encouraged to evaluate the duration of different perceptions resulting from the addition of sweeteners in the functional tamarind beverage or other fruits and vegetables.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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4. ARTIGO 2 – SUCROSE REPLACEMENT: A SENSORY PROFILE AND TIME INTENSITY ANALYSIS OF A TAMARIND FUNCTIONAL BEVERAGE WITH ARTIFICIAL AND NATURAL NON-NUTRITIVE SWEETENERS

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Sucrose replacement: A sensory profile and time-intensity analysis of a tamarind functional beverage with artificial and natural non-nutritive sweeteners

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ABSTRACT

BACKGROUND: Tamarind pulp contains polyphenolic compounds that exert antioxidant and anti-inflammatory activities with a positive impact on human health. The elaboration of a tamarind based functional beverage, without the addition of sucrose, can be an alternative to traditional caloric beverages. This study aimed to evaluate the sensory profile and time intensity of tamarind functional beverage containing artificial and natural non-nutritive sweeteners.

RESULTS: The results of the acceptance test, check-all-that-apply, and time-intensity test showed that the sample sweetened with sucralose presented no statistically significant difference between the obtained means for the attributes relating to appearance, aroma, flavor, texture, and overall impression compared to those of the sample with sucrose. Samples with natural sweeteners had lower means of overall product impression and lower percentage of purchase intention. The perception of astringency, bitter taste, and bitter aftertaste may be linked to the lower global impression of the product. The descriptors “tamarind flavor” and “refreshment sensation” were higher in products that were more liked. The stevia sweetened sample showed higher levels of sweetness, bitterness, and longer sweet stimulus duration in the time-intensity test.

CONCLUSION: The sample sweetened with sucralose was the best alternative to sucrose in the functional tamarind beverage. The analyzed sweeteners did not show changes in the perception of natural characteristics of the used fruit, such as tamarind flavor, refreshment sensation and astringency. However, the attributes related to sweet and bitter aftertaste experienced in samples with natural sweeteners may have influenced the decrease in the product purchase intention.

Keywords: Time-intensity. Acceptance. Sweeteners. Functional food.

4.1 INTRODUCTION

Tropical countries produce a large number of native and exotic fruit species that generate great interest in the food industry, especially because of the presence of phenolic compounds[1]. Tamarind (*Tamarindus indica* L.) is a tropical fruit with a distinctive bittersweet flavor and contains phenolic and aromatic compounds; has gathered commercial interest because of its antioxidant properties that can benefit human health[2].

Several studies have shown the positive effects of phenolic compounds of tamarind such as catenin, procyanidin, epicatechin, tartaric acid, and triterpenoids, in human health[3,4]. These substances, which are also present in vegetables act as antioxidant and anti-inflammatory, agents and help to reduce the risk of chronic non-communicable diseases, cardiovascular disease and cancer[4].

Therefore, the inclusion of these antioxidants compounds in beverages is associated with caloric reduction and may be an alternative to caloric beverages without added nutritional value, which are currently showing a reduction in consumption because of new market trends related to dietary changes in search of healthy alternatives[5].

The current trend is toward the development of exotic fruit-based functional food products, which demonstrate bioactive activities with a positive impact on chronic diseases[1,5]. Fruit juice blends are also gaining importance in the market, probably because of the public perception of juices as a healthy natural source of nutrients, and the increasing consumers demand for considering health benefits[6].

Therefore, to meet this new market, the research and development sectors of the food industry have made changes in the sector of ingredients with sensory and quality functions. A frequent example is the use of sweeteners as sucrose substitutes, as studies have shown that the consumption of non-caloric beverages has a beneficial effect on weight management[8,9,10].

The most important aspect of sweeteners is undoubtedly their sweetness potency. Thus, sweeteners have been used as alternatives for common sugar, such as sucrose and fructose in the food industries, in response to the increased prevalence of diseases related to the overuse of sucrose. Various studies have been carried out on sweeteners to evaluate the sweetness potency in different products, and their impact on consumers' health and economy[10].

However, substituting sucrose is challenging, as alternative sweeteners can change the physical characteristics of beverages and the perception of bitter and sweet taste. In this context, sensory analysis research is important in providing strategic data for the preparation of a functional low-calorie beverage retaining the characteristic appearance, aroma, flavor, and texture as close as possible to those of a traditional product to be to the consumers[11].

Considering the challenge involved in replacing sucrose by sweeteners for the product's acceptance, this study aimed to evaluate the sensory profile and time-intensity in a sucrose-free tamarind functional beverage with artificial and natural non-nutritive sweeteners.

4.2 MATERIALS AND METHODS

4.2.1 Ethical aspects

The research was approved by the Research Ethics Committee of the University of Campinas - UNICAMP under number CAAE: 84575518.0.0000.5404.

4.2.2 Sampling

Tamarind functional beverages were prepared using frozen tamarind pulp (Ricaeli®, Cabreúva, SP - Brazil, 2018) as the basic formulation, with the addition of fructooligosaccharides (FOS) (Sweet Mix, Sorocaba, SP - Brazil). The pulps were purchased from the local market and stored in a freezer at -20 °C, according to the manufacturer's instructions. A dilution ratio of 100g of pulp per 200mL of water was used. The amount of fructooligosaccharide was standardized to 1.5g/ 100mL, according to the recommendations for liquid foods of the Brazilian legislation[12].

The samples differed in the addition of sucrose (standard sample) and 6 non-nutritive sweeteners (4 artificial and 2 natural), selected as the most used in the low-calorie food industry[10]. For the control formulation, 10.70% sucrose (União®, São Paulo, SP - Brazil) was used, as calculated by the ideal sucrose test performed on 122 consumers who tested the ideal sweetness through just about right (JAR) based on 5 tamarind functional beverage samples with different sucrose concentrations (5.0; 7.5; 10.0; 12.5 and 15.0g/100mL)[13]. The results were analyzed through distribution histograms of the sensory responses as a function of the sucrose concentration, and by simple linear regression analysis between the hedonic values and concentration of sucrose[13].

The concentration of the sweeteners was calculated as the sweetness equivalence of 10.70% sucrose and was determined by the magnitude estimation method. Twelve consumers were pre-selected through WALD's sequential analysis[14], in which 12 triangle tests are applied (in complete balanced block design) to select subjects with a good ability to discriminate samples. A series of 12 triangular tests were conducted in which the candidates were provided 2 functional tamarind beverage samples: A (containing 3.5 g/100 mL sucrose) and B (containing 5.0 g/100 mL sucrose), with significant difference of 0.1% in relation to sweetness (firstly proven by paired difference test).

Then, the consumers participated in the Magnitude Estimation test with 5 different concentrations for each sweetener and elaborated based on the ideal sucrose value using a multiplying factor of 1.6 [13,14,15]. The selected and trained consumers received a 50mL reference sample, with an arbitrary value of 100 for sweetness intensity, followed by several samples (30mL each) in encoded and randomized cups, always with water to clean the palate.

Each sweetener was evaluated on different days, and the consumers estimated the sweetness intensities of the coded samples relative to the reference. For example, a sample that presented twice the sweetness from the reference received a value of 200, the one that presented half of the sweetness received a value of 50, and so on[16]. The obtained values from the magnitude scale were converted to logarithmic values expressed in geometric means. The relation between the concentration curves and the sensory response for each sweetener corresponded to a potency function[16].

As a result, the concentration in percentage for each sweetener was obtained. They were transformed in grams per 100 milliliters (g/100mL) of the tamarind functional beverage and the results are shown in Table 1.

Table 1. Equivalent concentration and sweetness potency of the tamarind functional beverage of 10.70% sucrose

Sweetener	Concentration (g/100mL)	Sweetness potency (times)
Sucralose*	0.18	535
Acesulfame-K*	0.31	315
Stevia 97% Rebaudioside A**	1.12	86
Saccharin*	0.31	315
Neohesperidin*	0.05	1,672
Neotame*	0.016	5,944

*Nutramax®, São Paulo, SP – Brazil. **Clariant®, São Paulo, SP - Brazil

4.2.3 Physicochemical characterization

The sucrose sample and the 6 samples sweetened with different sweeteners were analyzed for pH, titratable acidity, soluble solids, and color. The pH was determined by direct reading using a METTLER TOLEDO 8603 potentiometer calibrated with pH 4.0 and 7.0 buffer solutions according to AOAC[17]. The percentage of soluble solids (in °Brix) was determined using a HANNA HI96801 refractometer. The total titratable acidity was determined by titration with 0.1mol/L NaOH solution using phenolphthalein (1%) as an indicator, and the results have been expressed as percentages (g citric acid/100mL sample). Then, the SS/TA ratio was calculated using the results of soluble solids (°Brix) and titratable acidity.

The color of the samples (L*, a*, b*) was determined on a HUNTERLAB COLOR QUEST II COLORIMETER (Reston, Virginia, USA). The instrument was calibrated with the illuminant D65 (6900K), and the reading was acquired using a 10mm quartz cuvette, illuminant C and hue of 10°, using Regular Transmission at the moment of reading and a white reference plate (C6299 HUNTER COLOR STANDARD)[18,19].

4.2.4 Acceptance Tests

Acceptance tests were used to assess the acceptance of consumers considering their preferences and perceptions, using the product's hedonic scale and purchase intention test. Consumers received 30mL samples at 4 °C in a monadic manner in balanced complete blocks, and data collection was done using the Fizz Sensory Software model 2.47 B program[20]. The consumers were given water between samples to cleanse the palate.

The 7 tamarind functional beverage samples were evaluated by 113 consumers, female (57%) and male (43%) aged from 18 to 60 years old, with a mean age of 33 years (staff, undergraduate, and graduate students at UNICAMP who presented habitual consumption of tropical fruit), and who analyzed the appearance, aroma, flavor, texture, and overall liking of the samples. The test was applied using an unstructured 9cm linear scale anchored to the left and right extremes with the terms "extremely disliked" and "extremely liked", respectively. Additionally, consumers were questioned regarding the purchase intention for each sample, using a 5-point purchase attitude scale[14].

4.2.5 Check-All-That-Apply (CATA)

The same consumers who participated in the acceptance test (n=113) answered the CATA questionnaire. They received the CATA questionnaire with a list of sensory descriptors, which were generated by a group of trained assessors[21]. Twelve descriptors that were considered were as follows: brown color, watery, body, viscous, acid, sweet, bitter, tamarind, astringency, sweet aftertaste, bitter aftertaste, and refreshment sensation.

The consumers were asked to select all the descriptors they deemed necessary to describe the sensory characteristics of the samples. For this purpose, a list of descriptors organized in a balanced way was used, according to method recommendations[22,23].

4.2.6 Time-intensity analysis (TI)

A pre-selection of the assessors was performed previously by Wald's sequential test[14] to select potential assessors for the time-intensity test. Seventeen assessors were pre-selected and trained in 6 sessions of 1h each, to acquire sensory memory and agreement between the teams. The training was performed using the maximum intensity of the stimulus evaluated (sweet taste, acid taste, bitter taste, tamarind flavor, refreshment sensation, and astringency) according to Table 2, considering the descriptors and references established in a previous study on tamarind functional beverage with the same sweeteners used in this study[13]. The 6 attributes were evaluated in separate instances.

Table 2 – Description of the attributes and references definitions used for assessors' training of the tamarind functional beverage

Descriptor	Definition	Reference
Sweet taste	Sweet taste characteristic of added sucrose or sweetener	Tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 30% sucrose (União®)
Acid taste	Acid taste characteristic of the oxidation of tamarind juice	Unfrozen tamarind pulp (Ricaeli®)
Bitter taste	Bitter taste characteristic of caffeic acid	Tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 0.208% stevia (Clariant®)
Tamarind flavor	Characteristic flavor of natural tamarind	Unfrozen tamarind pulp (Ricaeli®)
Refreshment	Sensation of vigor, freshness	Tamarind pulp (Ricaeli®) 1-part pulp / 2-parts water / 0.011% neohesperidin (Nutramax®)
Astringency	Resulting sensation from the contraction of the mouth muscles caused by substances such as tannins	Unfrozen tamarind pulp (Ricaeli®)

The time-intensity test was carried out in the Laboratory of Sensory Science and Consumer Study of the School of Food Engineering at the University of Campinas, São Paulo, Brazil, in a controlled environment at 22 °C. The assessors evaluated 7 samples of the tamarind functional beverage (30mL), served in monadic sequence in disposable plastic cups coded with random 3-digit numbers in balanced complete blocks.

During training, the assessors performed the TI test, where they received each sample individually and evaluated it for 85 seconds. At the first signal given by the computer, the assessors were asked to place the total amount of the sample in the mouth, and then using the mouse, to start identifying the intensity of the specific sensory attribute on the structured linear scale from zero to nine (0 = none, 4.5 = moderate, 9 = strong). After 5 seconds, upon hearing the second signal, the assessors were told to swallow the sample while a third signal, 80 seconds after the second signal, indicated the end of the test[24,25].

Data were collected during each sensory evaluation, and the following parameters were evaluated: Imax (maximum intensity recorded); TImax (time at which the maximum intensity was recorded); Area (area of the time-intensity curve); DurPI (duration of maximum intensity), and Ttot (total duration of the stimulus).

Two TI tests were carried out, one to train the assessors and the other as a definitive test. The training was validated based on the assessors' capacity of reproducibility and repeatability of the TI test in triplicate on different days. Analysis of variance (ANOVA) was used for each assessor and parameter, and 10 participants were selected to participate according to their discriminatory ability ($p \leq 0.30$) and repeatability ($p \geq 0.05$).

Then the 10 selected assessors performed the TI test once again, evaluating 7 samples of the tamarind functional beverage (30mL), presented in monadic balanced complete blocks, and served in disposable plastic cups coded with random three-digit numbers. The test was conducted in triplicate on different days.

4.2.7 Statistical Analysis

The statistical analysis for the physicochemical properties of the samples (pH, titratable acidity, soluble solids, and color) was performed using Tukey's Honestly Significant Difference procedure (HSD). The acceptance test was analyzed by two-way ANOVA (consumer and sample) and Tukey's Honestly Significant Difference procedure ($P < 0.05$). The purchase intention data were analyzed by the histogram of purchase intent scores. Principal Component Analyses (PCA) was also performed by correlating the overall liking data with the descriptive results of CATA using XLSTAT for Windows[26].

In CATA questionnaire, the analysis of the results obtained based on the frequency of mention for each descriptor of the CATA question was determined by counting the number of consumers who used the descriptor to describe each sample. To evaluate whether the CATA questionnaire was able to detect the differences in consumers' perception, the Cochran's Q test was performed for each descriptor, considering the sample and consumption variation factors[27].

The TI test results were analyzed by Analysis of Variance (ANOVA), followed by Tukey's Honestly Significant Difference procedure, Principal Component Analysis, and represented by time-intensity curves. Correlation between the TI parameters and consumer test data was determined by Partial Least Squares (PLS) regression analysis using the software XLSTAT. The overall impression was the dependent variable (Y-matrix), and the TI parameters were the independent variables (X-matrix) [15,28].

4.3 RESULTS AND DISCUSSION

4.3.1 Physicochemical characterization

The results of the physicochemical characterization are shown in Table 3. Higher pH values were observed in the tamarind functional beverages than the passion fruit beverages[19], which were lower than those found in tropical fruit blends such as cashew, passion fruit, and guava (> pH 3.6)[29], and mixed beverages containing prebiotics[30]. The samples sweetened with saccharin and neohesperidin significantly differed from each other, having the highest and lowest pH values, respectively. A significant difference was observed for neohesperidin in the other samples, which had a lower titratable acidity. Regarding the total soluble solids and SS/TA ratio of the samples, significant differences were observed between sucrose and the other samples, which was expected since sucrose is a soluble solid[19,31].

Table 3 – Physicochemical characterization of the tamarind functional beverage samples

Samples	pH	Titratable acidity (%)	°Brix	Ratio¹	L*	a*	b*
Sucrose	2.86^{ab}	0.58^{ab}	13.1^{a}	22.29^{a}	41.82^{a}	4.91^{bc}	13.73^{ab}
Sucralose	2.88^{ab}	0.59^{ab}	4.36^{d}	7.33^{d}	41.75^{ab}	4.86^{cd}	13.59^{bc}
Acesulfame - K	2.87^{ab}	0.60^{a}	4.63^{bc}	7.64^{cd}	41.77^{ab}	5.00^{ab}	13.82^{a}
Stevia	2.86^{ab}	0.57^{b}	4.60^{bc}	8.00^{bc}	41.70^{ab}	4.79^{d}	13.55^{c}
Saccharin	2.91^{a}	0.60^{ab}	4.73^{b}	7.86^{cd}	41.83^{a}	5.03^{a}	13.84^{a}
Neohesperidin	2.83^{b}	0.53^{c}	4.60^{bc}	8.57^{b}	41.41^{b}	4.94^{abc}	13.68^{abc}
Neotame	2.86^{ab}	0.59^{ab}	4.50^{cd}	7.58^{cd}	41.41^{b}	4.92^{bc}	13.58^{bc}

Means with same letters in a same line each parameter indicate that samples do not have statistical difference at a significance level of 5% by Tukey's Honestly Significant Difference procedure (HSD).
*L = luminosity; +a = red, -a = green; +b = yellow, -b = blue.
¹Ratio of °Brix and titratable acidity (%).

Regarding the luminosity, the L* values were low (41.41 to 41.83) when compared to the luminosity scale ranging from 0 to 100[29]. The samples sweetened with stevia and sucralose had significantly lower averages when compared to the others, with a not extraordinarily strong red color but with a tendency toward yellow.

4.3.2 Acceptance Tests

The results of the acceptance test are shown in Table 4. Samples did not differ in liking based on the aroma. The flavor liking of the neohesperidin sweetened sample was significantly lower than the other samples. There was no significant difference in the overall or modality-specific liking of samples sweetened with sucralose and samples sweetened with sucrose. Overall, these samples were liked more than the samples sweetened with the other sweeteners. The neohesperidin sweetened sample were the least liked samples overall.

Table 4 – Means of Tukey's test for acceptance of differences of the tamarind functional beverage

Samples	Appearance	Aroma	Flavor	Texture	Overall liking
Sucrose	4.45a	4.50a	4.80a	5.53a	4.77a
Sucralose	4.20ab	4.47a	4.82a	5.37ab	4.70ab
Acesulfame - K	3.99b	4.93a	3.68bc	4.91bc	3.95cd
Stevia	4.08ab	4.35a	3.27bc	4.89bc	3.57de
Saccharin	4.17ab	4.51a	3.96b	5.14abc	4.18bc
Neohesperidin	4.01b	4.33a	2.99d	4.66bc	3.38e
Neotame	4.12ab	4.40a	3.90bc	5.13abc	4.14c
MSD**	0.41	0.52	0.63	0.54	0.53

* Means with same letters in a same column each parameter indicate that samples do not have statistical difference at a significance level of 5% by Tukey's Honestly Significant Difference procedure (HSD).

** Minimum significant difference.

The lowest overall liking of the samples may be because of the characteristic bitterness and bitter aftertaste of stevia[10,31] and the slow sweetness of neohesperidin along with a refreshing aftertaste[10]. The similar scores observed for sucralose and sucrose may be probably because of the stability of the sucralose in beverages and not having any interaction with the other sample ingredients, and thus not showing a marked bitter taste of the other sweeteners[13,32,33]. In addition, sucralose is widely used in the food industry (approved in more than 60 countries) for its acid taste and heat stability[32]. Thus, this sweetener may have maintained the characteristic acidity of tamarind.

To better understand the consumers' perception, a purchase intention distribution was also performed, as shown in Figure 1. It is possible to verify that the samples sweetened with stevia and neohesperidin were the least accepted by the consumers, and once they know that, more than 30% of them certainly would not buy these products. The sucralose-sweetened sample was closer to the control sample.

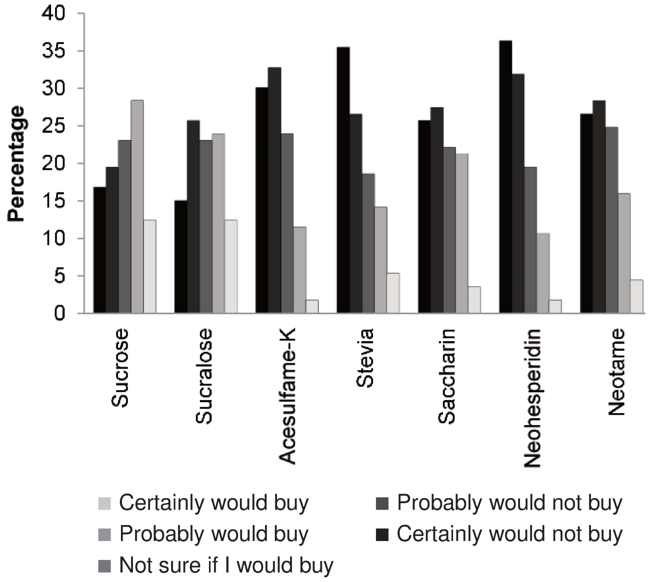


Figure 1 – Frequency distribution of scores corresponding to the scale used for the evaluation of the purchase intention of the tamarind functional beverage samples (%)

4.3.3 Check-All-That-Apply (CATA)

Figure 2(A) shows the positioning of each sample to the descriptors established by CATA. The sucralose sweetened sample was characterized by the attributes related to “body”, “tamarind flavor”, and “refreshment sensation”, similar to those found on the sucrose control sample. However, the samples sweetened with stevia and neotame were characterized by two descriptors, “sweet” and “sweet aftertaste”, whereas the samples sweetened with acesulfame-K and neohesperidin showed descriptors that are considered as negative attributes in a beverage, such as “bitter aftertaste”, “bitter”, and “watery”.

Figure 2(B) shows the distribution of the descriptors regarding the overall liking of the product. It is possible to observe that the samples described by the attributes “tamarind flavor” and “refreshment sensation” were closer to the overall liking. De Carvalho et al.[34] used consumers’ test to evaluate passion fruit nectar and reported that the most common descriptors were “characteristic of fresh fruit”, “color”, and “acid taste”, that is, the characteristics of fresh fruit were expected in the product. In this research, although the descriptors related to the flavor and color of the tamarind fruit were also important, the descriptor "refreshment sensation" emerged as a differential for the tamarind beverage.

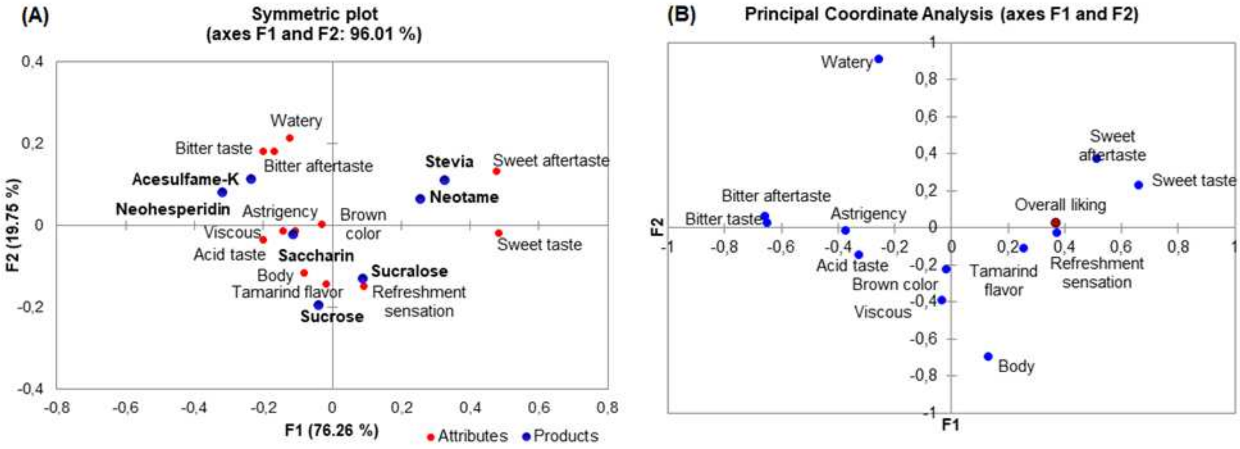


Figure 2 - (A) Distribution of CATA questionnaire's descriptive terms considering different evaluated sweeteners samples. (B) Correlation between the CATA descriptive terms and overall liking of the tamarind functional beverage

Figure 3 shows the positive and negative CATA descriptors of a specific sample of tamarind functional beverage. The tamarind flavor and refreshment sensation were possibly the most appreciated characteristics by consumers, whereas the attributes related to astringency, bitter taste, and bitter aftertaste were considered negative for a product's choice.

It is worth mentioning that although bitter taste is considered a negative factor, studies have shown that health-claiming products with bitter taste are well accepted by consumers. The product's health and/or functionality claim seems to interfere with the positive acceptance, possibly because of the consumers' belief in feeling healthier when consuming the product[35]. Therefore, consumer behavior studies towards health-claiming products should be developed to expand knowledge on factors that may interfere with consumers' perceptions.

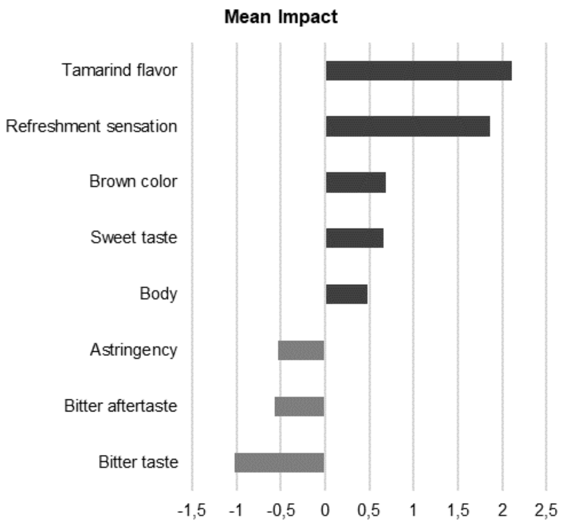


Figure 3 - Positive and negative descriptive terms for characterizing the tamarind functional beverage

4.3.4 Time-intensity analysis

Table 5 shows the results of analysis of variance (ANOVA) and Tukey's test of the time-intensity curve parameters for the 6 attributes evaluated for each sample. Differences between samples were observed in the perception of the following attributes: sweetness, acid taste, and bitter taste, especially in relation to the Imax parameters. The samples sweetened with neotame, sucralose, and stevia had higher Imax value, differing statistically from the others. A higher Ttot value was observed for the samples sweetened with stevia, neotame, and saccharin. Regarding the acid taste, a significant difference was observed only between the samples sweetened with acesulfame and stevia, whereas regarding the Imax parameters, no difference was observed for the total duration of the stimulus.

Table 5 – Means of Tukey's test of the time-intensity curve parameters for the tamarind functional beverage samples' attributes

Attribute: Sweetness

Sample	Imax	TImax*	Area	DurPI*	Ttot*
Sucrose	6.27^{c}	8.2^{a}	240.15^{cd}	19.30^{ab}	61.10^{bc}
Sucralose	6.51^{abc}	8.5^{a}	242.15^{bc}	19.52^{ab}	57.12^{c}
Acesulfame - K	5.27^{d}	8.25^{a}	187.33^{d}	16.25^{b}	59.77^{bc}
Stevia	7.25^{a}	9.85^{a}	345.43^{a}	23.27^{a}	75.22^{a}
Saccharin	5.77^{dc}	10.9^{a}	228.20^{cd}	17.25^{ab}	65.72^{abc}
Neohesperidin	5.45^{d}	10.17^{a}	205.90^{cd}	16.62^{b}	57.62^{c}
Neotame	6.77^{ab}	9.37^{a}	283.70^{b}	19.30^{ab}	69.82^{ab}

Attribute: Acid taste

Sample	Imax	TImax*	Area	DurPI*	Ttot*
Sucrose	6.59^{ab}	8.87^{a}	215.10^{a}	17.30^{bc}	58.07^{a}
Sucralose	6.72^{ab}	8.65^{a}	210.85^{a}	16.70^{bc}	50.57^{a}
Acesulfame - K	6.91^{a}	10.35^{a}	217.18^{a}	15.60^{c}	58.67^{a}
Stevia	6.26^{b}	9.42^{a}	220.28^{a}	23.50^{a}	59.75^{a}
Saccharin	6.91^{a}	11.47^{a}	225.00^{a}	15.42^{c}	55.77^{a}
Neohesperidin	7.16^{a}	9.50^{a}	254.88^{a}	21.00^{ab}	61.72^{a}
Neotame	6.81^{ab}	9.70^{a}	233.95^{a}	16.17^{bc}	59.62^{a}

Attribute: Bitter taste

Sample	Imax	TImax*	Area	DurPI*	Ttot*
Sucrose	3.43^{c}	12.20^{a}	291.63^{a}	17.55^{a}	61.17^{a}
Sucralose	3.51^{c}	11.30^{a}	258.98^{a}	17.02^{a}	58.20^{a}
Acesulfame - K	4.68^{b}	12.40^{a}	255.25^{a}	16.12^{a}	58.10^{a}
Stevia	6.41^{a}	11.90^{a}	282.20^{a}	19.67^{a}	62.32^{a}
Saccharin	4.59^{b}	13.35^{a}	283.83^{a}	17.25^{a}	60.02^{a}

Neohesperidin	4.18^{bc}	14.77^{a}	269.93^{a}	18.92^{a}	60.27^{a}
Neotame	3.90^{bc}	13.35^{a}	272.65^{a}	17.67^{a}	59.72^{a}
Attribute: Tamarind flavor					
Sample	Imax	TImax*	Area	DurPI*	Ttot*
Sucrose	7.36^{a}	12.22^{a}	291.63^{a}	17.55^{a}	61.17^{a}
Sucralose	7.10^{a}	11.30^{a}	258.98^{a}	17.02^{a}	58.20^{a}
Acesulfame - K	7.22^{a}	12.40^{a}	255.25^{a}	16.12^{a}	58.10^{a}
Stevia	6.82^{a}	11.90^{a}	282.20^{a}	19.67^{a}	62.32^{a}
Saccharin	7.22^{a}	13.35^{a}	283.83^{a}	17.25^{a}	60.02^{a}
Neohesperidin	6.83^{a}	14.77^{a}	269.93^{a}	18.92^{a}	60.27^{a}
Neotame	7.07^{a}	13.55^{a}	272.65^{a}	17.67^{a}	59.72^{a}
Attribute: Refreshment sensation					
Sample	Imax	TImax*	Area	DurPI*	Ttot*
Sucrose	5.19^{a}	13.37^{a}	162.35^{a}	22.62^{a}	54.92^{a}
Sucralose	5.22^{a}	12.07^{a}	157.60^{a}	21.52^{a}	55.00^{a}
Acesulfame - K	4.68^{a}	13.87^{a}	169.63^{a}	24.52^{a}	57.12^{a}
Stevia	4.90^{a}	16.77^{a}	168.08^{a}	21.67^{a}	54.97^{a}
Saccharin	4.88^{a}	14.20^{a}	164.60^{a}	21.15^{a}	54.72^{a}
Neohesperidin	5.26^{a}	18.80^{a}	187.05^{a}	18.17^{a}	56.15^{a}
Neotame	4.82^{a}	14.00^{a}	158.25^{a}	21.87^{a}	57.80^{a}
Attribute: Astringency					
Sample	Imax	TImax*	Area	DurPI*	Ttot*
Sucrose	4.97^{a}	15.55^{a}	127.00^{ab}	17.30^{ab}	44.42^{a}
Sucralose	4.57^{a}	16.40^{a}	107.45^{b}	12.95^{b}	43.85^{a}
Acesulfame - K	5.32^{a}	15.45^{a}	136.78^{ab}	15.42^{ab}	43.67^{a}
Stevia	4.78^{a}	18.62^{a}	137.75^{ab}	20.85^{a}	47.95^{a}
Saccharin	5.11^{a}	15.20^{a}	133.25^{ab}	17.25^{ab}	45.85^{a}
Neohesperidin	5.05^{a}	16.65^{a}	144.38^{a}	20.47^{a}	50.15^{a}
Neotame	4.96^{a}	16.37^{a}	141.20^{ab}	15.85^{ab}	45.72^{a}

Means with common letters in the same column indicate that there is not a significant difference between samples ($p \leq 0.05$) from Tukey's Honestly Significant Difference procedure (HSD).

*Time in seconds

The analysis of the attribute bitter taste is important, as the perception of bitter taste and bitter aftertaste is expected when consuming sweetener-sweetened products[24,36]. The stevia-sweetened sample had higher Imax for the bitter taste, with no significant differences for the other parameters (TImax, Area, DurPI, and Ttot). Regarding the attributes specifically related to the tamarind fruit, such as tamarind flavor, freshness, and astringency, no significant differences were observed among samples when compared to the control, which may be a positive result as the sweeteners did not affect the perception of the fresh fruit characteristics.

It is well known that the food industry is increasingly concerned about developing beverages that can preserve the natural characteristics of fruits and vegetables, as this is one of the main demands of consumers[5,37].

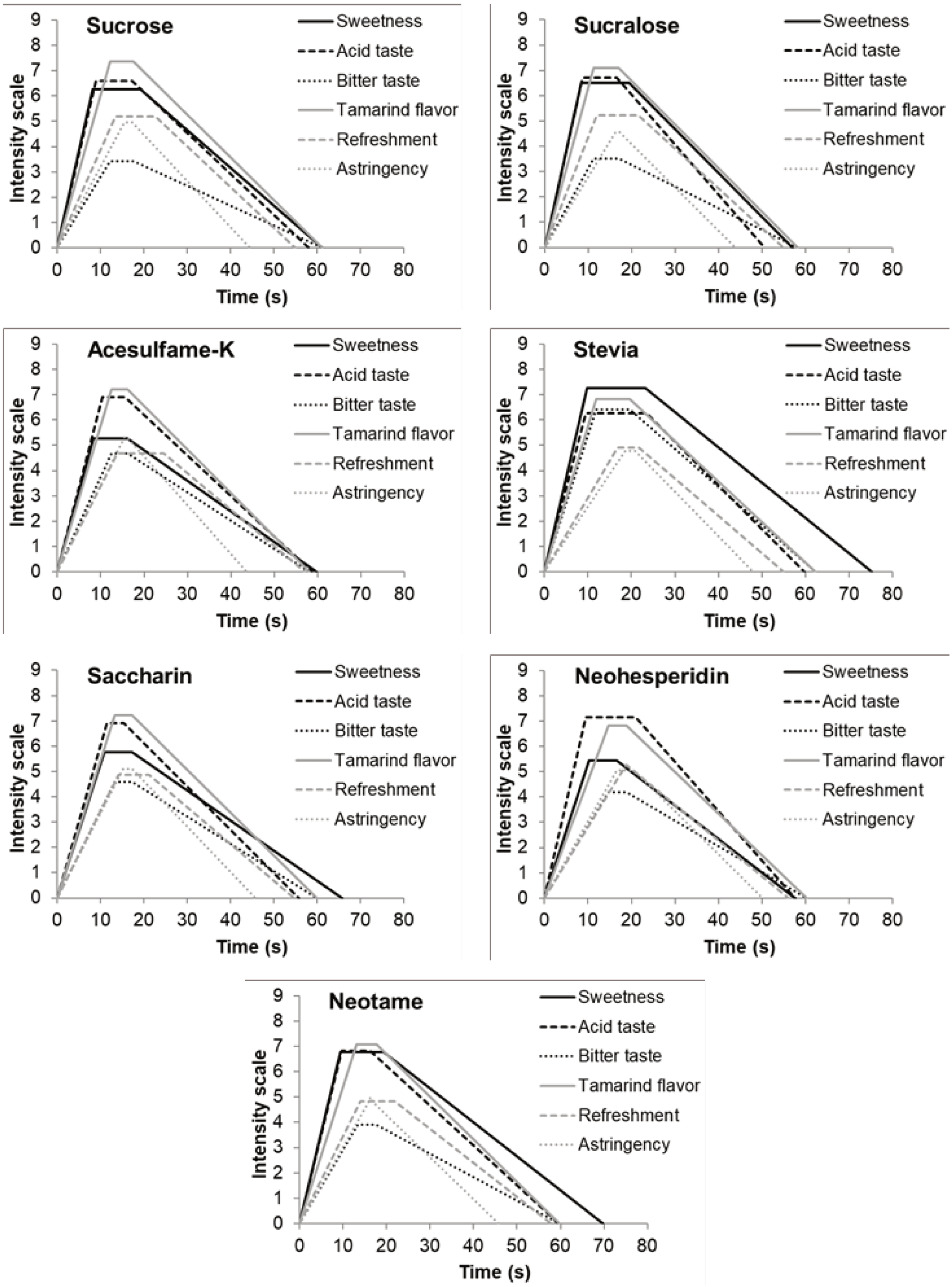
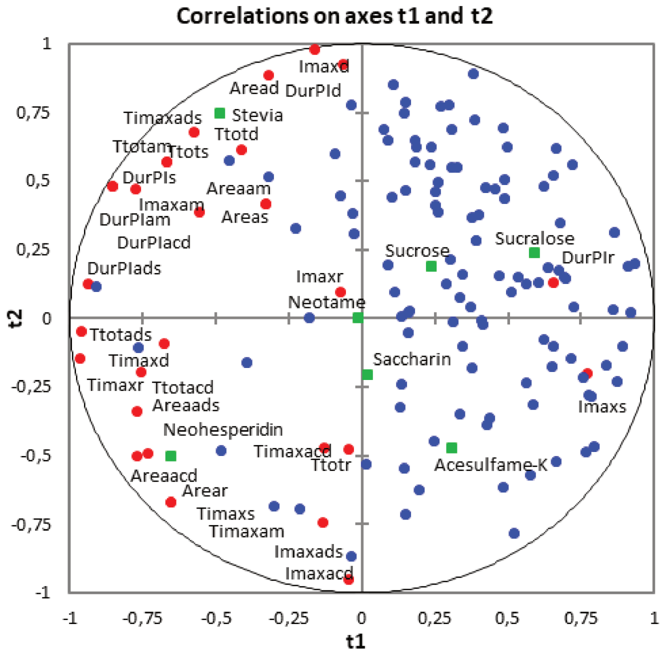


Figure 4 – Time-intensity profile of the tamarind functional beverage samples for sweetness, acid taste, bitter taste, tamarind flavor, refreshment, and astringency

To better understand the results, the 6 attributes are presented simultaneously for each sample in multiple time-intensity graphs, as shown in Figure 4. When comparing the 6 samples and the sucrose-sweetened sample, two important aspects were observed: the stevia-sweetened sample exhibited the highest peak for Imax, total duration of Imax, and total duration of the sweetness stimulus, whereas the sucralose-sweetened sample exhibited a similar profile to that observed for the sucrose-sweetened sample.

The TI curves were related to the results of the acceptance test (External Preference Mapping) and are shown in Figure 5, which are similar to CATA. The sucralose-sweetened sample is in the area with the highest concentration of consumers and close to the control sample, indicating greater consumer acceptance. The samples sweetened with natural sweetener (stevia and neohesperidin) are in the opposite locations with a lower concentration of consumers, and thus indicating lower acceptance.



Imax – Maximum intensity recorded
TImax – Time at which the maximum intensity was recorded
Area – Area of the time-intensity curve
DurPI – Duration of maximum intensity
Ttot – Total duration of the stimulus

r – Refreshment sensation
ads – Astringency
s – Tamarind flavor
am – Bitter taste
acd – Acid taste
d – Sweet taste

Figure 5 – External Preference Mapping (X and Y are horizontal and vertical axes, respectively) obtained by partial least squares regression of descriptive data and respondent´s overall liking scores for the tamarind functional beverage (square = samples; blue circle = consumers; red circle = time-intensity test parameters)

In recent years, there has been increase in consumers’ demand for natural sweeteners, including stevia10. However, the present results show a lower acceptance of these sweeteners. These data reinforce the importance of understanding the factors associated with the consumers’ low acceptance of natural sweeteners as they are considered healthier alternatives for sucrose replacement[10].

As also shown in Figure 5, the DurPI of the stimulus freshness and the Imax of the tamarind flavor were more important descriptors of greater consumer' acceptance. According to Labbe et al.[38], the expected acidity of the fruit can explain the perception of freshness by consumers, who considered the acid taste as a positive factor for the classification of a refreshing beverage.

However, the descriptors related to bitterness, sweetness, acidity, and refreshment sensation were more closely related to the samples sweetened with stevia and neohesperidin, which were the least accepted among the samples. It seems controversial when observing that the DurPI of refreshment sensation was associated with a better acceptance, despite this attribute being related to the less accepted stevia and neohesperidin sweeteners. However, this contrast is because of the positive refreshing sensation factors (coldness, mint odor, peach odor, or acidity), whereas some attributes appear to be negative factors for the refreshing sensation, such as sweetness, which is a strong characteristic of stevia and neohesperidin sweeteners[38].

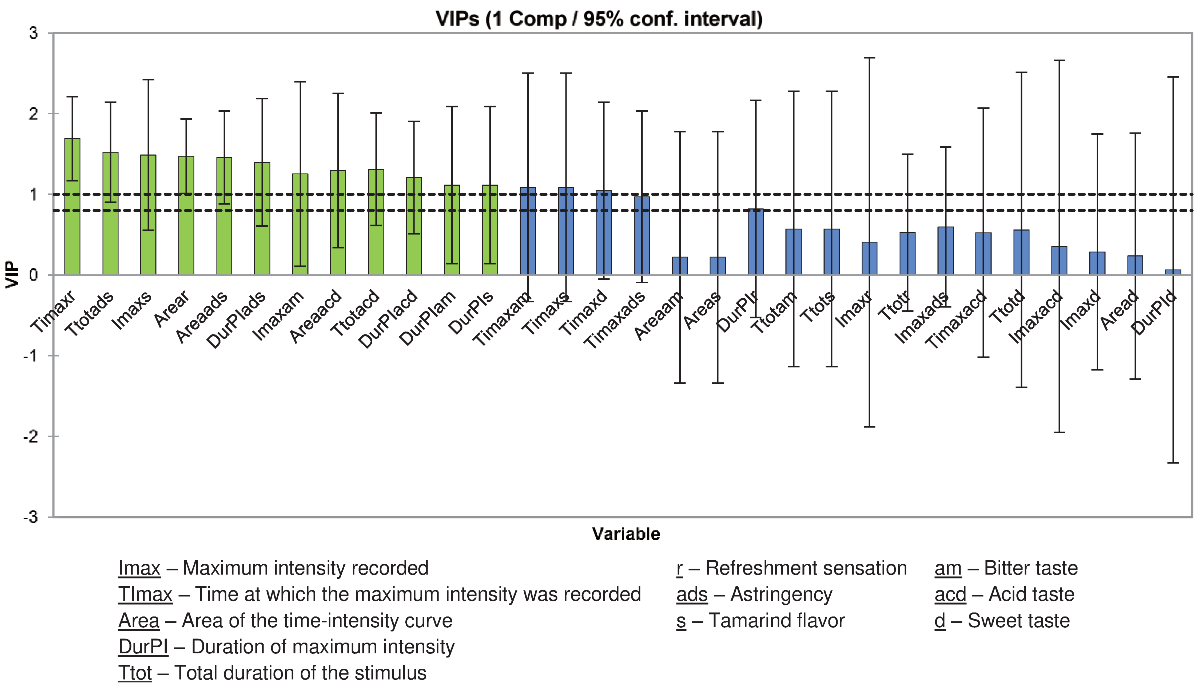


Figure 6 – Partial least squares standardized coefficients of the tamarind functional beverage (green = descriptor term with significant contribution to consumer acceptance)

A correlation was made between the global impression and the parameters analyzed in TI using PLS regression and the results are illustrated in Figure 6. PLS is a suitable multivariate method for the analysis of sensory descriptors and consumers' general impression. It may also be useful to guide the selection of a subset of relevant attributes from the full set of attributes[27].

Therefore, Figure 6 shows the 12 parameters of the TI curve that positively affected the acceptance of the samples, such as TImax for refreshment sensation and Imax for tamarind flavor. Thus, it is important to highlight that the results obtained in CATA and TI complemented each other. The CATA results showed the prevalent attributes linked to the global impression of the functional tamarind beverage, that is, refreshment sensation and flavor, whereas TI confirmed the presence and evidenced the intensity and duration of each attribute, indicating the duration of maximum intensity for refreshment sensation and tamarind flavor.

Thus, different sensory tests should be performed to understand the impact of the sensory attributes on consumers' choice of a food product. However, the test choice may depend on a food company's goal, mainly related to time and costs. If the company's decision is to conduct a study to describe specific characteristics of a product's sample set, to verify whether the differences between samples depend on the absence or presence of attributes, the CATA questionnaire should be preferred as it is simpler, less analytical, and more natural for consumers[39].

4.4 CONCLUSION

Sucrose-free tamarind functional beverages were evaluated by acceptance tests, CATA, and TI analysis. Thus, the sample sweetened with sucralose was the best alternative to sucrose in the tamarind functional beverage, whereas natural sweeteners may not be the best option for this product, as they show sweet aftertaste and bitter aftertaste characteristics. The CATA questionnaire identified 2 natural characteristics of fruit, refreshment sensation and fruit flavor, evidenced by TI test through duration of the refreshing stimulus and maximum intensity flavor. These factors were important for being associated with the overall impression of the product.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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5. ARTIGO 3 – DOES THE INDOOR THERMAL ENVIRONMENT INFLUENCE THE DOMINANT SENSATION IN A FUNCTIONAL BEVERAGE ATTRIBUTES?

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Does the indoor thermal environment influence the dominant sensation in a functional beverage attributes?

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ABSTRACT:

Different thermal environments can affect human productivity with repercussions on cognitive ability and physiological changes. However, the direct effect of room temperature on the sensations of food in the mouth during consumption is not yet well established. This study aimed to investigate the effect of indoor temperature on dominant sensations during intake of a beverage containing non-nutritive sweeteners. The Temporal Dominance of Sensations (TDS) technique was used to evaluate seven functional beverages with different non-nutritive sweeteners. Sixty consumers participated in the test, attending 3 days of laboratory analysis with strictly controlled indoor temperatures (20 °C, 24 °C, and 26 °C). The indoor temperature affected the TDS curves of four functional beverages, with emphasis on the colder environment, which accentuated the sensation of bitter taste in the samples sweetened with stevia and neohesperidin. In the warmer environment, a TDS peak of fruit flavor was observed for the sucrose-sweetened sample, while the neotame-sweetened sample presented lower dominance rate for sweetness. The sensory performance of the samples sweetened with non-nutritive sweeteners, determined by the dominance rate of attributes, can change over time in different indoor temperatures.

Keywords: *Sensory analysis. Dynamic sensory methodology. Temporal dominance techniques. Functional beverage.*

PRACTICAL APPLICATION:

Cold and warm environments can affect the consumer's decision to buy beverages, but the effect of the indoor temperature on the taste sensation of beverages has not been elucidated. The objective of this research was to evaluate whether the indoor temperature can affect the dominance of sensations during the consumption of a low-calorie beverage. The results showed that the cold environment prolonged the sensation of bitter taste in the beverages sweetened with natural sweeteners, which can help the non-alcoholic beverage industry to choose another type of sweetener, considering a new product sensory influence facto: room temperature.

5.1 INTRODUCTION

Human perceptions of foods and consumer products are the results of complex sensory and interpretation processes by the brain (Lawless & Heymann, 2010). These processes involve several factors with a determining role in the perception and acceptance of food products, including sensory factors intrinsic to food (color, aroma, texture, viscosity, etc.) and extrinsic factors (packaging properties, background music, lighting, and environment temperature) (Wang et al., 2019).

Some studies have shown the effect of food temperature on the basic tastes, mainly due to the thermal sensitivity of peripheral neural cells (Behrens & Meyerhof, 2006; Green & Andrew, 2017), like the increase in the sweetness of a certain food when consumed warm. For the detection of sweet, salty, and bitter stimuli, there is a concentration threshold that presents U-shaped dependence with temperature, with the lowest thresholds between 20 °C and 30 °C (McBurney et al., 1973; Talavera et al., 2007). Some authors have reported that the ion channels involved in the salty taste transduction are sensitive to cold (Askwith et al., 2001), while the non-selective sodium channel (coupled to the G-protein) is sensitive to heat (Green & Andrew, 2017).

Those studies were based on food temperature, but few studies have assessed the effect of indoor temperature on sensory perception. The room temperature is one of the factors that can interfere with the sensory evaluation of foods, thus the application of sensory tests in places with temperature between 20 °C and 22 °C, and relative humidity between 50% to 55% is recommended. These conditions let a comfortable environment for the participants in the sensory tests and prevent distraction (Lawless & Heymann, 2010).

Other studies have shown that different thermal environments can affect human performance and productivity with repercussions on cognitive ability (Zhang et al., 2019) and physiological changes such as increased heart rate (Wang et al., 2018). A study in Japan (Fujiyama & Toda, 2017) demonstrated a relationship between the taste sensation and the intraoral environment through two main mechanisms: the contrast effect between taste and somatosensation and the activation of the autonomic nervous system. However, the direct effect of room temperature on the sensations of food in the mouth during consumption has not been reported in the literature.

As most countries face a current scenario of climate change resulting from global warming, it is important to study the effect of indoor temperatures on the sensory perception and the acceptance of a product. Estimates show that 85% of the world population will live in developing countries by 2030, which are located in the hottest places of the globe (Mishra et al., 2016; Rupp et al., 2015). This will lead consumers to suffer the impact of room temperature on their physiology and behavior.

Considering the impact of temperature in physiological aspects, it is critical to evaluate its influence on food sensory aspects. Thus, the use of Temporal Dominance Sensation (TDS) method can provide information on the dominant sensory characteristics perceived during food consumption process. The method enables evaluating the influence of external factors, like temperature, throughout a product consumption period, with the advantage of the evaluation of several attributes simultaneously (Di Monaco et al., 2014).

The objective of this study was to evaluate the effect of the indoor temperature on the dominant sensations during the consumption of functional tamarind beverages containing non-nutritive sweeteners.

5.2 MATERIALS AND METHODS

5.2.1 Ethical aspects

The research was approved by the Research Ethics Committee of the University of Campinas - UNICAMP under CAAE #84575518.0.0000.5404.

5.2.2 Sampling

Tamarind functional beverages were prepared using frozen tamarind pulp (Ricaeli®, Cabreuva, SP – Brazil, 2018) as the basic formulation, using a dilution ratio considering 100g of pulp per 200mL of water, and addition of fructooligosaccharide (FOS, SweetMix, Sorocaba, SP – Brazil) at 1.5g/ 100mL (Brasil, 1999).

The samples differed in the addition of sucrose (standard sample) and different sweeteners. For the control formulation, 10.70% sucrose (União®, São Paulo, Brazil) was used. The non-caloric sweeteners were sucralose (Nutramax®, Sao Paulo, Brazil), acesulfame-K (Nutramax®), stevia with 97% rebaudioside A (Clariant®, Sao Paulo, Brazil), saccharin (Nutramax®), neohesperidin (Nutramax®), and neotame (Nutramax®) at concentrations to reach the sweetness equivalence to sucrose iso-sweetness level of 10.70% (de Oliveira Rocha

& Bolini, 2015; Lima & Bolini, 2020). The concentration values used for each sweetener are described in Table 1.

Table 1 Concentration of sweeteners used in the beverage samples

Sweetener	Concentration[1] (%)
Sucralose	0.0200
Acesulfame-K	0.0340
Stevia	0.1295
Saccharin	0.0340
Neohesperidin	0.0064
Neotame	0.0018

[1] Values calculated by sweet equivalence and ideal sweetness, considering 10.70% of sucrose (Lima & Bolini, 2020).

5.2.3 Indoor temperature control

Thermal comfort is defined as that condition of mind which expresses satisfaction with the thermal environment (ISO, 2005). It is a complex cognitive response that involves several factors (environmental, individual, among others) (Wang & Liu, 2020). The operative temperature limits defined by Standard 55 (ASHRAE, 2013) was established to control the indoor temperature in this experiment, for 90% of thermal acceptability in a thermal environment, according to the following equations:

$$Upper\ limit\ (°C) = 0.31\ \overline{t_{pma(out)}} + 20.3$$

$$Lower\ limit\ (°C) = 0.31\ \overline{t_{pma(out)}} + 15.3$$

Where $\overline{t_{pma(out)}}$: The prevailing mean outdoor air temperature

For the city of Campinas, São Paulo, Brazil, the annual mean outdoor air temperature of 20.7 °C was considered (INMET, 2018), thus the limit values for acceptable thermal comfort ranged from 21.7 °C and 26.7 °C. Whereas some studies have reported that in tropical regions, such as Brazil, people feel comfortable in more flexible temperature ranges at the upper limit, the temperature of 20 °C was considered the cold indoor temperature (below the lower limit) while temperatures of 24 °C and 26 °C were within the upper limit. Thus, the study was carried out at the Sensory Science and Consumer Study Laboratory of University of Campinas, with strict indoor temperature control (20 °C, 24 °C, and 26 °C) in three different days, defined according to the weather forecast from the Brazilian Institute of Meteorology (INMET). During those days, the outdoor temperature did not differ from the indoor temperature in ± 3 °C, from 14h to 16h (the period of the test application).

5.2.4 Temporal Dominance of Sensations (TDS) tests

Sixty untrained consumers aged 22 to 48 years, consumers of fruit-based beverages, of different types of gender identity, including undergraduate and graduate students and staff of the University of Campinas participated in the test. The consumers attended the Sensory Science and Consumer Study Laboratory on three different days (under different thermal conditions) to perform the TDS tests. The participants were asked to use the same type of clothing on the days of the tests, i.e., pants, tennis shoes and cotton shirts (or clothes they felt comfortable with). The participants were given at least 10 minutes to adjust to the room temperature before starting the test.

The TDS test was applied in individual computerized booths using the Fizz Sensory Software model 2.47b. The test was carried out using complete balanced blocks, in which all attributes were presented simultaneously to each consumer, on the computer screen in a different order, following the Williams' Latins square design. Seven attributes were defined through an indoor sensory panel composed of a team of trained advisors, according to the training procedures established in a previous study on tamarind functional beverages (Lima & Bolini, 2020).

Samples with 30mL were offered to the consumer monadically in disposable plastic cups at a temperature of 4 °C (ASTM, 2017), and the consumer should swallow the sample and click on the start button on the screen to begin the evaluation. The consumer should put the sample in their mouth and immediately start the evaluation, which lasted 1 minute and a half. During the evaluation, the consumer should select the attribute considered dominant, among the seven attributes (sweet taste, bitter taste, sour taste, astringency, refreshment sensation, tamarind flavor, and metallic flavor) or no alternatives available. When the dominant perception changed, the consumer had to click on another attribute that best described his feeling about the sample. The consumer had 1 minute and a half to click on the alternatives, with no frequency restriction (Pineau et al., 2009).

The following parameters were obtained during the test: the number of attributes, the number of times the attribute was selected, the duration of the first attribute, and the total duration of the TDS tests. To generate the results of the TDS, the dominance rates were calculated for 90 seconds, using the FIZZ software. The total number of selections for an attribute as dominant was divided by the total number of ratings (consumers) and converted into percentage dominance. A subject could select only one attribute at a time and, therefore, the data on the y-axis were presented as the percentage of subjects who selected a specific attribute at a given point in the evaluation sequence (Hutchings et al., 2014). TDS data were represented by dominance rate curves with the percentage of subjects that selected the attribute as dominant over time, for each product (Silva et al., 2018).

The points of the evolution curve were smoothed using a simple moving average. The curve showed the dominant attributes at the panel level over time. All attribute data were evaluated separately, thus the curves were overlaid and plotted as a function of time for each sample and compared at a level of significance to differentiate the results. An attribute was considered dominant when it was above the significance level. The random level was also considered, which refers to the dominance rate when a certain attribute may have been selected at random (Silva et al., 2018).

5.3 RESULTS AND DISCUSSION

The results showed that four out of seven samples presented significant results regarding the effect of the indoor temperature on the dominance of sensations in the consumption of functional tamarind beverages. The other three samples (sucralose, acesulfame-K, and saccharin) did not present attributes above the significance levels, and therefore were not included. The samples sweetened with sucrose, stevia, neohesperidin, and neotame exhibited variation in sensations such as tamarind flavor, bitter taste, and sweet taste in the face of different thermal conditions.

Figure 1 shows the variations in the dominance curves between the attributes of the sucrose-sweetened sample, considering different indoor temperatures. At 20 °C, a slight sensation of tamarind flavor was observed with a peak between 20% and 30% dominance and short duration of sensation (5 seconds), which was not observed at 24 °C. At 26 °C, the peak dominance of tamarind flavor was in the range of 30-40% with a longer duration when compared to the lower temperatures.

As reported by (Di Monaco et al., 2014), the fruit flavor was dominant in strawberry beverages sweetened with sucrose. None of the current low-calorie sweeteners match sucrose in terms of quality of sweet taste or other temporal characteristics, which may explain the dominance of sensation for the attribute tamarind fruit. However, the sensory performance of a sweetener changes over time and it can also interact with other stimuli (taste, smell, texture), emphasizing the importance of dynamic sensory techniques to describe these changes (Lawless & Heymann, 2010).

Figure 1 Temporal Dominance of Sensations curves of each attribute of tamarind beverages sweetened with sucrose at environmental temperatures of 20, 24, 26 °C.

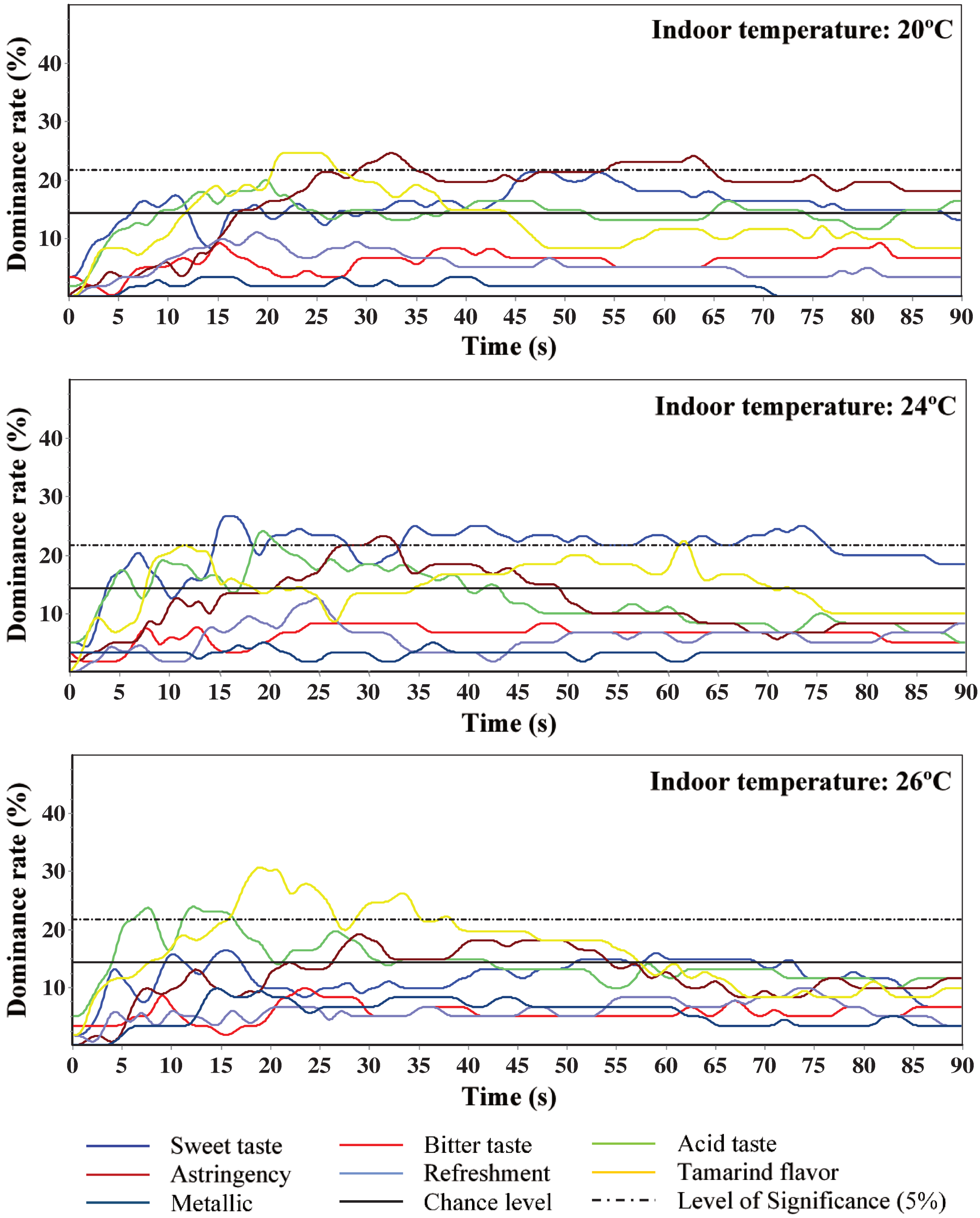
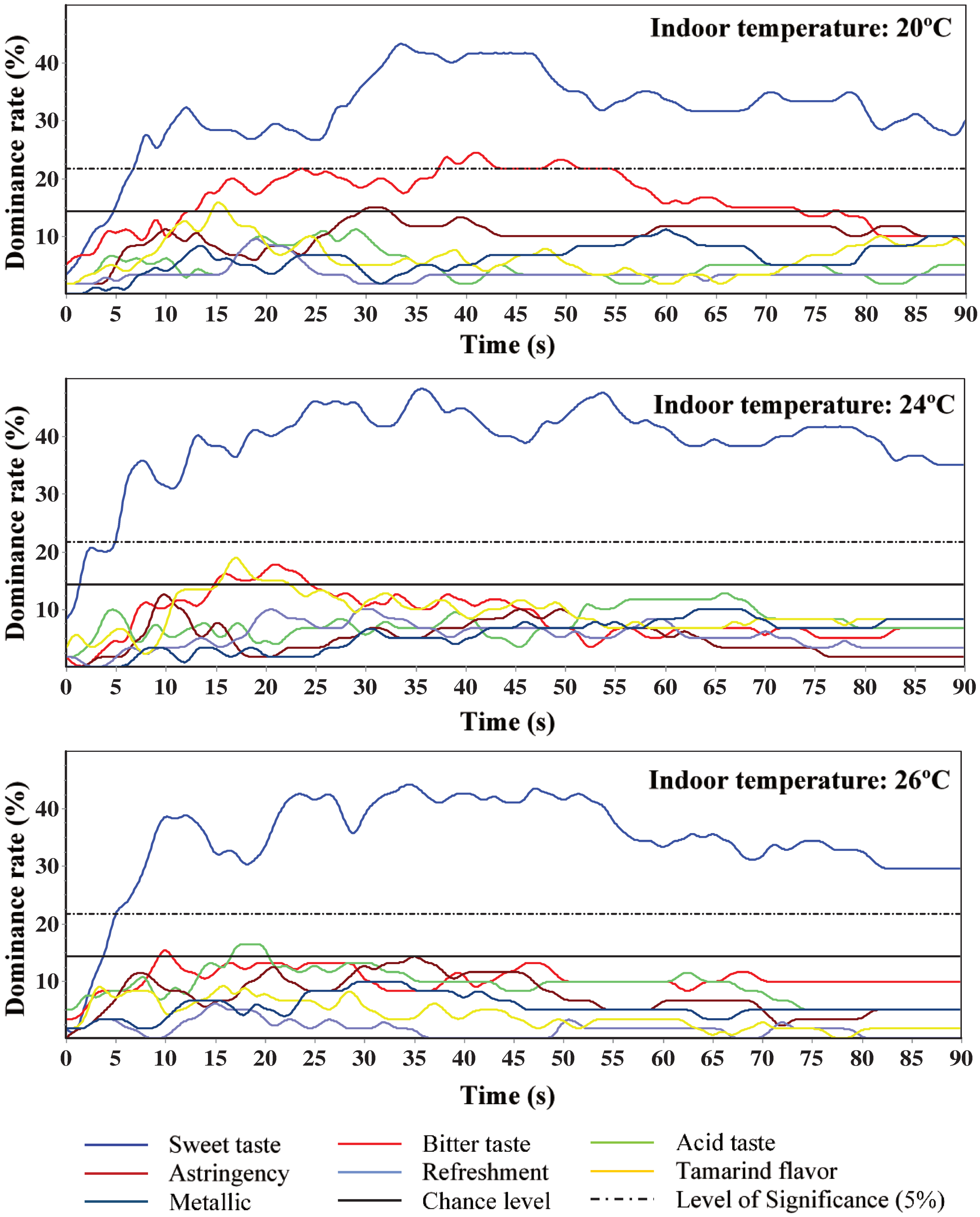


Figure 2 shows the variations in the dominance curves for the attributes of the stevia-sweetened sample. A significant variation was observed for the attributes sweet taste and bitter taste, and "sweet taste" was prevalent in all temperature conditions, with a higher dominance rate at the indoor temperature of 24 °C. Regarding the bitter taste, there was a slightly higher dominance rate (<30%) at 20 °C, without a long dominant sensation duration. At higher temperatures, the dominance rate is decreased, with no significant differences.

Figure 2 Temporal Dominance of Sensations curves of each attribute of tamarind beverages sweetened with stevia at environmental temperatures of 20, 24, 26 °C.



A quantitative descriptive analysis (QDA) was carried out previously with the same samples considering an indoor temperature of 22 °C (Lima & Bolini, 2020). The authors reported that the stevia-sweetened sample obtained the highest score for the attribute sweet taste and bitter taste. Those results corroborate with the values observed in the present study, which suggests a higher dominance rate and duration of sweet taste sensation at indoor temperatures ≤ 24 °C and dominance of bitter taste at temperatures of 20 °C.

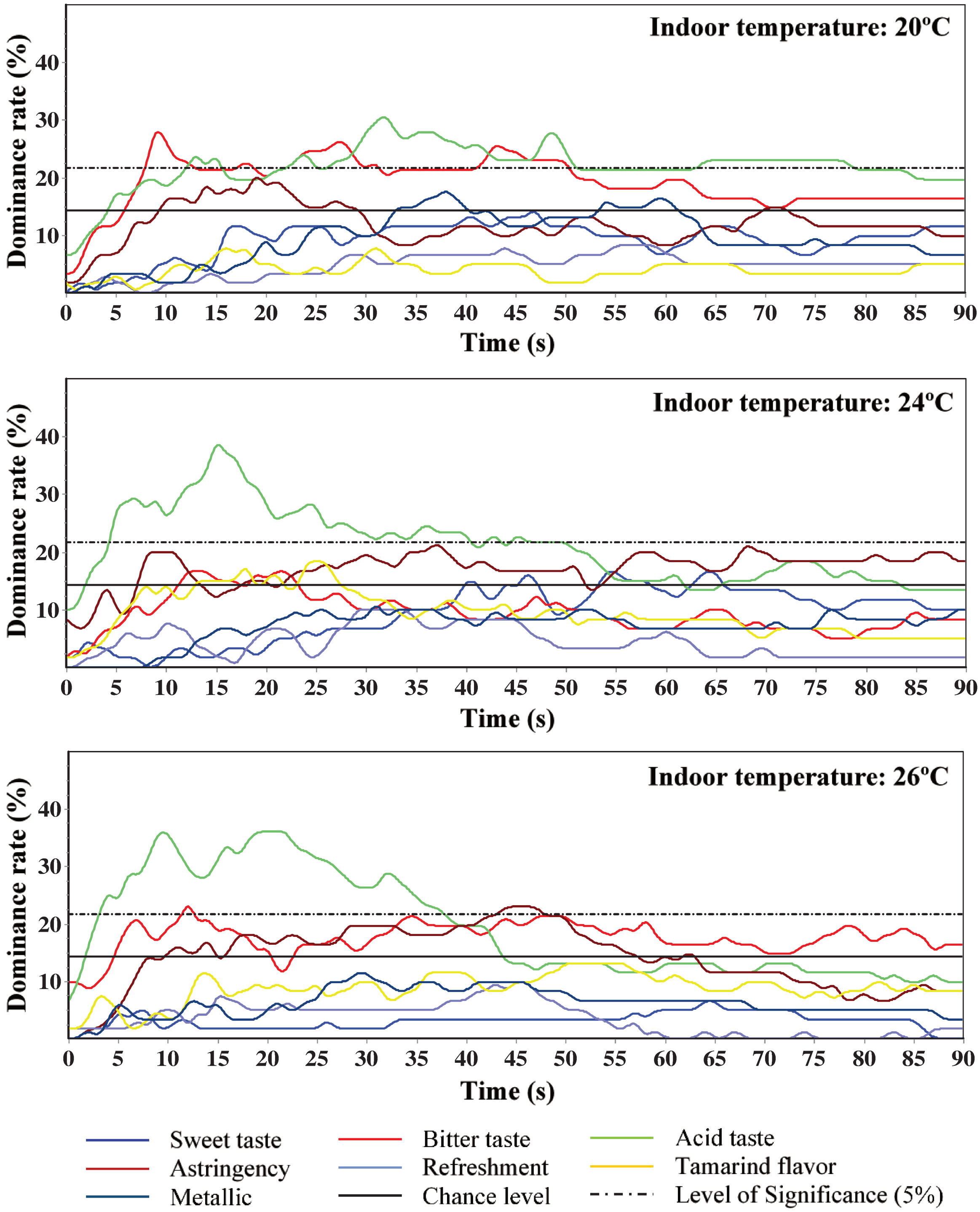
There are no studies on the direct effect of room temperature on the sensory responses, thus the dominance of bitter taste has been studied in a colder environment. The study reported by (Fujiyama & Toda, 2017) with healthy individuals in Japan demonstrated a relationship between the taste sensation and the intraoral environment. Statistical comparisons of the taste recognition thresholds before and after the cold stimulus showed greater sensitivity for all four basic tastes. According to the research, the cold stimulus increased the taste sensitivity possibly through two main mechanisms: 1- the contrast effect between taste and somatosensation, once the cold stimulus improved the sweet, bitter, and umami tastes that were modified by TRPM5, but also salty and sour tastes; 2- the activation of the autonomic nervous system, including the activation of the circulatory system.

Psychological and physiological changes due to different emotional states can also have an impact on the individuals' perception. Some authors investigated the effect of emotional state on the thermal perception and comfort state and reported that individuals in a boring mood state felt the heat more than others, besides presenting higher heart rate, and a state of discomfort (Wang et al., 2018). In addition, the higher temperatures cause discomfort (Kamalha et al., 2013) and imply the individual's unproductivity (Zhang et al., 2019), interfering with the individuals' ability to assess the sensations during the consumption of the beverage.

Another study used a heating system to study the impact of different thermal environments on the sensory evaluation, and found noticeable sensory differences between different temperatures, and consumer preferences were influenced by their thermal sensations in the environments (Petit et al., 2004). In addition, the trained assessors were able to describe and quantify those differences with high reliability.

Figure 3 shows the dominance curves of the sample sweetened with neohesperidin, with emphasis on the attributes sour taste and bitter taste. The dominance rate and the duration of sour taste increased with higher indoor temperatures, while several dominant peaks were observed for the bitter taste at an indoor temperature of 20 °C. The dominance peak for the bitter taste reverted with the increase in indoor temperature, below the significance level of 5%.

Figure 3 Temporal Dominance of Sensations curves of each attribute of tamarind beverages sweetened with neohesperidin at environmental temperatures of 20, 24, 26 °C.



The cold environment affected the dominance rate for the bitter taste of the sample sweetened with neohesperidin, as also observed for stevia. A difference in sour taste was observed, which was more dominant in the sample sweetened with neohesperidin, probably due to the contrast effect between taste and somatosensation, as previously reported.

Figure 4 Temporal Dominance of Sensations curves of each attribute of tamarind beverages sweetened with neotame at environmental temperatures of 20, 24, 26 °C.

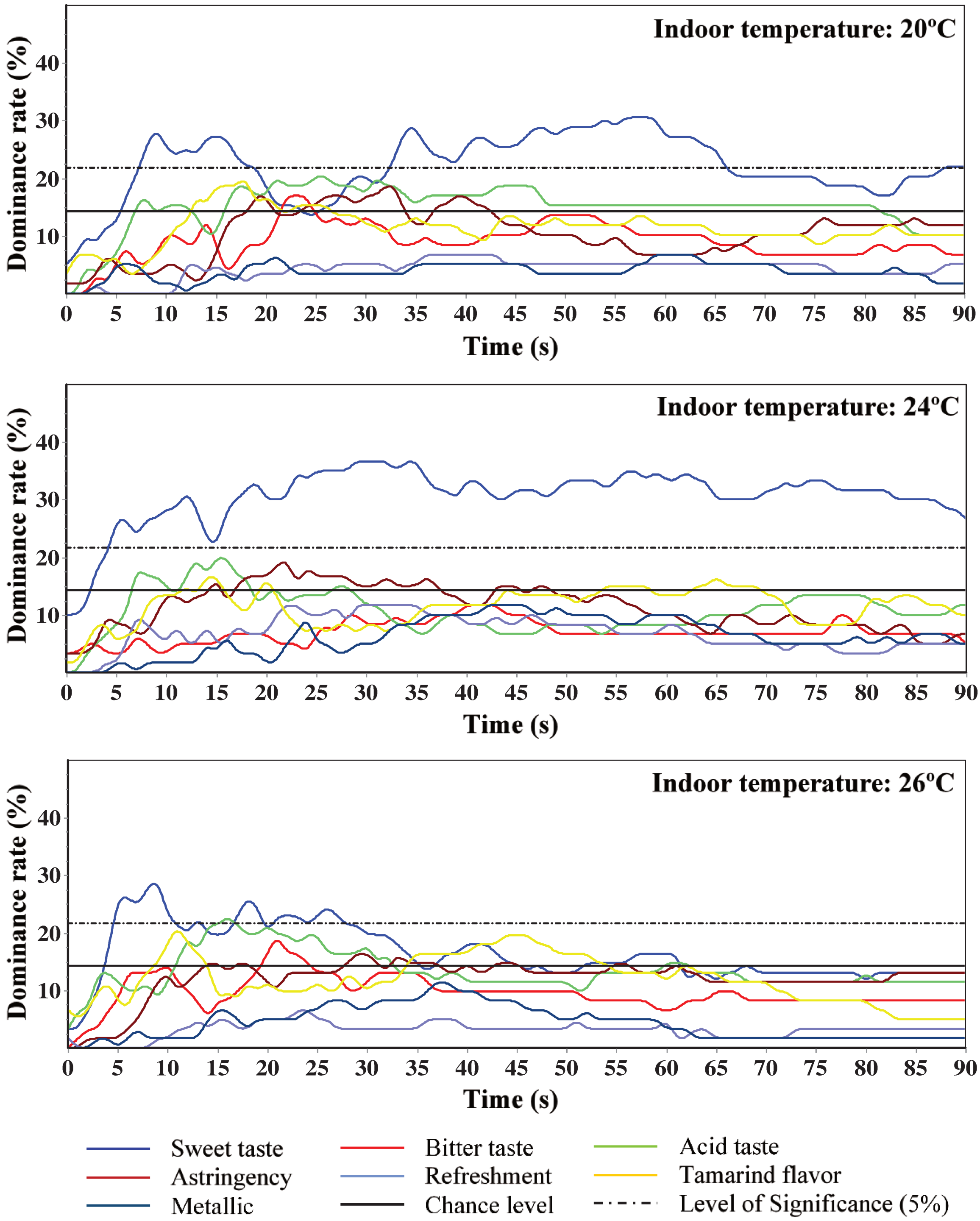


Figure 4 shows the TDS curves for the neotame-sweetened sample. The attribute sweet taste was the only one that presented a dominance rate above the significance level for the different temperatures when compared with all attributes evaluated. Although the initial peak of sweet taste occurred at similar times for the different conditions studied, the peaks were smaller at 26 °C with duration of less than 30 seconds.

Although the other sweeteners have common characteristics, such as the presence of bitter taste and sweet taste, other sensory characteristics may have affected the dominance of sensations among sweeteners. A recent study on Quantitative Descriptive Analysis (QDA) of tamarind functional beverage sweetened with different non-caloric sweeteners showed that beverages sweetened with neotame presented higher relative average regarding sensory characteristics of "sweet taste" and "sweet aftertaste", even though it was used in equivalent concentration of sucrose ideal (Lima & Bolini, 2020).

There is still a lack of sufficient information in the literature to explain how the perception of sweet taste of neotame decreased with higher temperature, weather there was a change in the perception of sweet aftertaste, or of another sensory attribute. However, it can be suggested that there is an impact of indoor temperature on physiological mechanisms (Zhang et al., 2019), which are also linked to the sensory perception in the mouth, as previously presented. Thus, higher temperatures of 26 °C may influence the physiological mechanisms of sweet taste perception. However, there is a need for further studies to analyze the direct influence of indoor temperature in sensory perception.

Another important aspect of this research is concerning the thermal comfort standards, as the thermal conditions of tropical countries, such as Brazil, are different from the countries with a temperate climate, so the application of sensory tests in environments with temperatures between 20 °C and 22 °C is recommended. However, the temperature of 20 °C is considered a cold environment in some regions of Brazil, which can cause local discomfort for the assessors.

The present results demonstrated that the environment temperature can affect the perception of various sensations during the consumption of a food product. Thus, this factor can interfere with the consumers' preference for a certain product, which should be a concern for the food industry, especially the beverage industry, once the majority of the world population will live in urban areas, which tend to be warmer than suburban and rural areas (Hajat et al., 2007).

5.4 CONCLUSION

The research showed that the indoor temperature affected the TDS curves for four functional beverages, with emphasis on the cold environment, which accentuated the sensation of bitter taste in the samples sweetened with stevia and neohesperidin. In the warm environment, a peak dominance of tamarind flavor and a reduction in sweetness dominance was observed for the sucrose-sweetened and neotame-sweetened samples, respectively. The sample sweetened with neohesperidin was also affected by the thermal variation, with an increase in the dominance peak and continuous duration of sour taste with an increase in the environment temperature.

Although the literature has reported two mechanisms to explain the effect of room temperature on the sensory perception, such as the contrast effect between taste and somatosensation and the activation of the autonomic nervous system, further studies are needed on the physiological sensory mechanisms. The sensory performance of the samples sweetened with sweetening agents, determined by the dominance rate of attributes, can change over time, besides interacting with other stimuli, reinforcing the importance of dynamic sensory techniques to describe these changes.

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AUTHOR CONTRIBUTIONS

Lima R. S. designed the study, conducted the test, collected/analyzed the data, and drafted the manuscript. Medeiros, A.C. worked on the study design process and analyzed the results. Bolini H.M.A. worked on the study design process, analyzed the results, edited the draft, reviewed all documents critically, and approved the final manuscript for submission to Journal of Food Science

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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6. DISCUSSÃO GERAL

6.1 ELABORAÇÃO DA BEBIDA FUNCIONAL DE TAMARINDO

As sete amostras de bebida funcional de tamarindo foram preparadas com polpa congelada de tamarindo com adição de FOS. A razão de diluição foi de 100 g de polpa por 200 mL de água. A quantidade de FOS foi de 1,5 g por 100 mL, de acordo com as recomendações da legislação brasileira para alimentos líquidos (BRASIL, 1999d). A concentração de sacarose e de diferentes edulcorantes das amostras da bebida funcional foi estabelecida por meio dos testes de doçura ideal de sacarose e teste de equivalência de doçura dos edulcorantes não calóricos (estévia com 97% de rebaudiosídeo A, sucralose, acessulfame-K, sacarina, neoesperidina e neotame).

6.1.1 Doçura ideal e equivalência de doçura

O teste de doçura ideal da bebida à base de tamarindo foi definido pela escala do ideal (*just-about-right scale*) e possibilitou identificar a quantidade de sacarose (10,7 g / 100 mL), utilizada como padrão para calcular a equivalência de doçura dos demais edulcorantes aplicados em substituição à sacarose. O método de estimativa de magnitude foi utilizado para estabelecer as concentrações equivalentes de cada edulcorante avaliado. A metodologia proposta constitui-se como padrão na quantificação de ingredientes substitutos de sacarose, portanto, possibilita a análise dos resultados em comparação com outras pesquisas aplicadas às bebidas de frutas.

Diversas pesquisas já realizadas com bebidas à base de diferentes frutas, como manga (CADENA; BOLINI, 2012), maracujá (DE OLIVEIRA ROCHA; BOLINI, 2015a), pêssego (CARDOSO; BOLINI, 2007) e pitanga (FREITAS; DUTRA; BOLINI, 2014) apresentaram valores de concentração de sacarose inferiores ao encontrado para a bebida funcional de tamarindo (<10,7g/100mL), possivelmente devido à propriedade ácida dessa fruta, estabelecida pela presença de ácido tartárico (OKELLO et al., 2017).

Em relação à concentração equivalente de edulcorantes, foi possível identificar que a potência de doçura encontrada nas amostras com acessulfame-K diferiu para mais, e de estévia diferiu para menos dos valores encontrados na literatura (CADENA; BOLINI, 2012; CAROCHO; MORALES; FERREIRA, 2017). Uma possível explicação para a menor potência de doçura da estévia é relacionada ao alto percentual de rebaudiosídeo e à presença de outras substâncias que podem interferir no mecanismo de percepção do gosto doce das amostras (AZEVEDO et al., 2016).

6.2 ANÁLISES FÍSICO-QUÍMICAS E COMPOSIÇÃO CENTESIMAL

De acordo com os resultados da determinação de composição centesimal referente à fórmula base da bebida funcional de tamarindo (sem sacarose), segundo a resolução brasileira (BRASIL, AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 2012), a bebida pode ser classificada como um alimento de baixo valor energético (<20 kcal / 100 mL), com quantidade não significativa de lipídeos (5 g / 100 mL). A composição centesimal não foi realizada para as demais amostras, pois os edulcorantes apresentaram contribuição calórica muito baixa (próxima de zero) (CAROCHO; MORALES; FERREIRA, 2017).

Esses resultados indicam que a bebida de tamarindo adicionada de edulcorantes em substituição à sacarose tem uma alegação de baixa caloria. Atualmente, existe um mercado crescente para produtos de baixo teor calórico e baixo teor de açúcar, especialmente bebidas com adoçantes não nutritivos (CARDOSO; BOLINI, 2007). Nos Estados Unidos, por exemplo, existe uma tendência para o mercado desses produtos, pois as políticas nacionais e os esforços da indústria incentivam os fabricantes a reformular e reduzir a densidade energética dos produtos alimentícios (PIERNAS; NG; POPKIN, 2013).

Os dados de análises físico-químicas foram importantes para comparar com as percepções sensoriais das amostras relatadas nos diferentes testes de análise sensorial. Portanto, observou-se que os valores médios de pH das amostras foram menores dos valores obtidos em bebida de maracujá (DE OLIVEIRA ROCHA; BOLINI, 2015b), menores do que os encontrados em publicações (< pH 3,6) com bebidas mistas à base de frutas tropicais, como caju, maracujá e goiaba (ABREU et al., 2011), e em bebidas mistas adicionadas de pré-bióticos (DE SOUSA et al., 2007). Os valores encontrados nas amostras adoçadas com sacarina e neohesperidina diferiram entre eles, sendo que o maior valor foi para sacarina e o menor para neohesperidina. Também se observou diferença da neohesperidina em relação às demais amostras, com menor valor para acidez titulável. Houve uma diferença expressiva entre a sacarose e as demais amostras em relação ao ºBrix e Ratio, mas isso era esperado justamente porque a sacarose é um sólido solúvel (CADENA et al., 2013; DE OLIVEIRA ROCHA; BOLINI, 2015a).

Em relação à luminosidade, os valores encontrados podem ser considerados baixos (41,41 a 41,83), quando comparados com a escala de luminosidade que varia de 0 a 100 (ABREU et al., 2011). Para coloração, as amostras acrescidas de estévia e sucralose apresentaram médias significativamente menores em relação às demais, com coloração não muito forte para vermelho com tendência à amarelo.

6.3 ANÁLISES SENSORIAL

6.3.1 Aceitação

Nos testes de aceitação realizados por consumidores, as amostras não diferiram quanto ao aroma e, em relação ao sabor, a amostra adoçada com neohesperidina obteve menor média, com diferença estatística entre as demais. Ao avaliar-se a impressão global, as amostras menos aceitas foram neohesperidina e estévia, enquanto a amostra com sucralose apresentou média estatisticamente semelhante à da sacarose.

As menores médias de impressão global podem estar relacionadas à presença do gosto amargo e residual de amargor característicos da estévia (CADENA et al., 2013) e a velocidade lenta de poder de doçura da neohesperidina juntamente com um residual refrescante (CAROCHO; MORALES; FERREIRA, 2017). A similaridade das médias observadas entre sucralose e sacarose pode ser explicada pelo poder adoçante sem apresentar o gosto amargo marcante, comum dos demais edulcorantes (BRUSICK et al., 2010; CAROCHO; MORALES; FERREIRA, 2017). Além disso, a sucralose é amplamente utilizada na indústria de alimentos mundial (aprovada em mais de 60 países) por apresentar estabilidade ao ácido e ao calor (BRUSICK et al., 2010) e, portanto pode ter apresentado maior estabilidade à acidez característica do tamarindo.

6.3.2 Análise Descritiva Quantitativa (ADQ)

Para melhor caracterização sensorial do produto, foi realizada a Análise Descritiva Quantitativa (ADQ), método em que 12 provadores avaliaram os atributos aparência, aroma, sabor e textura, contemplando 17 descritores identificados nesse contexto. Os resultados estatísticos mostraram que todos os edulcorantes, exceto estévia, não apresentaram diferença significativa para percepções importantes como aroma e sabor de tamarindo. A neohesperidina e o acessulfame-K apresentaram a menor intensidade para o gosto doce, enquanto a estévia apresentou a maior percepção do gosto doce e amargo. A sacarose e a sucralose foram os edulcorantes com menor percepção do gosto amargo, enquanto a neohesperidina apresentou a sensação refrescante de maior intensidade.

Esses resultados são suportados pela literatura. A sucralose, por exemplo, é conhecida por ter menos gosto amargo em comparação com outros adoçantes (BRUSICK et al., 2010), enquanto a estévia tem um componente amargo, e a neohesperidina tem uma velocidade de adoçamento muito lenta e sabor residual de mentol, que é responsável pela sensação de refresco (CAROCHO; MORALES; FERREIRA, 2017). A menor percepção do amargo da sucralose pode ser um fator determinante à sua escolha, uma vez que se trata do adoçante mais utilizado pelos consumidores (CARDOSO; BOLINI, 2007).

A representação bidimensional da Análise de Componente Principal (PCA) dos termos descritivos das amostras (componentes principais 1 e 2) explica 69,24% das variações entre elas. A amostra de estévia é isolada das demais, indicando diferença em relação aos atributos sensoriais. A proximidade da sacarina e da neohesperidina indica uma possível semelhança, principalmente quanto à aparência, caracterizada pela presença de partículas.

De acordo com o mapa de preferência externo, os consumidores (representados pelos círculos azuis) concentraram-se próximo às amostras (quadrados verdes) com maior aceitação. Há uma maior concentração de consumidores próximos às amostras de sacarose e sucralose, caracterizada por aroma ácido e viscosidade. Do lado oposto, a estévia apresentou a menor concentração de consumidores. A estévia é caracterizada pelo gosto amargo, doce residual e amargo residual, que possivelmente foram considerados como características negativas para a amostra.

6.3.3 Check-All-That-Apply (CATA)

Para o método CATA, foram considerados 12 termos: cor marrom, aguado, encorpado, viscoso, ácido, doce, amargo, sabor tamarindo, adstringente, residual doce, residual amargo e refrescante. A amostra preparada com sucralose foi caracterizada com os termos “encorpado”, “sabor de tamarindo” e “refrescante”. Por outro lado, as amostras com estévia e neotame apresentaram as caraterísticas “doce” e “residual doce”. As amostras com acessulfame-K e neohesperidina foram classificadas por termos geralmente consideradas não positivos em uma bebida: “residual amargo”, “amargo” e “aguado”.

Para melhor compreensão do impacto das características das amostras em relação à sua aceitação, foi realizada a correlação entre a impressão global e os descritores sensoriais utilizando a regressão dos mínimos quadrados parciais (PLS). A aplicação do método PLS multivariado é adequada para a análise de descritores sensoriais e impressão global pelos consumidores. Outro ponto importante diz respeito à utilidade do método, que pode orientar a seleção de um subconjunto de atributos relevantes dentro de um conjunto completo de atributos, e o número de componentes importantes a serem avaliados, que são geralmente determinados por um procedimento de validação cruzada (ROSSINI et al., 2012).

Desse modo, foi possível observar que as amostras descritas pelos atributos "sabor de tamarindo" e "sensação refrescante" estavam mais relacionadas à impressão global. De Carvalho et al. (2018) usaram testes de consumidor para avaliar néctar de fruta, e relataram que os descritores mais comuns eram "característica de fruta fresca", "cor" e "gosto ácido", isto é, características de frutas frescas eram esperadas no produto.

6.3.4 Tempo Intensidade - TI

Os resultados de TI foram avaliados por análise de variância (ANOVA) e Teste de Tukey dos parâmetros da curva TI para os 6 atributos avaliados para cada amostra. As diferenças entre as amostras foram observadas na percepção dos seguintes atributos: gosto doce, gosto ácido e gosto amargo, especialmente em relação à Intensidae Máxima (Imax) dos parâmetros. As amostras adoçadas com neotame, sucralose e estévia obtiveram valores mais elevados de Imax, diferindo estatisticamente das demais amostras. Um valor de Tempo Total de duração do estímulo (Ttot) alto foi observado para as amostras adoçadas com estévia, neotame e sacarina. Em relação ao gosto ácido, foi verificada uma diferença significativa apenas entre as amostras adoçadas com acessulfame e estévia.

Quanto aos atributos especificamente relacionados ao fruto tamarindo, como sabor, frescor e adstringência, não foram observadas diferenças significativas entre as amostras quando comparadas com a amostra padrão com sacarose, o que pode ser um resultado positivo, pois o os adoçantes não afetaram a percepção das características da fruta fresca. É sabido que a indústria alimentar está cada vez mais preocupada com o desenvolvimento de bebidas que podem preservar o sabor natural características de frutas e vegetais, já que esta é uma das principais demandas dos consumidores (ITAL, 2016; REGO; VIALTA; MADI, 2016).

6.3.5 Temporal Dominance of Sensation (TDS)

O método de TDS foi aplicado para verificar também a influência de fatores externos, como a temperatura ambiente em que pode ocorrer o consumo da bebida funcional de tamarindo. Desse modo, além de fornecer as características sensoriais que estão relacionadas com a aceitação da bebida, a pesquisa possibilita a compreensão de outros fatores que impactam na percepção dos atributos sensoriais durante o consumo da bebida funcional de tamarindo.

Os resultados da aplicação de três métodos de TDS, considerando diferentes temperaturas ambientes (20, 24 e 26ºC), mostraram que quatro das sete amostras apresentaram resultados significativos em relação ao efeito da temperatura interna sobre o domínio das sensações durante o consumo de bebidas de tamarindo. A pesquisa mostrou que a temperatura interna afetou as curvas TDS, com ênfase no ambiente frio, que acentuava a sensação de gosto amargo nas amostras adoçadas com estévia e neohesperidina. No ambiente quente, um domínio de pico de sabor de tamarindo e redução na dominância da doçura foi observada para a sacarose e amostras adoçadas com neotame, respectivamente. A amostra adoçada com neohesperidina também foi afetada pela variação de temperatura, com aumento do pico de dominância e contínua duração do gosto ácido com o aumento da temperatura ambiente.

Não há estudos sobre o efeito direto da temperatura ambiente sobre as respostas sensoriais, portanto, o domínio do gosto amargo foi estudado em um ambiente mais frio. Um estudo relatado por FUJIYAMA e TODA (2017) com indivíduos saudáveis no Japão demonstrou uma relação entre a sensação gustativa e o ambiente intraoral. Comparações estatísticas dos limites de reconhecimento de gosto antes e depois do estímulo frio mostraram maior sensibilidade para todos os cinco gostos básicos. Segundo a pesquisa, o estímulo ao frio aumentou a sensibilidade ao gosto possivelmente por meio de dois mecanismos principais: (1) o efeito de contraste entre paladar e somatossensibilidade, uma vez que o estímulo frio melhorou o gosto doce, amargo e umami, que foram modificados pelo TRPM5, mas também o gosto salgado e ácido; (2) a ativação do sistema nervoso autônomo, incluindo a ativação do sistema circulatório.

Mudanças psicológicas e fisiológicas devido a diferenças nos estados emocionais também podem ter um impacto na percepção dos indivíduos. Alguns autores investigaram o efeito da percepção de temperatura no estado emocional e estado de conforto, e verificaram que os indivíduos em um “mau” estado de humor sentiram o calor mais do que outros, além de apresentar frequência cardíaca mais elevada e um estado de desconforto (WANG et al., 2018). Além disso, outras pesquisas já demostraram que as temperaturas mais altas causam desconforto (KAMALHA et al., 2013) e implicam na improdutividade do indivíduo, interferindo na sua capacidade de avaliar as sensações durante o consumo de bebida.

7. CONCLUSÃO GERAL

A análise sensorial realizada para a bebida funcional de baixa caloria à base de tamarindo possibilitou identificar as características sensoriais de interesse comercial do produto, auxiliando dessa forma na determinação do tipo e quantidade de edulcorantes que podem ser utilizados em substituição à sacarose. Nesse processo, foram considerados os fatores que desempenham um papel determinante na percepção e aceitação sensorial: os fatores intrínsecos aos alimentos (cor, aroma, textura, viscosidade etc.) e os fatores extrínsecos (temperatura ambiente).

No estudo de aceitação pelo consumidor, a amostra adoçada com sucralose demostrou ser essa substância a melhor opção de substituta de sacarose na bebida de tamarindo, apresentando um perfil sensorial mais próximo à amostra com sacarose, sem apresentar diferença significativa em relação à aparência, aroma, sabor, textura e impressão global. As amostras adoçadas com edulcorantes naturais apresentaram menor impressão global e maior rejeição por percentual de intenção de compra.

Por meio da ADQ, foi possível identificar que a neohesperidina e o acessulfame-K apresentaram a menor intensidade para o gosto doce, enquanto a estévia apresentou a maior percepção do gosto doce e amargo. O questionário CATA também forneceu informações sobre a presença de gosto doce e residual doce na amostra com estévia, características que podem ter influenciado na diminuição de intenção de compra desse produto.

A aplicação do teste TI foi importante para avaliar a duração e intensidade de estímulo de duas características naturais da fruta, identificadas pela ADQ e CATA. A sensação de refrescância e sabor de tamarindo foram evidenciadas pelo teste TI por meio da duração do estímulo refrescante e intensidade máxima do sabor de tamarindo. Esses fatores foram considerados importantes por serem associados à impressão geral do produto.

A temperatura do laboratório, como fator extrínseco, também influenciou na percepção dos atributos sensoriais da bebida. A pesquisa mostrou que a temperatura interna afetou as curvas do TDS para quatro amostras de bebidas funcionais, com destaque para o ambiente frio, o que acentuou a sensação de gosto amargo nas amostras adoçadas com estévia e neohesperidina. No ambiente quente, foi observado um pico de dominância de sabor de tamarindo e uma redução na dominância de doçura para as amostras adoçadas com sacarose e com neotame, respectivamente.

Sugerem-se novos estudos sobre a abrangência da análise sensorial em relação ao impacto dos fatores extrínsecos, tais como embalagens, iluminação, música ambiente etc., na aceitação e no perfil sensorial de bebidas à base de frutas. Sabemos que a análise sensorial é primordial para o sucesso do produto no mercado, portanto, quanto mais abrangente for a pesquisa, maior será o suporte para se desenvolver um produto com qualidade aprimorada.

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