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Pryscilla Donato Casagrande

AVALIAÇÃO DOS EFEITOS DO USO DE AZEITE DE OLIVA EXTRAVIRGEM COMO SUBSTITUTO À GORDURA DE PALMA NA ELABORAÇÃO DE SORVETE DE BAUNILHA

RIO DE JANEIRO

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição, da Universidade Federal do Estado do Rio de Janeiro como requisito parcial para obtenção do título de Mestre em Alimentos e Nutrição.

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição da Universidade Federal do Estado do Rio de Janeiro (PPGAN/UNIRIO)

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BANCA EXAMINADORA

Prof. Dr. Rafael Siva Cadena

Universidade Federal do Estado do Rio de Janeiro – UNIRIO

Prof. Dr. Adriano Gomes da Cruz

Instituto Federal do Rio de Janeiro – IFRJ

Profª. Drª. Éllen Mayra Menezes Ayres

Universidade Federal do Rio de Janeiro – UNIRIO

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A menos que modifiquemos a nossa maneira de pensar, não seremos
capazes de resolver os problemas causados pela forma como nos
acostumamos a ver o mundo.

Albert Einstein

RESUMO

Sorvetes são amplamente apreciados em todo o mundo, independentemente da cultura, idade e nível socioeconômico, o uso de um ingrediente funcional pode agregar valor ao produto, fornecendo um apelo útil. A gordura tem papel fundamental no sorvete, além de ser um elemento estrutural também promove qualidades sensoriais a esse produto. Composto por mais de 30 tipos de polifenóis conhecidos como antioxidantes, além de vitamina E e caroteno, o azeite extravirgem (AOEV) tem se destacado como alimento funcional por ser uma excelente fonte de ácidos graxos monoinsaturados. Por isso, tem sido sugerido e recomendado no tratamento nutricional de doenças crônicas não transmissíveis (DCNT), doenças do sistema imune, cardiovasculares, entre outras, devido à possível redução da incidência dessas patologias relacionadas ao estresse oxidativo. O objetivo da pesquisa foi avaliar o impacto da substituição da gordura de palma por azeite de oliva extravirgem (AOEV) nas propriedades físicas (ácidos graxos, índices aterogênico e trombogênico, conteúdo fenólico total, atividade antioxidante, overrun, dureza, derretimento, cor, tamanho dos glóbulos de gordura, cristais de gelo e bolhas de ar) e sensoriais em sorvetes. Seis formulações com diferentes proporções de gordura de palma e AOEV foram desenvolvidas, variando de 100% gordura de palma (F1) a 100% AOEV (F6). As amostras com mistura de gorduras apresentaram melhor comportamento durante o teste de derretimento. Os resultados de overrun não foram muito altos e as amostras F1, F4 e F6 não apresentaram diferença significativa (P>0,05) entre elas, e o mesmo ocorreu com as amostras F2, F3 e F5. Além disso, foi possível observar uma tendência crescente na dureza e no croma à medida que a porcentagem de AOEV aumentava na amostra. Quanto à brancura, as amostras F1 e F2 foram responsáveis por valores mais altos, e a última mostrou menos impacto na mudança de cor durante o armazenamento. Amostras com alto teor de AOEV apresentaram alto conteúdo de ácido oleico, fenólicos totais e atividade antioxidante. Além disso, à medida que amentou o conteúdo de AOEE nas amostras, houve redução nos índices aterogênico e trombogênico. Em relação à análise sensorial, foram aplicados três testes, teste de aceitação (escala hedônica de 9 pontos), análise descritiva (lista livre) e o questionário do *Check-All-That-Apply* (CATA). Os resultados obtidos pelo teste de aceitação mostraram que a substituição da gordura de palma pelo AOEV não foi detectada pelos consumidores. Três clusters foram identificados com 51 (1), 60 (2) e 9 (3) consumidores cada. Percebeu-se que os membros do cluster 1 avaliaram homogeneamente a aceitação global para todas as amostras, uma vez que não foi observada diferença estatística (P>0,05) entre eles. Comparados com os outros dois clusters, os membros do cluster 1 apresentaram as maiores pontuações de aceitação global para todas as amostras (valores entre 8,039 (F3) e 7,529 (F6)). O Mapa Interno de Preferência definiu claramente dois grupos, um com duas amostras com baixo conteúdo de AOEV e outro com alto conteúdo de AOEV, o que sugere que o aumento da proporção de AOEV nas amostras de sorvete alterou as características do produto devido à formação de um grupo distinto da amostra controle. A metodologia *Check-All-That-Apply* (CATA) mostrou que os atributos sensoriais das amostras de sorvete podem variar de acordo com as diferentes proporções de gorduras utilizadas. Concluindo, a substituição da gordura de palma pelo AOEV na fabricação de sorvete pode ser uma alternativa eficaz para melhorar os aspectos nutricionais devido à adição do AOEV e os resultados sensoriais mostraram que as amostras com mistura de gorduras apresentaram comportamento positivo e semelhante ao da amostra controle para os consumidores.

Palavras-chave: Propriedades físico-químicas, *Check-All-That-Apply* (CATA), caracterização sensorial, Mapa de Preferência Interno, Análise de Cluster, Teste de Aceitação.

ABSTRACT

Ice creams are widely appreciated worldwide, regardless of culture, age and socioeconomic level, the use of a functional ingredient can add value to the product, providing useful appeal. Fat plays a fundamental role in ice cream, besides being a structural element also promotes sensory qualities to this product. Composed for more than 30 types of polyphenols known as antioxidants, in addition to vitamin E and carotene, extra virgin olive oil (EVOO) has been highlighted as a functional food for being an excellent source of monounsaturated fatty acids. Therefore, it has been suggested and recommended in the nutritional treatment of noncommunicable chronic diseases (NCDs), immune system diseases, cardiovascular diseases, among others, due to the possible reduction of the incidence of these pathologies related to oxidative stress. The objective of the research was to evaluate the impact of substituting palm fat by extra virgin olive oil (EVOO) on physical properties (fatty acids, atherogenic and thrombogenic index, total phenolic content, antioxidant activity, overrun, hardness, melting behavior, color, size of fat globules, ice crystals and air bubbles) and sensory properties in ice creams. To this aim, were developed six formulations with different proportion of palm fat and EVOO, ranging from 100% palm fat (F1) to 100% EVOO. The samples with mixture of fats showed better behavior during melting test. Overrun results were not too high, and samples F1, F4 and F6 did not show significate difference ($P>0.05$) among them, and the same happened with samples F2, F3 and F5. In addition, was possible to observe a tendency grow on the hardness and chroma as the percentage of EVOO increased in the sample. About whiteness, the samples F1 and F2 were responsible for higher values, and the last one showed less impact in color change during the storage. Samples with high content of EVOO presented higher content of oleic acid, total phenolic and antioxidant activity. In addition, as the content of EVOO increased in the samples, there was a reduction in atherogenic and thrombogenic indexes. Regarding sensory analysis, were applied three tests, acceptance test (nine-point hedonic scale), a descriptive analysis (free list) and the Check-All-That-Apply (CATA) questionnaire. Results obtained by the acceptance test showed that the replacement of palm fat by EVOO was not detected by consumers. Three clusters were identified with 51 (1), 60 (2), and 9 (3) consumers each. Were perceived that members of cluster 1 evaluated homogeneously the global acceptance for all samples, since no statistical difference ($P>0.05$) was observed between them. Compared with the other two clusters, members of cluster 1 gave the highest global acceptance scores for all samples (values between 8.039 (F3) and 7.529 (F6)). The internal preference mapping defined clearly two groups, one with two samples with low EVOO content and the other ones with high EVOO content, what suggest that the increase of the proportion of EVOO in the ice cream samples changed the characteristics of the product due to the formation of a distinct group than the control sample. The Check-All-That-Apply (CATA) methodology showed that the sensory attributes of ice cream samples may vary according to the different proportions of oils used. In conclusion, the replacement of palm fat by EVOO to manufacture ice cream can be an effective alternative to improve the nutritional aspects due to addition of EVOO and the sensory results showed that the samples with blend of oils had positive and similar behavior than control sample for consumers.

Key-words: Physicochemical properties, Check-All-That-Apply (CATA), sensory characterization, Internal Preference Mapping, Cluster Analyze, Acceptance Test.

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LISTA DE ABREVIATURAS E SIGLAS

ABIS	Associação Brasileira das Indústrias de Sorvetes
AGMI	Ácidos graxos monoinsaturados
AGS	Ácidos graxos saturados
ANOVA	Analysis of variances
Anvisa	Agência Nacional de Vigilância Sanitária
APHA	American Public Health Association
AI	Atherogenic index
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CATA	Check-All-That-Apply
GC / DIC	High resolution gas chromatography using flame ionization detector
GC / MS	High resolution gas chromatography coupled to mass spectrometry
DCNT	Doenças crônicas não transmissíveis
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EVOO	Extra virgin olive oil
FAO	Food and Agriculture Organization
FOSHU	Food for Specified Health Use
GA	Gallic acid
GAE	Gallic acid equivalents
GWL	Grape wine lees
HCA	Hierarchical Correspondence Analysis
HDL	High Density Lipoproteins
HPLC	High performance liquid chromatography
IBGE	Instituto Brasileiro de Geografia e Estatística
LBG	Locust bean gum
LCFA	Long-chain fatty acids
LDL	Low Density Lipoproteins
MCFA	Medium-chain fatty acids
MUFA	Monoinsaturated fatty acids
NASA	National Aeronautics and Space Administration
NCDs	Non-communicable chronic diseases
NI	Normative Instruction

POF	Pesquisa de Orçamento Familiar
PPE	Pomegranate peel extract
PUFA	Poliinsaturated fatty acids
RDC	Resolução da Diretoria Colegiada
SCFA	Short-chain fatty acids
SNGL	Sólidos não gordurosos do leite
TI	Thrombogenic index
TPTZ	2, 4, 6- tripyridyl-s-triazine
UERJ	State University of Rio de Janeiro
UNIRIO	Federal University of the State of Rio de Janeiro
UV	Ultraviolet
VCT	Valor calórico total diário
WI	Whiteness index

SUMÁRIO

1. REVISÃO BIBLIOGRÁFICA

De acordo com a Resolução da Diretoria Colegiada (RDC) nº 267 de 2003 da Agência Nacional de Vigilância Sanitária (Anvisa), gelados comestíveis são:

> *produtos alimentícios obtidos a partir de uma emulsão de gorduras e proteínas, com ou sem a adição de outros ingredientes e substâncias, ou de uma mistura de água, açúcares e outros ingredientes e substâncias que tenham sido submetidas ao congelamento, em condições que garantam a conservação do produto no estado congelado ou parcialmente congelado, durante o armazenamento, o transporte, a comercialização e a entrega ao consumo* (Brasil, 2003).

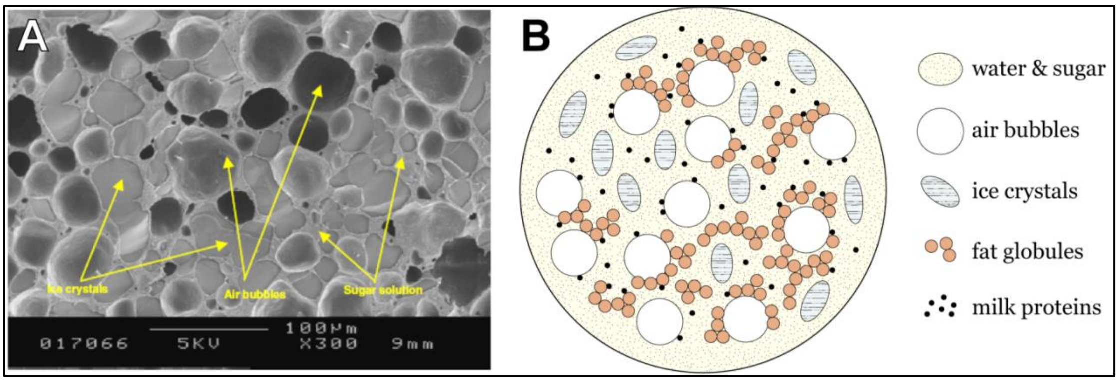
Em 2016, o brasileiro consumiu 1002 milhões de litros de sorvete e o consumo *per capita* foi de 4,86 litros de sorvete/ano por habitante nesse mesmo ano, de acordo com dados da Associação Brasileira das Indústrias de Sorvetes (ABIS, 2017). A Pesquisa de Orçamento Familiar (POF) realizada pelo Instituto Brasileiro de Geografia e Estatística (IBGE) em 2008 e 2009 identificou que o consumo *per capita* médio entre os adolescentes é de 9,2g/dia e entre os adultos 3,8g/dia (IBGE, 2011).

O sorvete é uma emulsão estabilizada, conhecida como calda, composta de uma mistura base de produtos lácteos, água, gordura, açúcar, aditivos, dentre eles estabilizante, emulsificante, corante e aromatizante, que primeiramente é pasteurizada e homogeneizada, logo após passa por um processo de congelamento sob contínua agitação (batimento) e incorporação de ar, com o objetivo de produzir uma substância aveludada, cremosa e muito agradável ao paladar (Marshall, Goff, & Hartel, 2003; Sawyer, 1969).

A estrutura física do sorvete é um sistema físico-químico complexo, composto por três fases: líquida, sólida e gasosa. As bolhas de ar e os cristais de gelo ficam dispersos numa fase líquida contínua. A gordura se apresenta na forma de emulsão, enquanto a proteína, estabilizantes e açúcares insolúveis se apresentam como uma suspensão coloidal. Em forma de dissolução verdadeira se tem a lactose e os sais. A água se encontra no estado líquido como solvente de sais e açúcares, e na forma sólida como cristais de gelo (Goff, 2017; Marshall, Goff, & Hartel, 2003).

Apesar de ser um alimento complexo onde a remoção de um ingrediente pode afetar múltiplas características importantes no produto (Rolon et al., 2016), é um alimento que pode sofrer a adição de novos ingredientes, especialmente com o objetivo de enriquecê-lo nutricionalmente, sem perder a palatabilidade, requisito muito exigido pelos consumidores, e outras características relativas à qualidade sensorial do alimento (Cruz et al., 2009).

A Figura 1 representa esquematicamente a microestrutura do sorvete e demonstra os principais elementos formadores da estrutura: glóbulos de gordura, proteínas, cristais de gelo, bolhas de ar, e a matriz não congelada (Roth-Johnson, 2013).



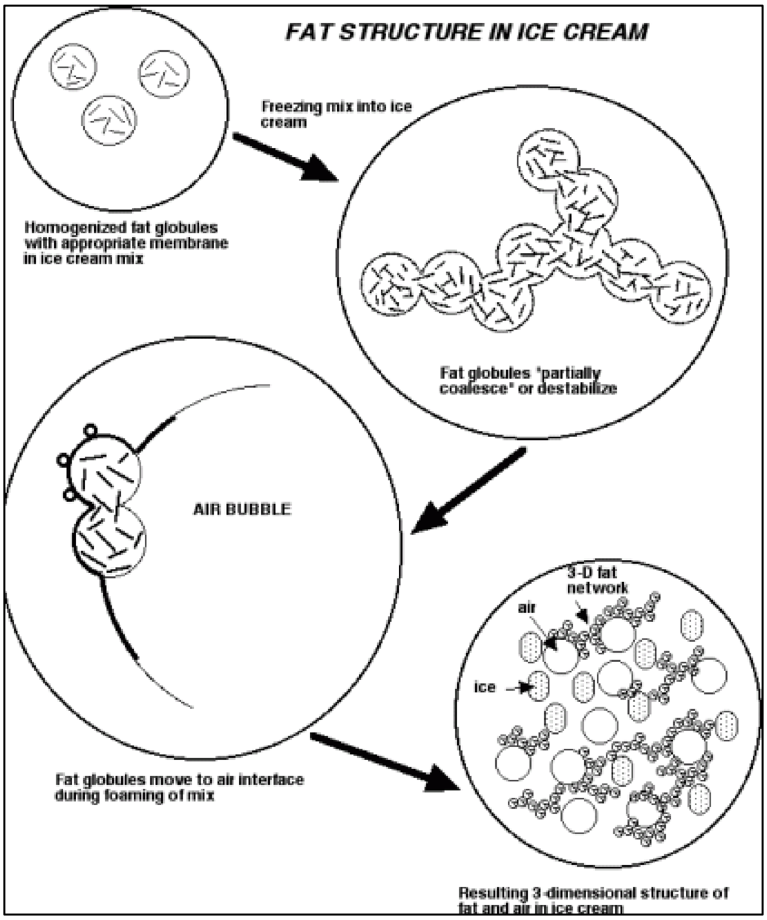
(A) uma micrografia eletrônica de sorvete que mostra bolhas de ar, cristais de gelo e a solução de açúcar. Os glóbulos gordurosos e as proteínas do leite não são visíveis nessa resolução. (B) Diagrama de estrutura de sorvete.
Fonte: Roth-Johnson (2013).

Fig. 1. Esquema da estrutura do sorvete

A gordura é um ingrediente de extrema importância no sorvete, pois ela reduz a velocidade de derretimento, melhora o corpo do produto e a sensação na boca, contribuindo para a obtenção de um produto com textura suave e agradável cremosidade (Clarke, 2012). A diferença mais marcante e facilmente observada entre sorvetes com baixo e alto teor de gordura é a sensação de frio. Os produtos com baixo teor parecem mais frios na boca, enquanto os produtos com maior quantidade reduzem a sensação de frio bucal, possuindo alta sensação lubrificante na boca e são mais macios e cremosos (Mikilita, 2002). Marshall, Goff, e Hartel (2003) esclarecem que o teor de gordura do leite pode variar de 0 a 24%, dependendo de fatores legais de cada país, qualidade do produto final, custo e ofertas/tendências do mercado.

Autores apontam que a presença da gordura implica na redução da formação de cristais de gelo formados no sorvete, bem como no tamanho desses cristais, já que ela se concentra nas superfícies das bolhas de ar durante o congelamento do produto e passa a ocupar os espaços vazios nos quais os cristais poderiam se formar (Silva Junior & Lannes, 2011; Marshall, Goff, & Hartel, 2003; Timm, 1989).

Os glóbulos de gordura se apresentam tanto individuais quanto agregados. Os últimos se desenvolvem através da aglomeração que ocorre quando os glóbulos individuais de gordura colidem durante a agitação rápida na etapa de congelamento (Fernandes, 2016). O glóbulo de gordura sofre duas modificações durante o processamento do sorvete: a formação da emulsão ou estabilização e a coalescência parcial ou desestabilização (Goff, 2017). A figura 2 representa a estrutura tridimensional do sorvete e transformação do glóbulo de gordura.



Fonte: Goff (2017).

Fig. 2. Transformações do glóbulo de gordura no sorvete

Na etapa de homogeneização se inicia o processo de formação da estrutura da gordura, nela ocorre a quebra dos glóbulos íntegros resultando em uma grande quantidade de pequenos glóbulos de gordura (de aproximadamente 1 μm de diâmetro) (Goff, 2017). Com o aumento da área superficial, apenas o material de membrana não é suficiente para recobrir todos os glóbulos recém-formados, por isso ocorre a adsorção de certas moléculas com características anfifílicas – molécula cuja estrutura possui uma parte hidrofílica (solúvel em água) e outra parte lipofílica (solúvel em lipídios e solventes orgânicos) –, como as caseínas, as proteínas do soro e os emulsificantes (Pereira et al., 2011).

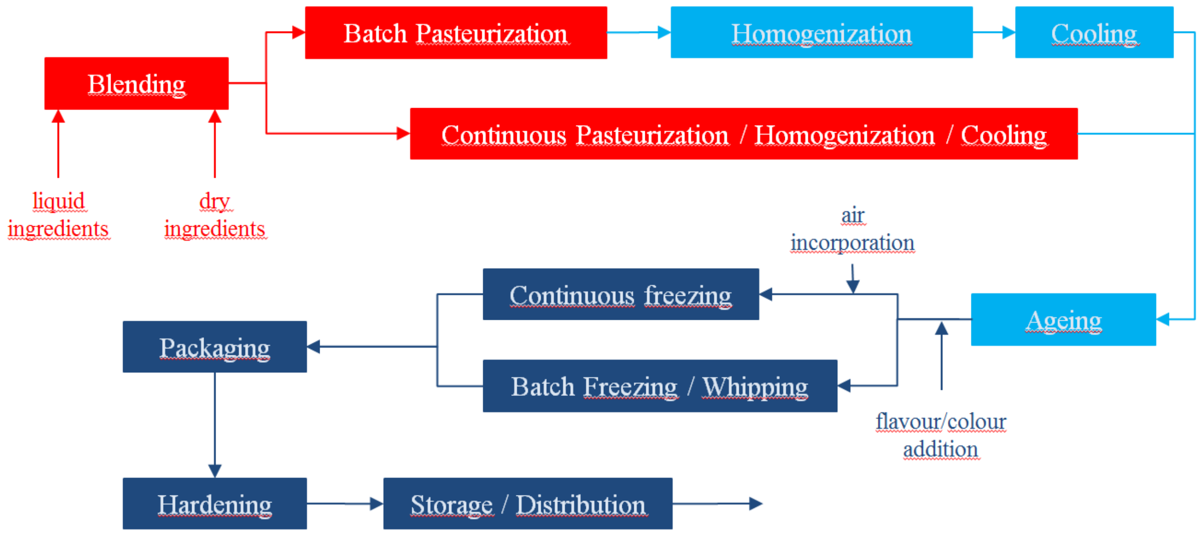
O uso de altos teores de gordura no produto é limitado por alguns fatores como, aumento do custo, redução da capacidade de incorporação de ar e alto valor calórico (Goff, 2017; Marshall, Goff, & Hartel, 2003). Por outro lado, muitos fabricantes têm sido motivados a reduzir custos na formulação dos produtos e o valor calórico, com isso diminuem o teor de gordura, já que a gordura fornece mais energia por grama do que outros macronutrientes e possui alto custo (Rolon et al., 2017). Reduzir o teor de gordura pode afetar características no produto como aparência, sabor, características adversas e reológicas (Tekin, Sahin, & Sumnu, 2017). Uma maneira de reduzir a gordura no sorvete envolve simplesmente em substituí-la por água. Esta estratégia resulta em uma mistura com baixo teor de sólidos, menor viscosidade e um produto com alta dureza e uma taxa de fusão mais rápida. Mais comumente, a gordura é substituída por agentes de volume, como carboidratos ou ingredientes proteicos, para fornecer suporte estrutural e melhorar as propriedades sensoriais. As maltodextrinas, polissacarídeos produzidos por hidrólise parcial do amido, são um ingrediente comumente utilizados em sobremesas congeladas, como o sorvete (Rolon et al, 2017).

Outro ponto relevante a ser mencionado é que para evitar que o sorvete se torne “arenoso”, ou seja, ocorra a cristalização do açúcar do leite (lactose) no produto final, na medida em que aumentar o teor de gordura do sorvete, o teor de sólidos não gordurosos do leite (SNGL) deve ser diminuído (Silva Junior & Lannes, 2011; Marshall, Goff, & Hartel, 2003).

O tipo de gordura, sua composição, a quantidade no produto e seu ponto de fusão têm forte influência sobre as características sensoriais e na estabilidade do sorvete durante sua conservação (Goff, 2017). Há várias fontes de gordura sendo utilizadas para fabricação de sorvetes. Pode ser fornecida através dos ingredientes lácteos como creme de leite – principal fonte de gordura utilizada nos Estados Unidos –, manteiga, leite em pó integral, ou de origem vegetal, derivada do coco, da palma, do algodão, do cacau e de colza (Marshall, Goff, & Hartel, 2003).

A formação da estrutura do sorvete começa desde o início do processo de fabricação, o qual consiste basicamente das etapas de mistura, pasteurização, homogeneização, resfriamento e maturação, seguido do congelamento e aeração (Shrivastav & Goswami, 2017; Goff, 1997). Nas últimas etapas citadas, eles são submetidos à alta condição de cisalhamento em um congelador de superfície raspada, também conhecido como “dasher” ou “batedeira” (Shrivastav & Goswami, 2017; Marshall, Goff, & Hartel, 2003). A etapa do congelamento combinada ao “chicoteamento” ou batedura – ambas acontecem simultaneamente no interior do *dasher* –, são as mais importantes para o desenvolvimento da microestrutura do produto final (Shrivastav & Goswami, 2017; Goff, 2002; Wildmoser, 2004). Por fim, acontecem o acondicionamento, congelamento final ou endurecimento e estocagem do produto.

Na figura 3 é apresentado o diagrama de fluxo do processo para a fabricação de sorvete, a seção vermelha representa as operações envolvendo a mistura “crua”, ainda não pasteurizada, a seção azul clara representa as operações envolvendo a mistura pasteurizada e a seção azul escuro representa as operações envolvendo o sorvete congelado.



Fonte: Goff (2017)

Fig. 3. Digrama de fluxo de fabricação do sorvete

Nos últimos anos têm surgido muitas inovações em sorvetes, a fim de atender às exigências e demanda dos consumidores, especialmente por alimentos com maior valor nutritivo, além de benefícios à saúde (Cummigs, Edmond, & Magee, 2004).

Algumas inovações em sorvetes estão apresentadas na tabela 1:

Tabela 1

Inovações em sorvetes

Inovação	Conclusão	Fonte
Sorbet de uva integral e adoçado com *blend* de maçã	O experimento com *blend* de polpas de uvas "Cora" e "Carmem" no tratamento com 70% de polpa apresentou maior impacto na aceitação global na análise sensorial, além de maior teor de bioativos	Lieira (2017)
Desenvolvimento de sorvete adicionado de kinkan (fruta cítrica originária da China)	Produto apresentou boa aceitação global na análise sensorial, alta atividade antioxidante e elevado teor de fenólicos totais	Lima et al. (2017)
Sorvete de chocolate com adição de biomassa de banana verde (*Musa spp*)	Produto apresentou características sensoriais satisfatórias de aceitação quanto aos atributos cor, sabor, textura, aparência e aceitação global	Wrobel e Teixeira (2017)
Sorvete adicionado de maltodextrina e farelo de mandioca	Sorvete obteve boa aceitação sensorial utilizando 5% de farelo e 50% de maltodextrina de mandioca, além de teor de fibras maior do que nos sorvetes tradicionais e reduzido teor de gordura	Fernandes (2016)
Sorvete tipo iogurte à base de soja com adição de microrganismos probióticos	Sorvete apresentou desempenho satisfatório, maior que o sorvete de base láctea, no processo de fermentação das cepas probióticas, além da análise sensorial apontar a amostra do sorvete de iogurte probiótico à base de soja como preferida em sua maioria	Arruda, Oliveira A. e Oliveira A. D. (2015)
Capacidade antioxidante e funcionalidade de farinha e crosta oleander (*Elaeagnus angustifolia L.*) em um novo tipo de sorvete frutado	As amostras contendo farinha *oleaster* foram preferidas pelos painelistas em relação às propriedades sensoriais. Os autores concluíram que a farinha *oleaster* pode ser um aditivo natural adequado na formulação de sorvete	Çakmakçi et al. (2015)
Sorvete sem lactose à base de vegetais, substituindo estabilizante e emulsificante	Os sorvetes elaborados com psyllium como substituinte integral do emulsificante e estabilizante não perderam qualidade sensorial e foram bem	Eiki et al. (2015)

por chia e psyllium tritutados	aceitas pelos consumidores	
Sorvete produzido com *amla* (groselha indiana)	A incorporação de *amla* processada aumentou a resistência ao derretimento do sorvete e reduziu o "*overrun*". As amostras com 5% de pedaços e polpa, 10% de conservas e doces e 0,5% de pó apresentaram maiores pontuações na aceitação global, na análise sensorial. A inclusão de *amla* em todas as formas melhorou as propriedades funcionais e o valor nutricional do sorvete	Goraya e Bajwa (2015)
Sorvete enriquecido com farinha da casca da jabuticaba (*Myrciaria cauliflora*)	O enriquecimento do sorvete com 5% de farinha da casca de jabuticaba resultou em produtos com elevado valor nutricional e não afetou negativamente as características sensoriais	Lamounier et al. (2015)
Sorvete com leite de cabra adicionado de mucilagem de chia (*Salvia hispânica L.*) e farinha de semente de alfarroba (*Serotonia siliqua L.*)	Produto com substituição total da gordura vegetal hidrogenada pela mucilagem de chia apresentou reduzidos teor de gordura e valor energético, além de maior taxa de "*overrun*"	Piati, Malacarne e Gall (2015)
Sorvete fortificado com proteína de peixe	Os sorvetes com 30 e 50 g/kg da proteína de peixe em pó apresentaram teores de proteínas e sólidos não gordurosos significativamente maiores do que os produtos com 0% da proteína. Na análise sensorial os produtos com a proteína de soja tiveram qualidade sensorial semelhante após a produção, exceto pela cor, porém após 2 meses as propriedades sensoriais das amostras fortificadas mudaram significativamente	Shaviklo et al. (2011)

De acordo com a legislação brasileira (Brasil, 2012), o azeite de oliva possui como principal ácido graxo monoinsaturado o ácido oleico (55 a 83%) e tem sido considerado um importante aliado na dieta mediterrânea, além disso, tem sido indicado e recomendado no tratamento nutricional das doenças crônicas não transmissíveis (DCNT), doenças do sistema imunológico, como câncer, aterosclerose, doenças cardiovasculares, entre outras, devido à possível redução da incidência dessas patologias relacionadas ao estresse oxidativo (Costa & Rosa, 2016; Salas-Salvadó & Mena-Sánchez, 2017).

De acordo com a *Food and Agriculture Organization* (WHO/FAO, 2015), existem evidências que comprovem que: a substituição de carboidratos por ácidos graxos monoinsaturados (AGMI) aumenta as concentrações de colesterol *High Density Lipoproteins* (HDL); a substituição de ácidos graxos saturados (C12:0 a C16:0) por AGMI reduz a concentração de colesterol *Low Density Lipoproteins* (LDL) e a relação colesterol total / HDL; a substituição de carboidratos por AGMI melhora a sensibilidade à insulina. E complementa que não há evidências suficientes para: relacionamentos de consumo de AGMI com pontos finais de doença crônica, como doenças cardíacas coronárias ou câncer; relacionamentos de consumo de AGMI e peso corporal e porcentagem de adiposidade; uma relação entre a ingestão de AGMI e o risco de diabetes.

De acordo com a mesma organização supracitada, a recomendação de consumo de gorduras totais é de 20 a 35% do valor calórico total diário (VCT), sendo que esse total deve ser distribuído em até 10% de ácidos graxos saturados (AGS), até 1% de ácidos graxos *trans* (AGt) e 6% a 11% de ácidos graxos poli-insaturados (AGPI). O total de ácidos graxos monoinsaturados (AGMI) é dado pela diferença: Ingestão total de gordura – (AGS + AGPI + AGt)[2]. De acordo com a ingestão total de gordura esse valor de AGMI pode ser de 15 a 20%.

Composto por mais de 30 tipos de polifenóis, reconhecidamente antioxidantes, além de vitamina E e caroteno, o azeite de oliva tem se destacado como um alimento funcional por ser uma excelente fonte de ácidos graxos monoinsaturados (Costa & Rosa, 2016).

Pestana-Bauer, Goularte-Dutra e Zambiazi (2011) em seu estudo de caracterização do fruto da oliveira (variedade Carolea) cultivada na região sul do Brasil, encontraram 71,34% de ácido oleico, 25,86% de palmítico e 2,11% de linoleico. Os autores atribuem essa diferença em relação aos parâmetros estabelecidos dos ácidos palmítico e linolênico, ao baixo índice de maturação da azeitona analisada, possivelmente não tendo concluído a síntese dos ácidos graxos, resultando em maior teor do ácido palmítico e menor teor do ácido linoleico.

O *International Olive Council* (2007) ressalta que a composição nutricional do azeite em ácidos graxos dependerá da zona de produção, espaço geográfico, clima, cultivar e da fase de amadurecimento do fruto.

Em estudo realizado por Franco et al. (2014) foi possível separar e identificar 24 compostos fenólicos nos azeites de oliva analisados. Em todos os produtos analisados, os derivados de secoutrosídeos foram os mais abundantes, seguidos de álcoois fenólicos, flavonoides e ácidos fenólicos. Outra conclusão dos autores é a de que quanto mais madura a oliva, menor a quantidade de compostos fenólicos no azeite. A tabela 2 mostra os principais compostos fenólicos encontrados no estudo, bem como suas respectivas faixas de concentrações.

Tabela 2

Principais compostos fenólicos encontrados no azeite de oliva e suas respectivas faixas de concentração

Composto fenólico	**Faixa de concentração (µg/mL)**
Hidroxitirosol	0,1 – 40
Tirosol	0,5 – 40
Ácido cafeico	0,05 – 30
Ácido vanílico	0,5 – 40
Vanilina	0,5 – 40
Ácido p-cumarínico	0,5 – 40
Ácido ferrúlico	0,05 – 30
Oleuropeína	2 – 1500
Luteolina	0,05 – 30
Apigenina	0,05 – 30

Fonte: Adaptada de Franco et al., 2014.

Segundo Kwak e Jukes (2001), a sociedade moderna tem mudado cada vez mais os padrões de vida. As pessoas comumente têm apresentado sintomas de estresse, cansaço e depressão. Anjo (2004) complementa que os vários fatores têm contribuído para que os consumidores estejam cada vez mais conscientes e busquem por alimentos com benefícios para a saúde.

Costa e Rosa (2016) informam que por conta disso, são inúmeros os esforços da indústria de alimentos, a fim de lançar produtos para atender à demanda, e do setor acadêmico, para ampliar os estudos na área dos alimentos e na sua interação com a saúde, como tem sido observado na última década. O mercado de alimentos funcionais no Brasil representa cerca de 15% do mercado de alimentos, com crescimento anual de aproximadamente 20%.

O consumo de alimentos funcionais está atrelado a efeitos benéficos à saúde, devido à presença de determinados compostos bioativos ou substâncias bioativas, como por exemplo, os compostos fenólicos, os quais são o maior grupo de antioxidantes naturais das dietas humanas (Martinis & Teixeira, 2015; Zhang & Tsao, 2016).

As substâncias bioativas têm sido amplamente estudadas, não só para atender a demanda atual, mas também às futuras necessidades de consumo, como mostra o estudo de Bermudez-Aguirre (2016), que desenvolveu novos alimentos de voo espacial com alto teor de ácidos graxos ômega-3, licopeno e flavonoides, com o objetivo de identificar produtos comerciais com esses compostos bioativos que atendam aos requisitos de voos espaciais, já que durante as missões espaciais da *National Aeronautics and Space Administration* (NASA) de longa duração, como as missões propostas para Marte, os astronautas podem sofrer efeitos fisiológicos negativos, como a perda óssea, que podem ser mitigados com alguns alimentos funcionais. O estudo concluiu que após 1 ano de armazenamento os compostos bioativos presentes nos alimentos desenvolvidos apresentaram resultados favoráveis, como o ômega-3 que mostrou excelente estabilidade, independentemente da temperatura de armazenamento; os compostos fenólicos também apresentaram boa estabilidade. O licopeno foi o mais estável em produtos à base de óleo em vez de produtos à base de água devido à proteção que os lipídios oferecem às moléculas de licopeno. Além disso, o licopeno foi mais estável em produtos liofilizados do que em alimentos com alto teor de umidade. Os autores pretendem continuar o estudo para avaliar a estabilidade por 2 até 5 anos.

Os “fenólicos” são fitoquímicos constituídos por um anel aromático com um grupo hidroxila, enquanto os “polifenóis” podem ter um ou mais anéis aromáticos que possuem mais de um grupo hidroxila. No entanto, ambos os termos são usualmente encontrados nos estudos científicos, sendo assim serão tratados de forma semelhante nesta revisão. Eles são amplamente encontrados na natureza, com mais de 8000 compostos detectados principalmente em vegetais, frutas, grãos, chá, óleos essenciais e seus alimentos e bebidas derivados. Os polifenóis podem ser classificados como diferuloilmetanos (curcuminas), stilbenos (resveratrol), flavonoides, ácidos fenólicos e taninos (Zhang & Tsao, 2016; Martinis & Teixeira, 2015; Pandareesh, Mythri, & Srinivas Bharath, 2015).

Segundo Scalbert e Williamson (2000), a ingestão total de alimentos ricos em compostos fenólicos chega a aproximadamente 1 g por dia, porém os autores concluem que ainda permanecem grandes incertezas desse número devido à falta de dados abrangentes sobre o conteúdo de algumas das principais classes de polifenóis nos alimentos.

Os teores de compostos fenólicos são influenciados por certos fatores e condições do solo nos seres vivos, por isso podem variar significativamente em um mesmo alimento (Martinis & Teixeira, 2015). Por exemplo, segundo Cordenunsi (2002) os teores de antocianinas de morango variam de 16 mg/100g na cultivar Campineiro a 30 mg/100g na cultivar Oso.

Estudos epidemiológicos, clínicos e nutricionais vêm mostrando os benefícios terapêuticos dos polifenóis, devido às suas propriedades antioxidantes e algumas evidências como redução do risco, prevenção e aparecimento de doenças degenerativas, cardiovasculares e distúrbios metabólicos, além do seu papel modulador na sinalização celular (Martinis & Teixeira, 2015; Kim, H., Quon, & Kim, J., 2014; Scoditi et al., 2014; Menard, Bastianetto, & Quirion, 2013).

No azeite, estudos vêm mostrando que estão presentes compostos fenólicos hidrofílicos como álcoois e ácidos fenólicos, flavonoides, secoiridóides e lignanos, e compostos lipofílicos como tocoferóis, comuns a outros óleos vegetais (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998; Servili et al., 2004).

O sorvete comercialmente disponível, em geral, é pobre em antioxidantes naturais como os polifenóis. Por isso, é interessante explorar novas oportunidades de aprimorar os atributos nutricionais utilizando ingredientes com benefícios à saúde, como compostos bioativos, antioxidantes naturais, vitaminas, corantes e aromas naturais, livres de aditivos sintéticos, a fim de atender às expectativas dos consumidores, que estão cada vez mais exigentes com sua forma de alimentação (Chanmchan et al., 2017; Sun-Waterhouse et al., 2013).

Sun-Waterhouse et al. (2013) examinaram a viabilidade de produzir um novo tipo de sorvete através da incorporação de uma quantidade substancial de frações aquosas de suco de kiwis com polpa verde, dourada e vermelha, sem aromatizantes. A mistura, com teor de gordura de 9,5%, foi pasteurizada a 80ºC por 30 segundos, resfriada a 4ºC, congelada a -80ºC$\pm$3ºC por 1,5 horas e estocada por 24 horas a -20ºC$\pm$2ºC, pontos críticos para retenção das substâncias bioativas. Como resultado, os autores conseguiriam detectar, nas três amostras, vários compostos fitoquímicos benéficos para saúde, como o hexosseto de ácido dimetil-cafeico, os derivados do ácido cafeico, o ácido protocatequico, o ácido siringídico, o ácido salicílico / ácido л-cumárico, a luteína e o beta-caroteno. Foi verificado, ainda, que o tipo de kiwi afetou a estabilidade da vitamina C na matriz do sorvete, além de algumas diferenças nas propriedades reológicas do sorvete também terem sido afetadas por esse fato.

Çakmakçi et al. (2015) estudaram a possibilidade de produzir sorvete com farinha e crosta de *oleaster*, uma fruta também conhecida como azeitona russa e nativa da Ásia ocidental e central, do sul da Rússia e do Cazaquistão ao mediterrâneo, Turquia e Irã. A mistura foi pasteurizada a 85ºC por 25 segundos e resfriada a 4ºC, congelada a -22ºC por 24 horas e estocada a -18ºC. Os autores indicaram que as amostras de sorvete contendo a farinha foram mais aceitas sensorialmente pelos avaliadores, além de melhorarem os valores nutricionais, capacidade antioxidante, cor e propriedades físico-químicas dos produtos. Além disso, concluíram que tanto a farinha quanto a crosta possuem propriedade antioxidante moderada e podem ser atribuídas à presença de compostos fenólicos e flavonoides.

Logo, para a prevenção de estresses oxidativos no corpo, substâncias com capacidades antioxidantes devem estar presentes em concentração suficiente. A estabilidade oxidativa dos alimentos é algo que precisa ser levado em consideração, pela sua extrema importância, visto que os estresses oxidativos podem levar a doenças crônicas como a diabetes, desenvolvimento de câncer, envelhecimento acelerado, aterogênese e mutação de DNA (Ullah, Nadeem, & Imran, 2017; Chanmchan et al., 2017; Sanguigni et al., 2016).

O estudo realizado pelos autores Ullah, Nadeem e Imran (2017) visou aumentar a concentração de ácidos graxos ômega-3 e a estabilidade oxidativa de sorvete, usando fração de oleína do óleo de chia, que possui aproximadamente 63-65% de ácidos graxos ômega-3. A mistura foi pasteurizada a 85ºC por 1 minuto, resfriada a 4ºC, congelada a -18ºC e estocada a mesma temperatura por 60 dias. Os autores concluíram que a suplementação de sorvete com fração de oleína do óleo de chia melhorou significativamente a concentração de ácidos graxos ômega-3 no sorvete. O conteúdo fenólico total, os flavonoides totais e a atividade de eliminação de radicais livres DPPH dos sorvetes suplementados foram maiores do que a amostra controle, sem suplementação.

Chanmchan et al. (2017) desenvolveram duas fórmulas de sorvete com teor reduzido em açúcares com ervas de gengibre e limão capim, usando xilitol como adoçante e determinaram os antioxidantes presentes no produto. A mistura foi pasteurizada a 87ºC por 2 minutos, após foi resfriada e mantida maturando por 24 horas. O produto foi mantido a -5ºC até completo congelamento. A conclusão do estudo foi que o enriquecimento de sorvete com extrato líquido de erva-limão ou extrato de gengibre apresentou maior conteúdo de compostos fenólicos e atividade antioxidante total.

O objetivo do estudo conduzido por Sanguigni et al. (2016) foi realizado com 14 indivíduos saudáveis randomizados para consumir 100 gramas de sorvete antioxidante contendo cacau amargo em pó e avelã e extrato de chá verde e sorvete de chocolate com leite (sorvete controle). Como resultado os autores sugeriram que a ingestão do sorvete antioxidante, que consistiu em uma mistura de alimentos selecionados com alto conteúdo de polifenóis, melhorou fortemente a função vascular e o desempenho físico, provavelmente através de um mecanismo mediado pelo combate ao estresse oxidativo.

Hwang, Shyu e Hsu (2009) utilizaram o sedimento de uva depositado no fundo dos barris de vinho (denominado por eles de "GWL" – *Grape wine lees*) para adicionar propriedades antioxidantes ao sorvete. A mistura foi pasteurizada a 80±2ºC por 20 minutos, após foi resfriada e mantida maturando a 4ºC. A temperatura de congelamento e estocagem foi de -25ºC. Como resultado foi demonstrado que a adição do GWL aumentou significativamente a atividade antioxidante do sorvete, e o antioxidante no GWL se mostrou estável durante o processo de fabricação.

Contudo, estudos têm demonstrado resultados favoráveis com a produção de sorvetes adicionados de matérias-primas com alto teor de substâncias bioativas a fim de melhorar as propriedades nutricionais do produto final.

Na maioria dos estudos é possível observar a utilização da análise sensorial dos produtos como ferramenta de caracterização e aceitação, visto que tem sido amplamente usada como uma importante avaliação no desenvolvimento de alimentos, para fins de controle de produtos, controle de qualidade na indústria de alimentos, marketing e pesquisa (Tham et al., 2016). De acordo com Dutcosky (2007), a análise sensorial representa a interação entre alimento/homem, nas quais as características intrínsecas do alimento interagem com as condições fisiológicas, fenômenos emocionais e sociais do indivíduo.

2. INTRODUÇÃO GERAL

Sorvete é uma emulsão aerada congelada, um alimento complexo, que contém glóbulos de gordura parcialmente coalescidos, cristais de gelo dispersos na fase sérica de proteínas, bolhas de ar, sais, açúcares dissolvidos e água. A produção industrial do sorvete é lucrativa e está em constante desenvolvimento, visto que é um dos alimentos preferidos das crianças, adultos e até dos idosos (Hartel, Rankin, e Bradley, 2017; Turgut & Cakmakcı, 2009; Marshall, Goff, e Hartel, 2003). Por isso, muitos pesquisadores recentes têm estudado o controle de suas microestruturas complexas para melhorar suas qualidades funcionais e nutricionais (Hartel, Rankin, & Bradley, 2017).

A gordura é um componente de grande funcionalidade no sorvete, porque além de ser um elemento estrutural, podendo afetar a resistência ao derretimento, a retenção da forma após o processo de congelamento e a suavidade após o derretimento também promove qualidades sensoriais características deste produto. É proveniente de fontes lácteas ou não e varia, normalmente, de 10 a 16% no produto (Akbari, Eskandari, e Davoudi, 2019; Rolon et al., 2017; Goff, 2002; Berger, 1990). No entanto, o uso de gorduras vegetais em sorvetes é muito comum em todo o mundo, alterando a proporção de gordura sólido/líquido em temperaturas de congelamento/chicoteamento, selecionando gorduras com diferentes taxas de triglicerídeos saturados:insaturados que podem afetar a quantidade e a força da desestabilização da gordura (Sung e Goff, 2010).

Composto por mais de 30 tipos de polifenóis conhecidos como antioxidantes, além de vitamina E e caroteno, o azeite de oliva extravirgem tem se destacado como alimento funcional por ser uma excelente fonte de ácidos graxos monoinsaturados (Costa e Rosa, 2016). Por isso, tem sido sugerido e recomendado no tratamento nutricional de doenças crônicas não transmissíveis (DCNT), doenças do sistema imune, cardiovasculares, entre outras, devido à possível redução da incidência dessas patologias relacionadas ao estresse oxidativo (Salas-Salvadó, J., e Mena-Sánchez, 2017; Costa e Rosa, 2016).

Alimento funcional é um termo usado por diferentes países e organizações para descrever alimentos com potenciais benefícios à saúde. Não existe uma definição universalmente aceita e determinada para um alimento funcional. Foi sugerido que alimentos funcionais têm o potencial de reduzir os riscos associados a várias doenças, como hiperlipidemia, diabetes, distúrbios ósseos, doenças cardiovasculares, hipertensão, doenças imunológicas, distúrbios digestivos e câncer (Crowe e Francis, 2013; Hasler et al., 2004; Zheng e Wang, 2001). Devido ao crescente interesse em seus potenciais benefícos à saúde, a produção e o consumo de alimentos funcionais têm aumentado nas últimas duas décadas (Zheng e Wang, 2001).

Os resultados obtidos no estudo estão apresentados nessa dissertação na forma de artigo, conforme descrição dos capítulos a seguir.

No primeiro capítulo deste trabalho é apresentado um artigo entitulado "E*ffects on* *physicochemical properties, acceptance and sensory product characterization using extra virgin olive oil as a substitute to palm fat on the preparation of vanilla ice cream*". Neste capítulo são apresentados os resultados das análises físico-químicas (ácidos graxos, conteúdo de fenólicos totais e atividade antioxidante) e as propriedades sensoriais das seis amostras de sorvete produzidas com diferentes proporções de gordura de palma e azeite de oliva extravirgem. Foram aplicados três testes sensoriais, teste de aceitação (escala hedônica de nove pontos), uma análise descritiva (lista livre) e o questionário *Check-All-That-Apply* (CATA). A análise de cluster foi realizada com os resultados da impressão global e o Mapa de Preferências Interno pelas duas primeiras dimensões de preferência do grupo de consumidores.

O capítulo 2 compreende o artigo "*Extra virgin olive oil addition in ice cream: The effects on the physical properties"*. Neste capítulo são apresentados os resultados das análises físicas: overrun, dureza, derretimento, cor e tamanho dos cristais de gelo, bolhas de ar e glóbulos de gordura para avaliar o comportamento do azeite de oliva extravirgem e da gordura de palma nas amostras desenvolvidas.

3. OBJETIVO GERAL

Avaliar os efeitos da substituição da gordura de palma por azeite de oliva extravirgem nas propriedades físicas, físico-químicas e sensoriais em sorvetes.

4. OBJETIVOS ESPECÍFICOS

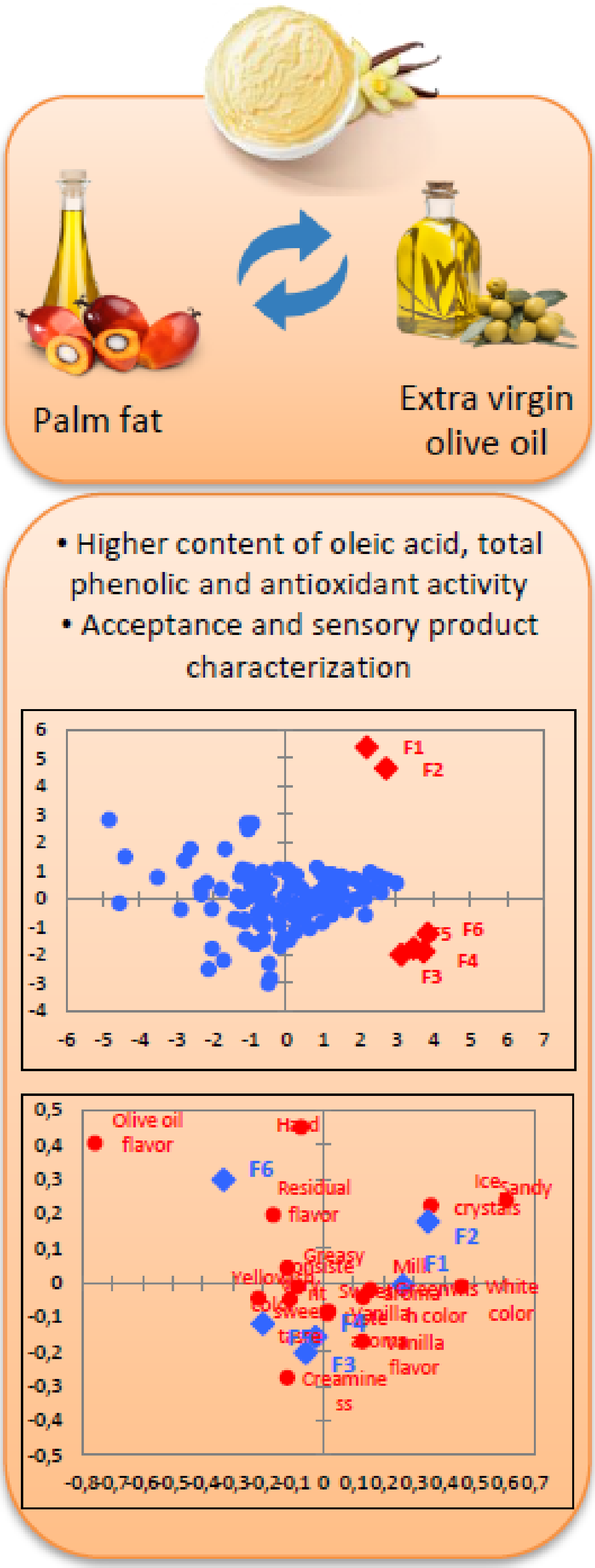
- Desenvolver sorvete contendo gordura de palma e azeite de oliva extravirgem, como fontes lipídicas, em diferentes proporções;
- Determinar o overrun, comportamento do derretimento, cor, textura e tamanho dos glóbulos de gordura, cristais de gelo e bolhas de ar, das amostras de sorvete, através de análises físicas;
- Qualificar e quantificar os ácidos graxos presentes nas gorduras utilizadas e nas amostras produzidas;
- Quantificar o conteúdo fenólico total e a atividade antioxidante das amostras;
- Avaliar a aceitação dos consumidores e realizar a caracterização sensorial das amostras de sorvete desenvolvidas.

CAPÍTULO 1 – EFFECTS ON PHYSICOCHEMICAL PROPERTIES, ACCEPTANCE AND SENSORY PRODUCT CHARACTERIZATION USING EXTRA VIRGIN OLIVE OIL AS A SUBSTITUTE TO PALM FAT ON THE PREPARATION OF VANILLA ICE CREAM

Pryscilla D. Casagrande[1], Iago S. Quintino[2], Guilherme M. Santos[2], Jaqueline P. Silva[2], Joel Abreu[3], Anderson Teodoro[3], Roberta F. Miyahira[4], Ricardo F.A. Moreira[5], Lilia Zago[4], Rafael S. Cadena[1]

[1] Food and Nutrition Program, Federal University of Rio de Janeiro State, Pasteur Avenue, 296 – Urca – Zip code 22290-240, BRA. [2] Nutrition School, Federal University of Rio de Janeiro State, Pasteur Avenue, 296 – Urca – Zip code 22290-240, BRA. [3] Nutrition Biochemistry Core, Laboratory of Functional Foods, Federal University of Rio de Janeiro State, Pasteur Avenue, 296 – Urca – Zip code 22290-240, BRA. [4] Nutrition Institute, University of Rio de Janeiro State, São Francisco Xavier, from 280 to 526 – Maracanã – Zip code 20550-013, BRA. [5] Center of Biological and Health Sciences, Biomedical Institute, Federal University of Rio de Janeiro State, Frei Caneca, 94 – Centro – Zip code 20211040, BRA.*

GRAPHIC ABSTRACT



ABSTRACT

The aim of this research was to evaluate the impact on physical (fatty acids, atherogenic and thrombogenic indexes, total phenolic content and antioxidant activity) and sensory properties of the ice cream with palm fat replacement of extra virgin olive oil (EVOO). Six formulations with different proportion of palm fat and EVOO were developed. Ice cream samples with high content of EVOO presented higher content of oleic acid, total phenolic and antioxidant activity. In addition, as the content of EVOO increased in the samples, there was a reduction in atherogenic and thrombogenic indexes. Three sensory tests were applied: liking (nine-point hedonic scale), free listing and Check-All-That-Apply (CATA) descriptive analysis. Cluster analysis was performed on consumers liking scores prior to internal preference mapping using Hierarchical Correspondence Analysis (HCA). Results showed that the replacement of palm fat by EVOO was not detected or poorly evaluated by consumers. Three clusters were identified with 51 (1), 60 (2), and 9 (3) consumers in each. Cluster 1 evaluated homogeneously the overall liking for all samples, since no statistical difference ($P>0.05$) was observed in between them and consumers gave the highest overall liking scores for all samples (7.53 to 8.04) compared to the other two clusters. The internal preference mapping defined clearly two groups, one with two samples with low EVOO content and the other ones with high EVOO content, what suggest that the increase of the proportion of EVOO in the ice cream samples changed the characteristics of the product due to the formation of a distinct group than the control sample. Sensory attributes of ice cream samples may vary according to the different proportions of oils used. In conclusion, the replacement of palm fat by EVOO to produce ice cream can be an effective alternative to improve the nutritional aspects due to functionality provided by EVOO and the sensory results showed that the samples with blend of oils had positive and similar behavior than control sample for consumers.

Keywords: Extra virgin olive oil, palm fat, ice cream, Check-All-That-Apply (CATA), Free Listing, Internal Preference Mapping, Cluster Analyze, Acceptance Test.

1. Introduction

The concept of functional foods emerged in Japan in the mid-1980s as a result of collaborative studies between government, universities, and food manufacturers: Food for Specified Health Use (FOSHU) as part of a normal diet that demonstrate physiological benefits or reduce the risk of chronic diseases, in addition to their basic nutritional functions. Only in Japan, the term "functional food" is defined by law (Baptista et al., 2013; Costa & Rosa, 2016). In Brazil, there is no such a policy functional food, but the Government Health Ministry (Anvisa), evaluates and approves the claim of functional and/or health property and establishes the guidelines for its use, as well as the registration conditions for this food claiming (Brasil, 1999; Costa & Rosa, 2016).

According to Kwak and Jukes (2001), the modern society has increasingly changed the living standards. People commonly have symptoms of stress, fatigue and depression. Anjo (2004) complements that many factors have contributed to consumers becoming more conscious and secking for foods with health benefits. Due to this in the last decade, the efforts of the food industry in order to launch products to meet such demand, and the academic society to expand studies in the area of food and its interaction, have been observed. The functional food market in Brazil represents about 15% of the food market, with annual growth of approximately 20% (Costa & Rosa, 2016).

The aforementioned authors complemented that there is a great diversity of bioactive compounds in foods and also an extensive therapeutic application of these compounds, which makes a great challenge in the approach of functional foods. According to the Food and Agriculture Organization (WHO/FAO, 2015), there is evidence showing that replacing carbohydrates by monounsaturated fatty acid (mainly oleic acid) increases High Density Lipoproteins (HDL) cholesterol concentrations and improves insulin sensitivity and the replacement of saturated fatty acids (C12:0 to C16:0) by monounsaturated fatty acid reduces Low Density Lipoproteins (LDL) cholesterol concentration and total/ HDL cholesterol ratio.

Composed of more than 30 types of polyphenols known as antioxidants, besides vitamin E and carotene, EVOO has been outstanding as a functional food because it is an excellent source of monounsaturated fatty acids (Costa & Rosa, 2016). According to the International Olive Council (2017), world production in 2017/2018 is estimated at about 2,854,000 tons, which would represent an increase of approximately 12% over the previous year – 2016 harvest / 2017. Consumption is estimated at 2,889,000 tons, which would indicate an increase of 5% in relation to the previous agricultural year. For imports and exports is expected to exceed 830,000 tons.

EVOO has, in its fatty acid composition, basically oleic acid as the main source of monounsaturated fatty acid, this quantity can reach 83% in the product, followed by linoleic and palmitic acid, according to the Brazilian Guidelines (Brasil, 2012) and the Codex Alimentarius (WHO/FAO, 2015). Because of this, the EVOO has been suggested and recommended in the nutritional treatment of non-communicable chronic diseases (NCDs), diseases of the immune system, such as cancer, atherosclerosis, cardiovascular diseases, among others, due to the possible reduction of the incidence of these pathologies related to oxidative stress (Costa & Rosa, 2016; Salas-Salvadó & Mena-Sánchez, 2017).

Ice creams are extensively appreciated worldwide, irrespective of culture, age, and socioeconomic level, the use of a functional ingredient can add value to the product by providing an useful appeal (Cruz et al., 2009; Soukoulis, Lebesi, & Tzia, 2009; Soukoulis, Fisk, & Bohn, 2014). There is e a complex system, consisting of a frozen matrix containing air bubbles, fat globules, ice crystals, and an unfrozen serum phase. Moreover, it is the most known dairy dessert in the entire world. Many types are made with milk, fruits, low fat, low sugar, so it may be considered a nutritive food, representing a source of proteins, some vitamins, minerals, and some phytochemicals (Soukoulis, Fisk, & Bohn, 2014; Bahram-Parvar, 2015). Fat plays a fundamental role in ice cream, besides being a structural element it also promotes, improvement of the sensory qualities this product (Rolon et al., 2017).

In view of the above, the main objective of this research was to evaluate the effects of the addition of extra virgin olive oil as a lipid source in ice cream samples, on some physicochemical and sensory properties.

2. Materials and methods

2.1 Materials

The ingredients of ice cream mix included extra virgin olive oil (Borriello®, Minas Gerais, Brazil), palm fat (Agropalma®, São Paulo, Brazil), locust bean gum (LBG) (Dupont®, São Paulo, Brazil), vanilla flavor identical to natural (Givaudan®, São Paulo, Brazil). The skimmed milk powder (Nestlé®, São Paulo, Brazil) and sucrose (Caravelas®, São Paulo, Brazil) were purchased from local supermarket.

2.2 Ice cream production

Six formulations were developed at the Dietetic Techniques Laboratory of Rio de Janeiro State – UNIRIO, Rio de Janeiro, Brazil, approximately 11 kg/sample by using a stainless steel ice cream maker with self-cooled compressor (Tramontina by Breville® 1L).

In all formations the amount of all ingredients was maintained, except palm fat and EVOO which had undergone changes in their proportions in each formula, but keeping the total fat at 10%, according to the Table 1.

Table 1

Ice cream formulations (%)

Ingredients	F1	F2	F3	F4	F5	F6
Water	54.7	54.7	54.7	54.7	54.7	54.7
Extra virgin olive oil (EVOO)	-	2	4	6	8	10
Palm fat	10	8	6	4	2	-
Skimmed milk	22	22	22	22	22	22
Sucrose	13	13	13	13	13	13
LBG	0.05	0.05	0.05	0.05	0.05	0.05
Vanilla flavor	0.23	0.23	0.23	0.23	0.23	0.23
Total	100	100	100	100	100	100

The ice cream processing was carried out in a batch type system, producing approximately 800 mL/hour. For the sample production, an adaptation of the method proposed by Clarke (2012) was used as described below.

Each ingredient was weighted in a semi-analytical scale. At room temperature sugar and emulsifier (LBG) were mixed. The water was heated to 35°C and held at that temperature until all ingredients were dissolved. Subsequently, the skimmed milk powder was added, and after complete dissolution the sugar and emulsifier mixture was added, followed by fat (EVOO and/or palm), while maintaining constant stirring. The blend was homogenized in a household blender for 3 minutes, in the first level (the slower). Afterwards, it was transferred to a plastic Becker and cooled in an ice bath under constant stirring at 20°C. After this step, the Becker was sealed with foil and kept in a refrigerated environment at 10°C for at least 12 hours. After that time the flavor was weighed and added to the mixture before starting the first freezing step.

The mixture was frozen and aerated in the ice cream maker and cooled at -4±1°C, filled in plastic packages, hardened in a cabinet freezer at -18 to -20°C for at least 24 hours and stored at this temperature until analysis.

2.3. Microbiologic analyses

Microbiological analyzes had as objective to verify if the elaborated products were suitable for consumption and therefore suitable for the sensorial tests. To guarantee the sanity safety of the samples produced, products were analyzed. All analyses were carried out at the Bioactivity and Food Safety Laboratory of Nutrition Institute in State University of Rio de Janeiro (UERJ) according to the methodology of the American Public Health Association (APHA, 2001) and the results were compared with the current Brazilian legislation (Brasil, 2001). Salmonella sp., Thermotolerant coliforms and Coagulase positive Staphylococcus were analysed. All microorganisms were below the maximum values allowed by the legislation for the six formulations elaborated.

2.4. Extraction and determination of fatty acids

Lipid fractions from freeze-dried ice cream samples (500 mg) were cold-extracted according to the method of Bligh and Dye (1959). Analysis of the fatty acids of these lipid fractions was performed according to the method described by Huang et al. (2006), with minor modifications. The triglycerides of these lipid fractions (100 mg) were initially subjected to alkaline derivatization with methanolic solution of sodium methoxide (2.5 M) for the conversion of fatty acids to methyl esters. The hexane extracts obtained at the end of this derivatization process were evaluated by high resolution gas chromatography using flame ionization detector (GC / FID) and by high resolution gas chromatography coupled to mass spectrometry (GC / MS). These extracts (1 μL) were injected into the GC / FID (GC-2010 Plus of Shimadzu, Japan) in split mode (1:20). Chromatographic separation was developed with the aid of a fused silica capillary column of 30 m x 0.25 mm i.d., coated with 0.25 μm of polyethylene glycol (Supelcowax-10TM, Supelco, USA). The chromatographic column was initially maintained at a temperature of 100°C for 5 minutes. Then, the temperature of the chromatographic oven was raised at a constant rate of 5°C/minute until the final temperature of 230°C was reached, where it remained for an additional 11 minutes. Both the injector and the detector were maintained at 280°C. Helium gas was used as carrier gas in a flow of 1 mL/minute. The extracts were also injected into a GC/MS system (GC-2010Plus / GCMS-QP2010 from Shimadzu, Japan). The column and the chromatographic conditions were the same as those described for GC/FID analyzes. The mass spectrometer operated at an ionization voltage of 70 eV, scanning the fragments in the range of 30 to 400 m/z, in cycles of 3 tenths of a second. The temperatures of the ion source and interface with the GC were maintained at 280°C. Identification of the fatty acid esters produced during the derivatization process was based on the comparison of their mass spectra with the data available on the NIST12.lib and NIST62.lib spectral libraries available in the manager software of that GC/MS system. The identification was complemented with coelution with external standards available in the laboratory (FAME-37, Supelco, USA) and by comparing the retention rates calculated with those available in the literature. These retention indices of the compounds in the column were estimated by the modified Kovats method (Van Den Dol & Kratz, 1963) with the aid of a mixture of saturated alkanes (C9-C26; 1000 μg mL-1 of each component in hexane). The concentrations of the fatty acid esters of the samples were estimated by the area normalization method. All analyzes were performed in triplicate.

Atherogenicity and thrombogenicity indexes were calculated according to Albenzio et al. (2013) from the determination of shortchain fatty acids (SCFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), MUFA (monoinsaturated fatty acids) and PUFA (poliinsaturated fatty acids).

2.5 Physicochemical analyses

2.5.1 Extraction of sample

Sample of ice cream (1 g) was mixed with 10 mL extractors solutions: (I) ethanol, (II) ethanol:water (50:50), (III) acetone:water (70:30) and (IV) water wrapped with aluminum foil. Then, the mixture was added in an ultrasound bath for 30 minutes in ambient temperature. After that, the mixture was poured into centrifuge tubes and centrifuged in a centrifuge (Heraeus Multifuge X3FR Centrifuge Thermo Fisher Scientific) at 10,000 G for 10 minutes and 10°C to obtain a clear solution. Light exposure was prevented throughout the extraction process (Wanyo, Chomnawang, & Siriamornpun, 2009; Vizzotto & Pereira, 2011). The extract obtained was used for total phenolic content, antioxidant value 2,2-diphenyl-1-picrylhydrazyl DPPH, 2,2 acid'-azino-bis 3-6-etilbenzotiazolin-sulfonic acid, and FRAP assay.

2.5.2 Determination of total phenolic content

Total phenolic content was determined by the Folin–Ciocalteu method, which was adapted by Rocchetti et al. (2017). The extract and 2.5 mL Ciocalteu reagent solution 10% were combined and mixed using a Vortex. The mixture was allowed to react for 5 minutes, and then 2 mL of sodium carbonate 4% solution was added and mixed. After that, the solution was incubated at room temperature (25°C) in a dark condition for 2 hours. The absorbance was measured at 750 nm using a spectrophotometer (Shimadzu UV-2700) and the results were expressed in gallic acid equivalents, mg/100 g ice cream using gallic acid (2.5–50 µg/µL) standard curve. The analyzes were carried out on High performance liquid chromatography (HPLC) system (HP 1100 model), column Lichrospher RP18 (5 µm) equipped with ultraviolet (UV) detector at 210 nm and quaternary pump system. Reverse phase analysis consisted of solvent A (Milli-Q water with 1% phosphoric acid) and solvent B (acetonitrile). The mobile phase pumping system was a gradient, with 90% of the solvent A from 0 to 5 minutes, 60% A from 5 to 40 minutes and 90% A from 45 to 50 minutes. The default flow was maintained at 0.5 mL/min. The diluted samples were filtered Nylon membranes 0.45 µm in pore diameter. The phenolic compounds were identified according to their elution order and by comparing their retention time with those of its pure standards. The quantification was performed by the external standardization method, through the correlation of the area (mAU*s) of the compound peak to the standard curve performed with each standard evaluated (gallic acid, catechin, epicatechin, rutin, ferulic acid, naringin, hesperidin, myricetin, resveratrol, quercetin, vitexin, apigenin and canferol).

2.5.3 Determination of antioxidant activity

The antioxidant capacity of the samples was evaluated using two different methods: FRAP and ORAC.

FRAP assay

For preparation of solutions using FRAP test, was used according Benzie and Strain (1996) method with some modifications. The stock solutions included 0.3 M acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution, and 20 mM ferric chloride solution. The fresh working solution was obtained by mixing 25 mL acetate buffer 0.3 M, 2.5 mL of TPTZ solution, and 2.5 mL ferric chloride solution. Ice cream samples (three different concentrations in triplicate) were allowed to react with 2.7 mL of the FRAP solution for 30 min in dark condition and warmed at 37°C. Interpretations of the colored product [ferrous tripyridyltriazine complex] were taken at 595 nm in a spectrophotometer (Shimadzu UV-2700). The standard curve was linear between 500 µM and 2.000 µM ferrous sulfate. Results are expressed in µM ferrous sulfate/g ice cream.

ORAC assay

For ORAC analyses the procedure followed the method according to Prior et al. (2003). The analyze used an automated plate reader (SpectraMax i3x) with 96-well plates. Analysis were conducted in phosphate buffer pH 7.4 at 37°C. Peroxyl radical was generated using 2, 2'-azobis (2-amidino-propane) dihydrochloride which was prepared fresh for each run. The substrate used was fluorescein. The fluorescence conditions used were excitation at 485 nm and emission at 520 nm. The standard curve was linear between 1 µM and 90 µM Trolox. Results are expressed as µM (TE)/g of ice cream.

2.6 Hedonic Test and Sensory Characterization

Sensory evaluation was carried out in 2 non-consecutive days. Tests were conducted in individual booths with adequate temperature and lighting, ensuring the comfort and privacy of consumers. Sessions were conducted at the Sensory Analysis Laboratory of the Federal University of Rio de Janeiro State (UNIRIO). The evaluations were performed after approval by the Research Ethics Committee of UNIRIO (CAAE 39693914.8.0000.5285). Before the tests, a written consent form was given to all volunteers, explaining details about the analysis, being signed if the person wished to continue participating in the process. Consumers were provided with water for palate cleansing. Samples were coded with 3 random digits in monadic way and balanced for order presentation to avoid carryover effects (Macfie et al., 1989). To avoid bias, no information about the samples was given to the consumers (Thompson et al., 2009).

In the first day were applied two tests, the acceptance test (nine-point hedonic scale) and a descriptive analysis (Free List). One hundred twenty (n = 120) frequent vanilla ice cream consumers and non-lactose, milk and milk products intolerant persons were recruited on campus. The recruitment was done through posters and invitations via social networks. The samples were served in disposable plastic cups (capacity of 50 mL) with a clear plastic spoon with around 20g of each sample. The products were served at a temperature of -10°C±2°C (Cadena et al., 2012).

The six vanilla ice cream samples were served to consumers in a monadic way, and in the first moment, they were asked to answer the acceptance test. For this test was used a nine-point hedonic scale in which ‘1’ indicated “dislike extremely”, ‘5’ indicated “neither like nor dislike” and ‘9’ indicated “like extremely”. The consumers evaluated their preferences on product appearance, aroma, flavor, texture and overall liking (Stone & Sidel, 2004).

Right after complete the acceptance test, the consumers were asked to assess the free listing questionnaire, which was used to Check-All-That-Apply (CATA) questionnaire. The consumers were asked, in a free manner, to write all terms that were related to each of the six samples of ice cream with respect to the appearance, aroma, flavor and texture. They were given a sheet of paper with written instructions and asked to complete the task (Dos Santos et al., 2015). The terms were selected based on a previous free listing study, the similar ones were grouped and the terms that appeared most frequently, above 5%, were selected. The terms "greenish color", "olive oil taste" and "white color" presented frequency less than 5%, but were selected due to the addition of EVOO.

On the second day, eighty one consumers (n = 81) were asked to complete a Check-All-That-Apply (CATA) questionnaire with 16 terms related to the sensory characteristics of the vanilla ice cream samples.

According to Ares et al. (2010), the consumers were requested to check all the terms that they considered appropriate to describe each ice cream sample. The sensory terms listed were balanced within and across consumers, following William's Latin Square experimental design. This indicates that each consumer received the Check-All-That-Apply (CATA) questionnaire with the terms in different order and this order was modified from sample to sample along the test. The terms considered were the following: sandy, white color, very sweet taste, milk aroma, olive oil flavor, consistent, greenish color, creamy, greasy, residual flavor, yellowish color, vanilla aroma, sweet taste, ice crystal, hard, vanilla flavor.

2.7 Statistical analyses

Data on total phenolic content, antioxidant activity, sensory acceptance, overrun, color and texture were studied using one-way analysis of variances (ANOVA) and the comparison of means was conducted using the Tukey test at 95% confidence level (Cadena et al., 2012) using the statistical program XLSTAT (version 2014.6.04, Addinsoft; Paris, France). Check-All-That-Apply (CATA) data, in sensory analysis, was analyzed according to the procedures of Alcaire et al. (2017). The frequency of use of each term to describe each sample was calculated by counting the number of consumers who selected the term to describe each sample. To identify the significant differences between the samples, the Cochran Q Test was performed. Post hoc multiple pairwise comparisons were performed with Bonferroni alpha adjustment (Pramudya & Seo, 2018). The proportions with different letters within each row represent a significant difference at $P<0.05$. Cluster analysis was performed on consumer liking scores prior to internal preference mapping using Hierarchical Cluster Analysis (HCA), in order to obtain a two-dimensional representation between the samples and the terms, based on the chi-square distances in the frequency tables. In the HCA, Euclidean distances (dissimilarity) and Ward's method (agglomeration method) were used, for this was used the statistical program XLSTAT (version 2014.6.04, Addinsoft; Paris, France).

3. Results and discussion

3.1 Fatty Acids of ice creams

Firstly were analyzed the fatty acids compositions of fats used in the ice cream samples. Results of fatty acid profile of ice cream samples and fats (palm and extra virgin olive oil) are presented in Table 2. Palm oil showed the prevalence of two fatty acids with quantities close to each other, palmitic acid (42.47±0.73 %) and oleic acid (41.77±1.24 %). In contrast the sample of EVOO showed a much higher amount of the oleic acid (76.54±1.37 %) and the second larger fatty acid was the palmitic acid (14.50±0.64 %).

Table 2

Fatty Acids profile, atherogenic and thrombogenic indexes and amount (%) of the added ingredients and formulated ice creams

Fatty Acids (%)	Palm fat	EVOO	F1	F2	F3	F4	F5	F6
Methyl laurate	0.23 ± 0.05	0.00	0.16 ± 0.04	0.13 ± 0.02	0.12 ± 0.02	0.09 ± 0.01	0.04 ± 0.00	0.01 ± 0.01
Methyl myristate	0.86 ± 0.12	0.00	0.72 ± 0.11	0.61 ± 0.04	0.48 ± 0.05	0.37 ± 0.01	0.21 ± 0.02	0.08 ± 0.02
Methyl palmitate	42.47 ± 0.73	14.50 ± 0.64	41.85 ± 1.01	37.12 ± 0.53	31.64 ± 0.73	27.15 ± 0.15	21.38 ± 0.51	15.67 ± 1.09
Methyl palmitoleate	0.08 ± 0.01	1.00 ± 0.14	0.03 ± 0.03	0.19 ± 0.02	0.42 ± 0.07	0.75 ± 0.04	0.74 ± 0.07	1.10 ± 0.14
Methyl heptadecanoate	0.08 ± 0.01	0.00	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.09 ± 0.01	0.06 ± 0.00	0.08 ± 0.02
Methyl stearate	5.65 ± 0.19	1.61 ± 0.14	5.29 ± 0.31	4.65 ± 0.09	4.02 ± 0.16	3.39 ± 0.05	2.44 ± 0.09	1.70 ± 0.23
Methyl oleato	41.77 ± 1.24	76.54 ± 1.37	43.98 ± 0.37	49.24 ± 0.87	55.24 ± 1.64	59.72 ± 0.18	68.18 ± 0.64	74.70 ± 1.78
Methyl linoleate	8.30 ± 0.40	5.60 ± 0.47	7.46 ± 0.72	7.47 ± 0.16	7.33 ± 0.58	7.54 ± 0.13	6.23 ± 0.05	5.84 ± 0.79
Methyl linolenate	0.10 ± 0.01	0.31 ± 0.06	0.06 ± 0.05	0.12 ± 0.01	0.20 ± 0.05	0.34 ± 0.02	0.29 ± 0.01	0.36 ± 0.12
Methyl arachidate	0.35 ± 0.04	0.28 ± 0.06	0.32 ± 0.06	0.31 ± 0.00	0.34 ± 0.01	0.35 ± 0.03	0.29 ± 0.02	0.30 ± 0.09
Methyl cis-11-eicosenoate	0.10 ± 0.01	0.16 ± 0.08	0.06 ± 0.05	0.09 ± 0.02	0.14 ± 0.02	0.21 ± 0.01	0.15 ± 0.03	0.18 ± 0.10
Atherogenic index	0.92 ± 0.03	0.17 ± 0.01	0.87 ± 0.02	0.70 ± 0.02	0.53 ± 0.02	0,42 ± 0.00	0.29 ± 0.01	0.19 ± 0.01
Thrombogenic index	1.93 ± 0.07	0.38 ± 0.01	1.85 ± 0.04	1.47 ± 0.03	1.12 ± 0.03	0.88 ± 0.00	0.62 ± 0.01	0.42 ± 0.03

Values are Mean ± Standard Deviation (%).
EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.
Atherogenic index = (C12:0 + 4 × C14:0 + C16:0) / [∑ MUFA + ∑ PUFA(n-6) and (n-3)];
Thrombogenic index = (C14:0 + C16:0 + C18:0) / [0.5 × ∑ MUFA + 0.5 × ∑ PUFA(n-6) + 3 × ∑ PUFA(n-3) + (n-3)/(n-6)].
The fatty acid composition was classified as reported by Florence et al. (2009), who described SCFA as C2:0 to C4:0, MCFA as C6:0 to C12:0, and LCFA as C14: to C24:0.

These results are in agreement with the literature. According to NI n° 1 of 2012 (Brasil, 2012) and Codex Alimentarius (WHO/FAO, 2015) oleic acid is the main source of monounsaturated fatty acid in EVOO, this amount can vary from 55 to 83% in the product, followed by palmitic and linoleic acids. As stated by Prada et al. (2011) and Sambanthamurthi, Sundram, and Tan (2000) the palm oil contains approximately 50% saturated fatty acids, with 44% palmitic acid and 5% stearic acid. The unsaturated fatty acids are approximately 40% oleic acid and 10% polyunsaturated linoleic acid and linolenic acid.

From these information it is possible to observe in the results of ice cream samples that as the amount of EVOO increased (F1 not containing EVOO and F6 contains 100% EVOO) the concentration of oleic acid increased proportionally with the values, ranging from 43.98±0.37% (F1) to 74.70±1.78% (F6). Nevertheless, for palmitic, stearic and linoleic acids, the concentrations decreased as palm fat content in the ice cream decreased.

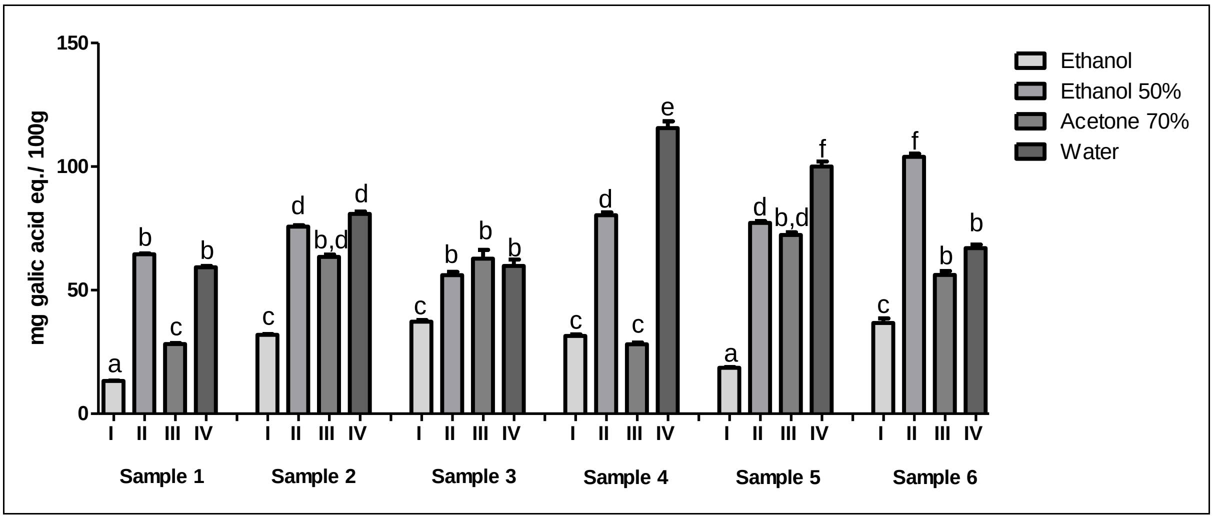
This may suggest that the EVOO used can affect the fatty acids concentration in samples of ice cream (F2 to F6) because oleic acid comes mainly from EVOO. The EVOO is considered as one of the most health-promoting nutritional habits worldwide, essential in the Mediterranean diet characterized by a high intake of monounsaturated fatty acids, which have indicated to help in lowering cholesterol and heart disease (Estruch et al., 2013).

Through the fatty acid profile was possible to measure atherogenic (AI) and thrombogenic (TI) indexes. Atherosclerosis and coronary thrombosis can be prevented or caused by some fatty acids based on their effects on serum cholesterol and low density lipoprotein (LDL) cholesterol concentrations (Vargas-Bello-Pérez et al., 2015). These indexes consider the different implications that a single fatty acid might have on human health, in this case increasing the occurrence of pathogenic phenomena such as atheroma or thrombus formation (Garaffo et al., 2011). Therefore, AI and TI constitute an important tool to verify the nutritional quality of a product. These indexes indicate the potential of stimulating platelet aggregation, that means, the lower the AI and TI values, the greater the amount for anti-atherogenic fatty acids present in an oil / fat and, consequently, the greater the potential for coronary disease prevention (Turan, Sönmez & Kaya, 2007). The atherogenic index is linked to the possibility of blockage of arteries, a high AI can cause cell adhesion of the immune and circulatory systems. On the other hand, a low AI is related to healthy lipid indexes (Ross, Van Nieuwenhove & Gonzalez, 2012), because it avoids the occurrence of micro and macrocoronary diseases (Matheus, Cobas & Gomes, 2008). In the present study, we observed a decrease in both AI and TI indexes as the amount of EVOO in the product increased, showing that the addition of EVOO in ice cream may have a beneficial effect on human health based on its fatty acid profile. Therefore, based on the results, EVOO added ice cream can be characterized as a food with low atherogenic and thrombogenic risk due to its healthy lipid composition (Ross, Van Nieuwenhove & Gonzalez, 2012). Balthazar et al. (2016) evaluated sheep milk yogurts containing different levels of inulin and the results showed that the product can be characterized as a food with low atherogenic and thrombogenic risk because of its healthy lipid composition. Further studies are important to evaluate clinical performance and consequently the possible benefits to consumer health (Morato et al., 2015).

3.2 Total phenolic content and antioxidant activity

The human diet contains different compounds that possess antioxidant activities. Phenolic compounds are considered as an integral part of both human and animal diets (Çakmakçi et al., 2015). Genovese et al. (2018) proved that phenolic compounds play an important role in the intensity and timing of the release of certain aroma compounds during the consumption of virgin olive oil.

Our results indicated that ice cream samples with high EVOO content contain remarkable phenolic compounds. For determining total phenolic contents, results were obtained using known quantities of standard gallic acid, according to each extract solutions (ethanol, ethanol 50%, acetone 70% and water), analyzed by HPLC and are showed on figure 1.

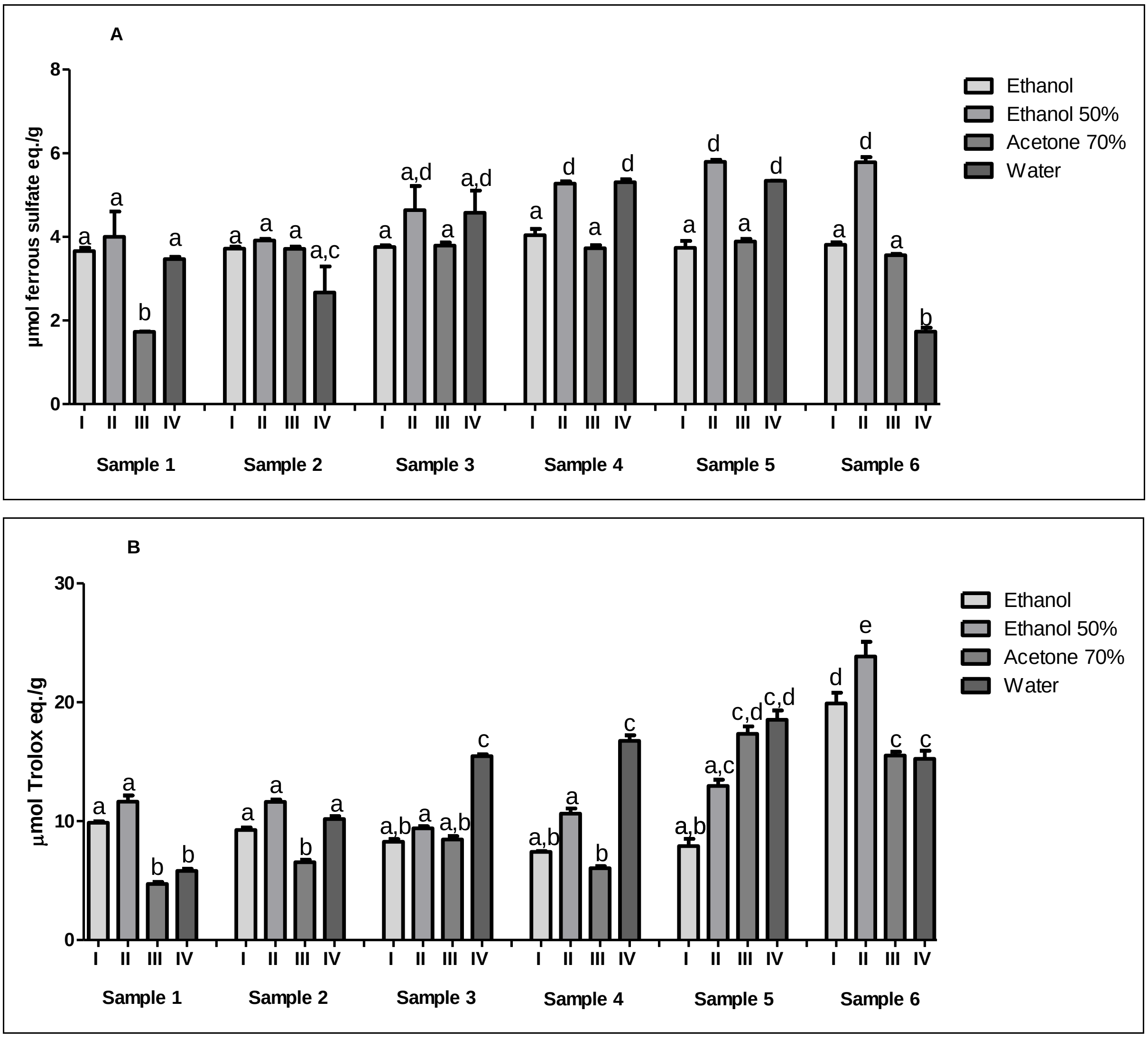


Sample 1: 100% palm fat; Sample 2: 80% palm fat + 20% EVOO; Sample 3: 60% palm fat + 40% EVOO; Sample 4: 40% palm fat + 60% EVOO; Sample 5: 20% palm fat + 80% EVOO; Sample 6: 100% EVOO.

Fig. 1. Total phenolic content of ice cream samples in mg gallic acid equivalents (GAE)/100g of ice cream. Total phenolic content ice cream, (I) Ethanol, (II) Ethanol 50%, (III) Acetone 70% and (IV) Water. Data are expressed as means ± SD, ($P<0.05$) by Tukey test.

All the samples presented variation in the results of each extract solutions and in some cases, there were no significant difference between them ($P<0.05$). Sample F4 in water (ranged from 115.61±4.68 mg GAE/100g), the samples F5 (ranged from 100.09±3.48 mg GAE/100g) in water and F6 (ranged from 103.98±2.04 mg GAE/100g) in ethanol 50%, showed the highest amount of phenolic content, comparing with the other samples and extract solutions, being that the last two cited did not have significant difference between them ($P<0.05$). It can be explained because of phenolic compounds present in EVOO. In a study by Franco et al. (2014) it was possible to separate and identify 24 phenolic compounds in the extra virgin olive oils analyzed. In all the analyzed products, the secoiridoid derivates were the most abundant, followed by phenolic alcohols, flavonoids and phenolic acids. Another conclusion of the authors is that the more mature the olive, the lower the amount of phenolic compounds in the EVOO. The phenolic compounds have been associated with the health benefits attributed to extra virgin olive oil, such as reduced risk of developing cardiovascular disease, neurological disorders and breast cancer. Moreover, these compounds are also associated with oxidative stability and sensory characteristics (flavor and aroma) of EVOO (Frankel et al., 2013). Hwang et al. (2009) added grape wine lees (GWL) in ice cream samples and the study estimated the amount of total phenolic content in ice cream with 150 g/kg GWL was 242 mg/100ml, what resulted in the increase of total phenolic content. Ullah, Nadeem, and Imran (2017) supplemented the ice cream with olein fraction of chia oil and increase the total phenolic contents from 12 (control), 165 (5% chia oil), 317 (10%), 519 (15%) and 748 (20%) mg GAE/100mL ice cream.

In our study, the antioxidant activity of ice creams using some bioanalytical methods like by FRAP, an indirect method, and ORAC assay, a direct method. The results are shown in figure 2.



Sample 1: 100% palm fat; Sample 2: 80% palm fat + 20% EVOO; Sample 3: 60% palm fat + 40% EVOO; Sample 4: 40% palm fat + 60% EVOO; Sample 5: 20% palm fat + 80% EVOO; Sample 6: 100% EVOO.

Fig. 2. Antioxidant capacity FRAP (A) and ORAC (B) of ice cream samples, (I) Ethanol, (II) Ethanol 50%, (III) Acetone 70% and (IV) Water. Data are expressed as means ± SD, (P<0.05) by Tukey test.

A linear correlation was observed among the concentration of EVOO in the samples, phenolic compounds and antioxidant activity, is possible to note a tendency of growth in the antioxidant activity and phenolic content of the samples that were added higher amount of EVOO in the composition, especially in ORAC assay. The highest antioxidant activity of ice cream by ORAC were 23.84±1.75 µM Trolox/g in sample F6 extracted by ethanol 50% and 17.34±0.89 and 18.53±1.11 µM Trolox/g in sample F5 extracted by acetone 70% and water respectively, that did not present significant difference between them ($P<0.05$). In all the cases the amounts were higher than control sample (F1) (ranged from 4.72±0.25 to 11.64±0.73) in ORAC assay.

Danneskiold-Samsøe et al. (2019) evidenced that the reciprocal interactions between gut microbiota and dietary phenolic compounds influence the bioavailability of the compounds and their effects on human health, what shows the importance of our results for health. Goraya and Bajwa (2015) concluded that amla (Indian gooseberry) being a rich source of total phenols and tannins resulted in increase of these substances in ice cream on its inclusion. Hwang et al. (2009) detected that addition of grape wine lee resulted in increase of total phenols and tannins in ice cream.

3.3 Sensory properties

Ice creams prepared with proportions of EVOO and palm fat. The acceptance test results showed that the replacement of palm fat by extra virgin olive oil did not affect the appearance and aroma of the ice creams studied ($P>0.05$), however influenced flavor, texture and overall liking of some ice cream samples ($P<0.05$). The scores on the attributes of flavor, texture and overall liking did not show a tendency to increase or decrease, with the increase of the proportion of extra virgin olive oil in the formulations.

Table 3 presents the acceptance means for appearance, aroma, flavor, texture and overall linking of the samples.

Table 3

Means liking hedonic scores for appearance, aroma, flavor, texture and overall liking ice creams with different ratios of EVOO and palm fat (n = 120)

Sample	Appearance	Aroma	Flavor	Texture	Overall linking
F1	7.39[a]	6.99[a]	7.12[a]	6.92[a]	7.19[a]
F2	7.04[a]	6.99[a]	7.04[a]	6.10[b]	6.81[ab]
F3	7.41[a]	6.77[a]	7.02[a]	6.79[ab]	6.97[ab]
F4	7.23[a]	6.84[a]	6.68[ab]	6.59[ab]	6.71[ab]
F5	7.55[a]	7.00[a]	6.95[a]	7.07[a]	7.05[a]
F6	7.06[a]	6.74[a]	6.07[b]	6.46[ab]	6.41[b]

[a–b] Means with the different letters in the same column are significantly different (P<0.05) using Tukey's test.
EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.
Using a 9-point hedonic scale

In relation to the flavor attribute, only the F6 sample presented a significant difference in relation to the other samples, except for the F4 sample. In this attribute the average scores ranged from 6.07 (F6) to 7.12 (F1). As for the texture, which can be directly affected by the type and content of fat (Soukoulis et al., 2010), only the F2 sample presented a significant difference in relation to the control and F5 samples (P<0.05). A similar case occurred for these samples (F1 and F5) in relation to the overall liking, which differed significantly in relation to the F6 sample (P<0.05). All the other samples did not differ among the other two, for texture and overall liking.

An important observation was that the Tukey test revealed that the control (F1), F3, F4 and F5 samples did not present significant differences (P>0.05) in all attributes, appearance, aroma, flavor, texture and overall liking, suggesting that samples with a high content of EVOO (except the F6 sample) did not differ significantly from the control sample. Matsakidou, Blekas, and Paraskevopoulou (2010) prepared cakes by replacing margarine with EVOO and concluded that the use of the extra virgin / margarine oil blend improved cake nutrition characteristics and revealed similar sensory scores as the control cake.

Cluster analysis was performed with the goal of evaluate consumers based on their global acceptability. Three clusters were identified with 51, 60, and 9 consumers each, corresponding to 42.5, 50.0, and 7.5% of the participants, respectively (Table 4). The dendrogram graph (Figure 3) shows the clusters obtained with the results of the overall liking for the ice cream samples.

Table 4

Means of the overall liking scores for the six samples of ice cream per cluster[1]

Cluster	Number of Consumers (% of consumers)	Sample	Mean Scores
1	51 (42.5%)	F1	7.88[a]
		F2	7.59[a]
		F3	8.04[a]
		F4	7.88[a]
		F5	8.04[a]
		F6	7.53[a]
2	60 (50.0%)	F1	6.65[ab]
		F2	6.12[bc]
		F3	6.35[abc]
		F4	6.08[bc]
		F5	6.95[a]
		F6	5.77[c]
3	9 (7.5%)	F1	6.78[a]
		F2	7.00[a]
		F3	5.00[ab]
		F4	4.22[ab]
		F5	2.22[b]
		F6	4.33[ab]

[a–c] Means with the different letters in the same columns in the same cluster are significantly different ($P<0.05$) using Tukey's test.

[1] Data represent 120 consumers reflecting the evaluation of overall liking. Overall liking in clusters 1, 2, and 3 were scored on a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely.

EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.

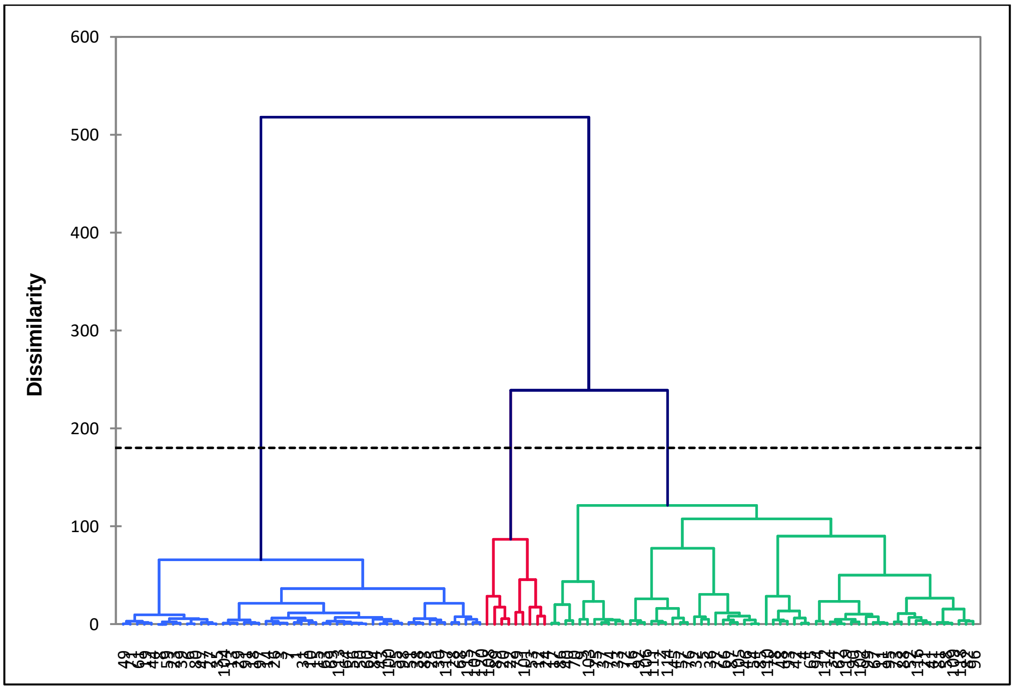


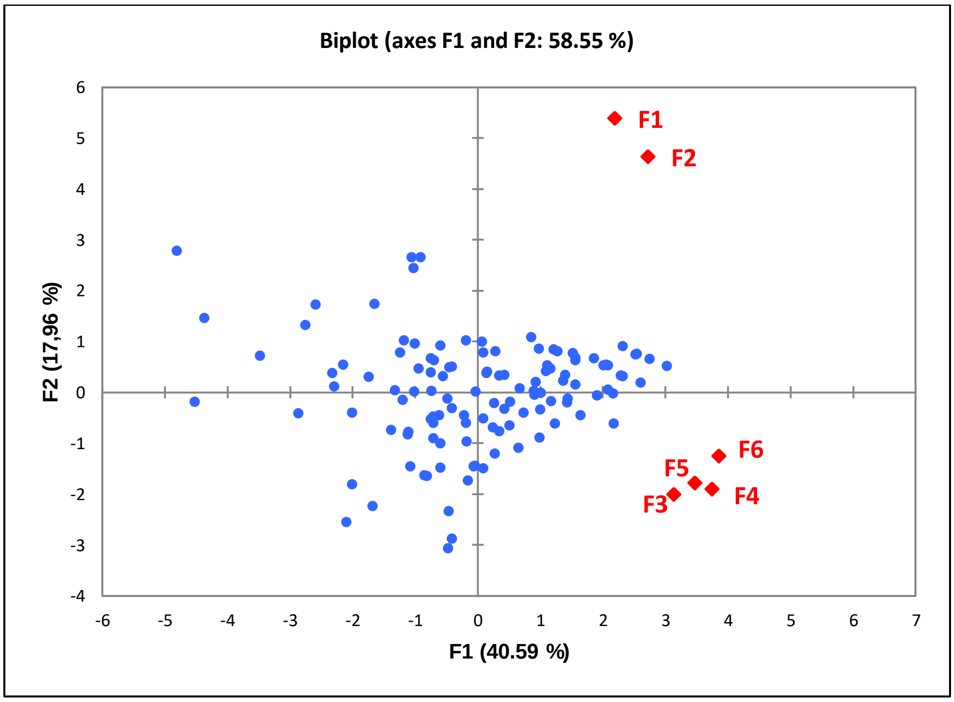
Fig. 3. Dendogram of overall liking of ice cream by consumers

Analyzing the differences between clusters for each sample, it is observed that consumers of cluster 1 evaluated homogeneously the overall liking for all samples, since no statistical difference (P>0.05) was observed between them. Compared with the other two clusters, participants of cluster 1 gave the highest overall liking scores for all six ice cream samples (values between 8.04 (F3) and 7.53 (F6)).

The relevance of cluster 1 is the high concentration of consumers (42.5%), but above this is the cluster 2 with half of consumers. The members of this cluster did not observed significant difference (P>0.05) between control, F3 and F5 samples, as well as control, F2, F3 and F4 samples, and still F2, F3, F4 and F6 samples. The most distant scores were between F5 (6.95) and F6 (5.77) samples.

The group with the lowest number of consumers, cluster 3, only 7.5% of the respondents, observed differences (P<0.05) between samples F2 and F5, and between the control sample (F1) and F5, this last one was not observed by any other clusters. In addition, this group had the lowest average scores, ranging from 7.00 (F2) to 2.22 (F5).

From the data obtained in the acceptance test of the six ice cream samples, the Internal Preference Mapping was generated and analyzed, with the objective to visualize the general behavior of the groups of consumers and maybe seek for groups for consumers (Figure 4).



EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.

Fig. 4. Internal preference mapping defined by the two first preference dimensions from the consumers (n = 120). Ice cream samples (lozenges) and consumers (circles)

Consumers were separated in two groups by the Internal Preference Mapping. Groups are represented in the positive values of the first dimension, one group formed by samples F1 (control) and F2, is situated in the higher half of the second dimension, clearly separated from the second group formed by the other samples, F3, F4, F5 and F6. This second group is positioned in the lower half of the second dimension, opposite a larger group of consumers indicating a relatively lower preference for those samples of the group.

Furthermore, it is possible to notice that samples are divided by the first dimension in two well-defined groups, where in the higher half of the second dimension are the control sample (F1), using only palm fat and F2, with only 20% of EVOO and 80% of palm fat in its composition, which are clearly distant from the other samples with higher proportion of EVOO. Therefore, it can be suggested that the increase of the proportion of EVOO in the ice cream samples changed the characteristics of the product due to the formation of a distinct group than the control sample. This fact graphically showed the tendency of consumers to give higher scores to the samples containing greater proportion of palm fat in their composition.

According to Pramudya and Seo (2018), the CATA method can be useful for characterizing variations in sensory attributes with temperatures of cooked rice samples. In this project we applied the CATA method for ice cream and observed important results. CATA was developed using 16 sensory attributes, 3 of which described appearance, 2 for aroma, 5 for flavor, and 6 for texture (Table 5). The most common attributes cited for control sample (F1), with 100% palm fat, were vanilla flavor (56), sweet taste (53) and yellowish color (50), the first two did not show significant difference ($P<0.05$) among all samples and the last one, yellowish color, just showed significant difference ($P>0.05$) between the samples F4, F5 and F1, what shows that the consumers characterized the samples as similar attributes than the control sample. On the other extreme with 100% of EVOO (F6) the attributes yellowish color (64), consistent (43), residual flavor (41) and sweet taste (40) were the most cited by consumers. The terms yellowish color (55) and sandy (55) characterized the samples containing 80% palm fat and 20% EVOO (F2) , whereas creamy (55), vanilla flavor (54) and yellowish color (53) characterized the samples produced with the blend 60% palm fat and 40% EVOO (F3). The most cited attributes for sample F4 were yellowish color (67) and creamy (60) and for sample F5 were yellowish color (68), creamy (52), sweet taste (50) and consistent (50). The attributes very sweet taste, sweet taste, white color, greenish color, milk aroma, vanilla aroma and greasy did not show significant difference ($P<0.05$) between the six samples.

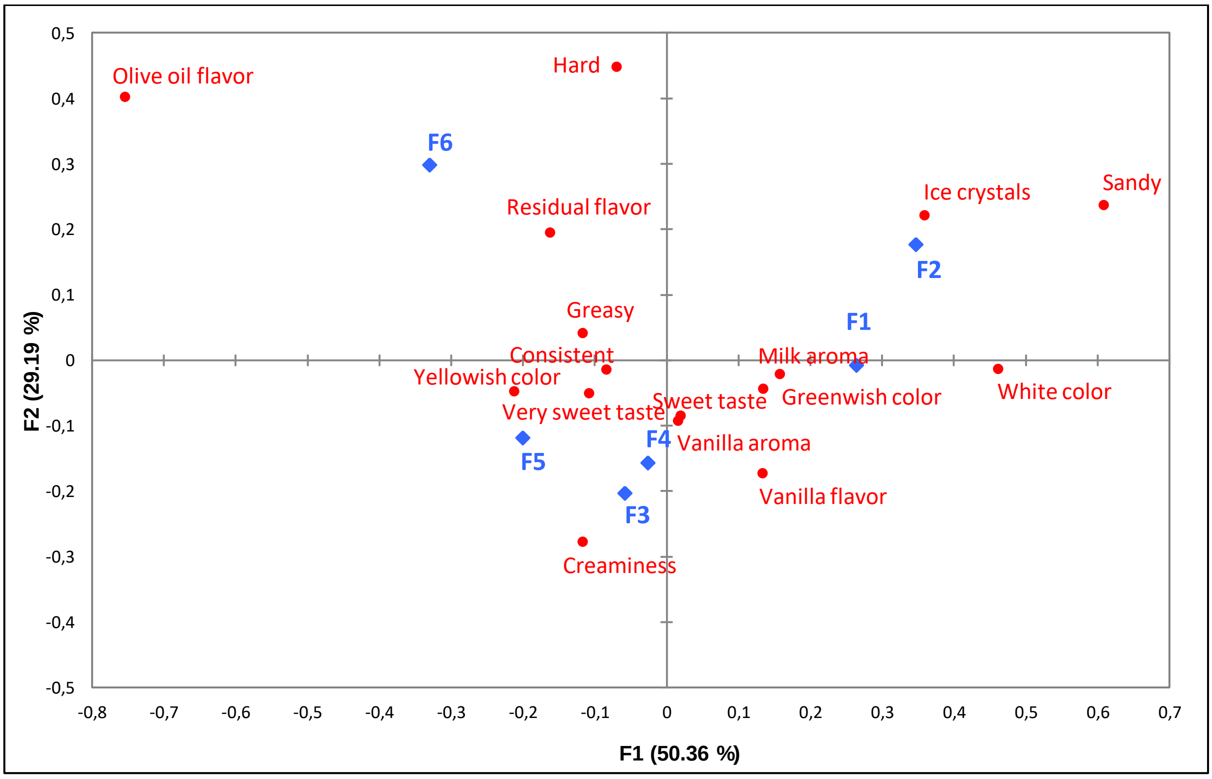
Table 5

A contingency table of the frequency of selection by 81consumers across all 6 ice cream samples for individual terms of the Check-All-That-Apply (CATA) question

	p-value	F1	F2	F3	F4	F5	F6
White color	0.0004	15^{a}	15^{a}	12^{a}	6^{a}	4^{a}	4^{a}
Greenish color	0.1326	7^{a}	1^{a}	3^{a}	2^{a}	2^{a}	3^{a}
Yellowish color	0.0002	50^{b}	55^{ab}	53^{ab}	67^{a}	68^{a}	64^{ab}
Milk aroma	0.0281	41^{a}	32^{a}	29^{a}	24^{a}	27^{a}	23^{a}
Vanilla aroma	0.7345	28^{a}	28^{a}	30^{a}	29^{a}	32^{a}	23^{a}
Very sweet taste	0.2229	23^{a}	11^{a}	19^{a}	20^{a}	20^{a}	22^{a}
Olive oil flavor	<0.0001	1^{c}	4^{bc}	10^{bc}	11^{bc}	15^{b}	35^{a}
Vanilla flavor	<0.0001	56^{a}	43^{ab}	54^{a}	48^{a}	41^{ab}	26^{b}
Sweet taste	0.2034	53^{a}	42^{a}	49^{a}	47^{a}	50^{a}	40^{a}
Residual flavor	0.0001	14^{b}	31^{ab}	23^{ab}	26^{ab}	26^{ab}	41^{a}
Ice crystals	<0.0001	38^{a}	33^{a}	13^{b}	13^{b}	13^{b}	20^{ab}
Sandy	<0.0001	22^{bc}	55^{a}	9^{c}	27^{b}	7^{c}	10^{bc}
Hard	<0.0001	23^{abc}	25^{ab}	12^{bc}	7^{c}	17^{bc}	38^{a}
Consistent	0.0004	28^{b}	25^{b}	36^{ab}	36^{ab}	50^{a}	43^{ab}
Greasy	0.2264	16^{a}	19^{a}	21^{a}	25^{a}	18^{a}	27^{a}
Creaminess	<0.0001	26^{b}	31^{b}	55^{a}	60^{a}	52^{a}	29^{b}

[a–c] Post hoc multiple pairwise comparisons were performed with Bonferroni alpha adjustment. The proportions with different letters within each row represent a significant difference at P<0.05.
EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.

A bi-plot of correspondence analysis (Figure 5), explaining 79.56% of the experimental data in two dimension, with 50.36% and 29.19%, respectively, this illustrates the associations between samples with different concentrations of palm fat and EVOO and sensory attributes. The first dimension was positively represented by attributes “sandy”, “white color” and "ice crystals" and there was not negatively attribute. On the other hand, the second dimension was positively correlated with the descriptors “hard” and "olive oil flavor", and negatively correlated with the descriptors “creamy” and "vanilla flavor". More specifically, while the control sample (F1) was associated with "milk aroma" and "greenwish color", the sample F2 was associated with "ice crystals" and "white color". The samples F3 and F4 were associated with similar attributes as "creamy" and "vanilla aroma", F5 was characterized as "very sweet taste", "yellowish color" and "consistent" and F6 as "residual flavor". Based on the Check-All-That-Apply (CATA) terms selected by consumers, ice cream samples could be classified in to three groups, i.e., the first one with samples F1 and F2, the second with samples F3, F4 and F5 and the third with sample F6, mainly by the X-axis that explains 50.36% of the total variance. This result indicates that sensory attributes are perceived by different forms depending on the concentration of palm fat and EVOO in the product.



EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.

Fig. 5. A biplot drawn by the correspondence analysis, explaining 79.56% of total variance, in the association among ice cream samples (lozenges) and sensory attributes (circles)

4. Conclusion

The addition of EVOO increased the content of oleic acid, total phenolic and antioxidant activity of ice cream samples. Therefore, EVOO has the potential to be used as a value-added ingredient in ice cream industry to enhance the monounsaturated fatty acid (oleic acid), total phenolic and antioxidant activity. Moreover, the addition of EVOO may contribute to the prevention of cardiovascular diseases, as verified by the atherogenic and thrombogenic indices, what can be interesting for the functional foods market because of the beneficial effects of this fat.

The acceptance test suggested that the samples with high content of EVOO (except F6 sample) did not showed significant difference with control sample, so the replacement was not detected or poorly evaluated by consumers. The cluster analysis identified three clusters, the first with 42.5% of consumers did not observed difference between all samples, but the second cluster with 50% of consumers detected difference between samples with high and low EVOO content. The internal preference mapping defined clearly two groups, one with control and F2 samples (low EVOO content) and the other one with F3, F4, F5 and F6 samples (high EVOO content), what suggested that the increase of the proportion of EVOO in the ice cream samples changed the characteristics of the product due to the formation of a distinct group than the control sample. The results of this study using the Check-All-That-Apply (CATA) methodology showed that seven of the sixteen attributes evaluated showed no significant difference ($P<0.05$) between the six samples, but the sensory attributes of ice cream may vary according to the different proportions of palm fat and EVOO in the samples.

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Author contributions

PDC, LZ and RSC drafted the manuscript. PDC, ISQ, GMS and JPS prepared all samples and conducted all the sensory tests. JA, AT, PDC and JPS realized the determination of total phenolic content and antioxidant activity, RFM the microbiologic analyses and RFAM, PDC and JPS the extraction and determination of fatty acids. RSC and PDC made extensive restructuring and revisions of the manuscript.

CAPÍTULO 2 – EXTRA VIRGIN OLIVE OIL ADDITION IN ICE CREAM: THE EFFECTS ON THE PHYSICAL PROPERTIES

Pryscilla D. Casagrande[1*], Iago S. Quintino[2], Guilherme M. Santos[2], Jaqueline P. Silva[2], Denes K. A. Rosário[3], Angela C. P. Duncke[5], Márcio Nele[5], Carlos A. Conte-Junior[3,4], Lilia Zago[6], Rafael S. Cadena[1,2]

[1] Food and Nutrition Program, Federal University of Rio de Janeiro State, Pasteur Avenue, 296 – Urca – Zip code 22290-240, BRA. [2] Nutrition School, Federal University of Rio de Janeiro State, Pasteur Avenue, 296 – Urca – Zip code 22290-240, BRA. [3] Food Science Program, Institute of Chemistry, Federal University of Rio de Janeiro State, Athos da Silveira Ramos Avenue, 149, Cidade Universitária, Zip code 21941-909, BRA. [4] Center of Medical Sciences, Veterinary University, Fluminense Federal University, Vital Brasil Filho Street – Niterói – Rio de Janeiro – Zip code 24230-340, BRA. [5] Chemistry School, Federal University of Rio de Janeiro. Av. Athos da Silveira Ramos, 149 – Cidade Universitária – Zip code 21941-909, BRA. [6] Nutrition Institute, University of Rio de Janeiro State, São Francisco Xavier Street, from 280 to 526 – Maracanã – Zip code 20550-013, BRA.*

ABSTRACT

The objective of the research was to contribute to the analysis of food in ice cream by evaluating the effects of extra virgin olive oil (EVOO) on the physical characteristics of ice cream. Therefore, 6 formulations with different proportions of palm fat and EVOO were developed, ranging from 100% palm fat (F1) to 100% EVOO (F6). Analysis of overrun, hardness, melt, color and size of ice crystals, air bubbles and fat globules were applied. The samples with mixture of fats showed better behavior during melting test. Overrun results were not too high, and samples F1, F4 and F6 did not show significate difference (P>0.05) among them, and the same happened with samples F2, F3 and F5. In addition, was possible to observe a tendency grow on the hardness and chroma as the percentage of EVOO increased in the sample. About whiteness, the samples F1 and F2 were responsible for higher values, and the last one showed less impact in color change during the storage.

Keywords: ice cream, extra virgin olive oil, palm fat, physical properties, overrun, melting, color, texture, ice crystals, fat globules, air bubbles.

1. Introduction

Ice cream is basically composed of dairy products, water, fat, sugar and additives (Goff & Hartel, 2013). Recently, consumers are searching for natural foods, natural additives such as fruits and dietary fiber, since these additives can increase the nutritional value of the food, its metabolic qualities, bringing benefits to the health, as well as sensory enrichment (Diplock et al., 1999). New ingredients may be added, especially with the objective of enriching the nutritionally, without losing palatability (Cruz et al., 2009). However, ice cream is a complex food matrix, where the removal of an ingredient can affect multiple important characteristics in the product (Rolon et al., 2016).

Ice cream consists of a highly complex colloidal structure composed of the main elements: fat globules, proteins, ice crystals, air bubbles, and the non-frozen matrix that consists of polysaccharides, proteins, fat droplets, lactose and minerals (Roth-Johnson, 2013). According to Goff and Hartel (2013), the composition of the ice cream interferes on its physical characteristics, because it relates to the process involved in manufacturing and thus will directly influence the aggregation state of the fat globules, the viscosity of the aqueous phase, the size and amount of the air bubbles and the size and state of aggregation of the ice crystals.

Fat performs a fundamental role in ice cream, besides being a structural element also promotes sensory quality characteristics of this product (Rolon et al., 2017). According to the Brazilian policies (Brasil, 2012), EVOO has as main monounsaturated fatty acid oleic acid (55 to 83%) and has been considered important in the Mediterranean diet. In addition, it has been suggested and recommended in the nutritional treatment of non-communicable chronic diseases (NCDs), diseases of the immune system, such as cancer, atherosclerosis, cardiovascular diseases, among others, due to the possible reduction of the incidence of these pathologies related to oxidative stress (Costa & Rosa, 2016; Salas-Salvadó & Mena-Sánchez, 2017). The use of vegetable fats in ice creams is very common around the world, though, changing the solid-to-liquid fat ratio at freezing/whipping temperatures by selecting fats with differing saturated:unsaturated triglyceride ratios can affect the amount and strength of fat destabilization (Sung & Goff, 2010).

According to Costa and Rosa (2016), functional foods have increased their importance in the market, in studies and in the media, both nationally and internationally, since consumers are interested to meet their nutritional needs and the constant search for knowledge by their promising properties. A regular basis of functional foods consumption is important to consumers achieve the health benefits, derived from them, so the sensory characteristics of functional foods must not dishearten sustained consumption (Sarubin, 2000; Siró et al., 2008). Therefore, the development of product should rely on valid methodologies to measure the impact of functional ingredients on the sensory characteristics of the products (Cadena et al., 2014).

In this sense, this study aimed to contribute to the food analysis of ice creams, assessing the effects of EVOO on physical characteristics of ice cream.

2. Materials and methods

2.1. Ice cream production

The ice cream was manufactured with the replacement of palm fat by EVOO in different proportions. The EVOO was obtained from Borriello®, located in Minas Gerais, Brazil. Six ice cream formulations (11 kg/each batch) were processed at the Dietetic Laboratory (Faculdade de Nutrição da Universidade Federal do Estado do Rio de Janeiro – UNIRIO, Rio de Janeiro, Brazil), using an ice cream maker in stainless steel with self-cooled compressor (Tramontina by Breville 1L). For the samples manufacture an adaptation of the method proposed by Clarke (2012) was used.

In all formulations the amount of all ingredients was maintained, except palm fat and EVOO which has gone through change in their proportions in each formula, but kept the total fat at 10%, according to the Table 1.

Table 1

Ice cream formulations (%)

Ingredients	F1	F2	F3	F4	F5	F6
Water	54.7	54.7	54.7	54.7	54.7	54.7
Extra virgin olive oil (EVOO)	-	2	4	6	8	10
Palm fat	10	8	6	4	2	-
Skimmed milk	22	22	22	22	22	22
Sucrose	13	13	13	13	13	13
LBG	0.05	0.05	0.05	0.05	0.05	0.05
Vanilla flavor	0.23	0.23	0.23	0.23	0.23	0.23
Total	100	100	100	100	100	100

Besides, the ice cream included palm fat (Agropalma®, São Paulo, Brazil), locust bean gum (LBG) (Dupont®, São Paulo, Brazil), vanilla flavor identical to natural (Givaudan®, São Paulo, Brazil). The skimmed milk powder (Nestlé®, São Paulo, Brazil) and sucrose (Caravelas®, São Paulo, Brazil) were purchased from local supermarket. All the ingredients were weighted in semi-analytical balance separately. In the beginning, at room temperature sugar and emulsifier (LBG) were mixed to guarantee a good mixing and dispersion. The water was heated to 35°C and held at that temperature until all ingredients were dissolved. Then, the skimmed milk powder was added, and after complete disbanding the mixture of sugar and emulsifier was added. After completely dissolution fat (EVOO and/or palm fat) were added, while maintaining constant moving. In a household blender, the blend was homogenized for 3 minutes. Subsequently, the mix was transferred to a plastic Becker and cooled in an ice bath under constant moving until 20°C. After this step, the Becker was sealed with foil and kept in a refrigerated environment at 10°C for at least 12 hours. After that time the flavor was weighed and added to the mixture before starting the first freezing step. The air incorporation procedure was conducted at -4±1°C in a self-cooled compressor for approximately 1 hour. After that, ice cream mixes were packed into 145 mL plastic packages and frozen at -18 to -20°C for at least 24 hours and stored at this temperature for further studies. The manufacture of ice cream formulations was performed at laboratory scale.

2.2. Physical analyses

2.2.1. Overrun

The determination of the overrun (%) of ice cream samples was performed according to Segall and Goff (2002), measuring the weight of a known volume of the base for the production of ice cream (initial) (*mi*) and the weight of the same volume of the final product (ice cream) (*mf*). The percentage of overrun was calculated according to Eq. 1. Analysis were carried out in triplicate for each sample:

$$\text{Overrun (\%)} = \frac{(mi - mf)}{mi} x\ 100 \qquad (1)$$

2.2.2. Melting behavior

For the accomplishment of the test of melting behavior the method was adapted from Granger et al. (2005). The ice cream samples were stored at -18°C for 3 weeks. Melting rate was determined by carefully cutting the plastic cups from the 100±2 g ice cream samples that were suspended on a wire mesh screen (wire thickness = 2.3 mm) and left to melting in a graduated test tube of 50 mL at room temperature (24±1°C). The volume of the melted ice cream was recorded every 5 minutes until the volume of sample melted reached 50 mL. A plot was constructed of drained volume versus melting time, in order to obtain a sigmoidal curve with a linear region of maximal melting rate. The melt test was done in triplicate for each sample.

2.2.3. Color measurements

Color measurements were performed using a spectrophotometer CM-600D (Konica Minolta Sensing Inc., Osaka, Japan) equipped with illuminant A (D65), 8 mm aperture, and 10° standard observer, at -10±2°C, two days after the manufacture of ice creams. The coordinates, L*, a*, b*, chroma (C*), hue (h) and whiteness index (WI) were obtained using software SpectraMagic NX version 2.70 (Konica Minolta Inc., Osaka, Japan), where L* is a measure of the luminosity and ranges from black (L*=0) to white (L*=100); a* denoting the intensity of the color varies from green (-) to red (+); b* varies from blue (-) to yellow (+) of the CIE color scale. Chroma (saturation/intensity) and hue denoting the tonality; WI is the whiteness index. Readings were carried out in analytical and experimental triplicate. Additionally, total color difference (ΔE; Eq. 2) was calculated as the following:

$$\Delta E = \sqrt{(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2} \qquad (2)$$

Where greater ΔE values indicates larger color change during storage. Similar procedures were performed by Balthazar et al. (2015).

2.2.4. Textural analyses

Hardness was evaluated on frozen ice cream samples two days after the manufacture, they were tempered to about -10±2°C before analyses. Penetration test was performed at room temperature using a Texture Analyzer TA-XT Plus, Stable Micro Systems Ltd. (Surrey, England) equipped with a 50Kg load cell and 36 mm cylindrical probe (P/36), based on the modified method recommended by Guinard et al. (1997). The tests were carried out in the compression power mode with a pretest speed of 1 mm/s, test speed of 2 mm/s and post-test speed of 10 mm/s with a penetration distance of 15 mm. Hardness was calculated using the Exponent software package, version 6.1.9.1 (Stable Micro Systems, Surrey, England). For each sample a total of seven measurements for hardness were performed. The hardness was measured as the peak compression force (g) during probe penetration.

2.2.5. Size of ice crystals, air bubbles and fat globules

The size of ice crystals, air bubbles and fat globules were measured by optical light microscopy (Axio Imager A2m, Carl Zeiss, Germany) at -10°C using 50x objective lens. Samples were moved directly from the freezer to the chilled microscope slides (-18°C), based on the modified method proposed by Warren and Hartel (2014). Air bubbles, ice crystals and fat globules images were captured by a digital camera (AxioCamMRc 5, Zeiss, Germany) and edited using the Axio Vision Rel. 4.8 software (Microscope Software AxioVision LE, Zeiss, Germany). For each samples, 10 structures were measured (µm) in 5 images.

2.3. Statistical analyses

One-way analysis of variances (ANOVA) was applied to the results of overrun, hardness and color. The comparison of means was conducted using the Tukey test obtaining for each attribute at a 95% significance level (Cadena et al., 2012) using the statistical program XLSTAT (version 2014.6.04, Addinsoft; Paris, France).

3. Results and discussion

3.1. Overrun

The air incorporation in the ice cream provides a softer texture and influences the physical properties like texture, softness and stability. When enough air is whipped into a mix during freezing to cause doubling of the volume, 100% overrun has been obtained and is generated an economy or standard ice cream (Goff & Hartel, 2013). However, it is not only the amount of overrun, but also the distribution and size of the molecules that influence these properties. Several factors are determinant for the development of air entrainment in ice creams, such as: total solids content (the higher the total solids content, the greater the incorporation of air into the ice cream); of the type and amount of fat (the higher fat content, the lower air incorporated); the type and amount of emulsifiers and stabilizers; and the type of the freezing equipment (the horizontal (continuous) producers incorporate larger amounts of air than the vertical producers (batch) (Chang & Hartel, 2002a; Chang & Hartel, 2002b; Sofjan & Hartel, 2004; Pereira et al., 2011). As a result of the project, ice cream containing only palm fat (F1), mix of 40% palm fat and 60% EVOO (F4) and only EVOO (F6) presented an overrun higher than 23% (Table 2), ranging from 23,2 to 25,2%, and did not show significant difference between them (P>0.05). The other samples (F2, F3 and F5) showed lower air incorporation, ranging from 17.6 to 20.1% and did not show significant difference between them (P>0.05). The fact that there is no significant difference in the overrun of the samples that has been added EVOO may be suggested by the difficulties of maintaining, measuring and controlling the parameters in the craft process that was carried out on a laboratory scale. Rolon et al. (2017) investigated the effect of fat reduction in ice cream using maltodextrin as a bulking agent and showed that overrun did not have significantly difference between samples, they ranged from 63 to 66%. Silva Junior and Lannes (2011) produced ice cream using different sugar blends and fat types, the results presented the overrun was comprised between 35 and 40% and comparing the ice creams, it seems that the fat type did not influence the air incorporation.

Table 2

Physical properties of ice cream made with different fats (palm fat and EVOO)

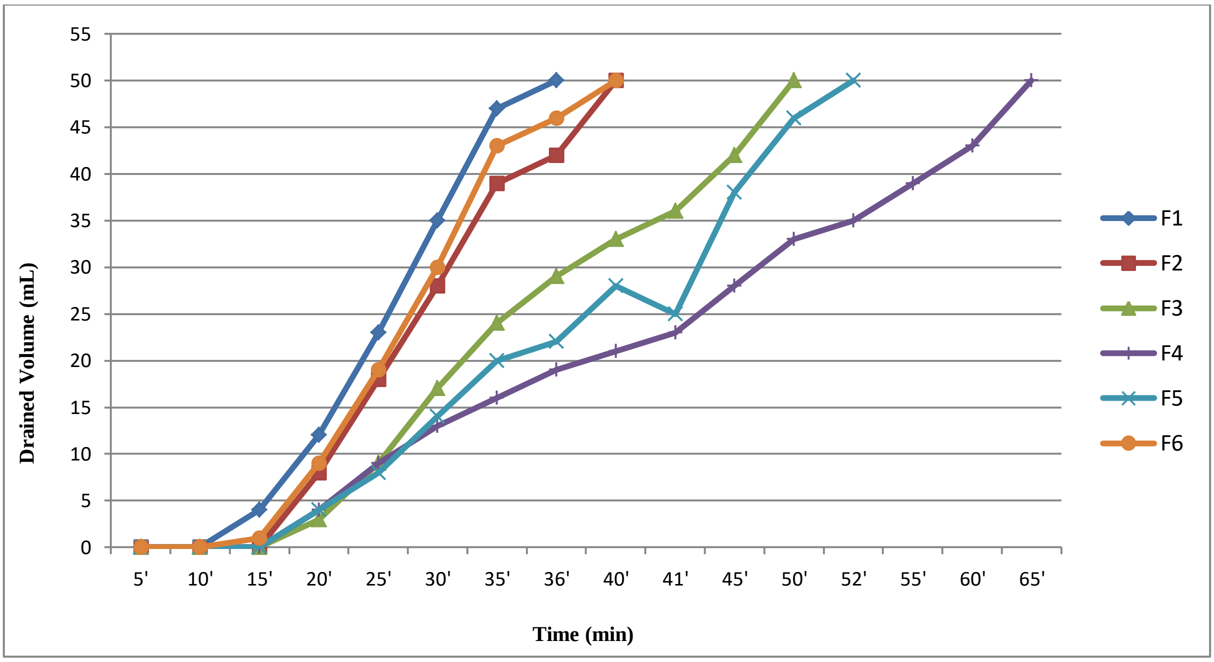
Sample	F1	F2	F3	F4	F5	F6
Overrun (%)	25.2^{a}	20.1^{bc}	17.6^{c}	23.2^{ab}	18.6^{c}	23.7^{ab}
Hardness (N)	29.05^{d}	40.66^{cd}	66.22^{abc}	45.70^{bcd}	78.20^{ab}	92.18^{a}
L*	86.65^{a}	86.42^{a}	84.22^{b}	84.68^{b}	84.84^{b}	84.91^{b}
a*	-3.36^{f}	-2.73^{e}	-2.55^{d}	-2.36^{c}	-2.17^{b}	-1.90^{a}
C*	17.45^{d}	17.56^{d}	18.77^{c}	19.43^{b}	20.20^{a}	19.62^{ab}
h	101.12^{a}	98.93^{b}	97.82^{c}	96.98^{d}	96.16^{e}	95.56^{f}
WI	-15.36^{a}	-16.96^{a}	-29.41^{b}	-31.45^{bc}	-34.88^{c}	-31.85^{bc}
b*	17.13^{d}	17.35^{d}	18.60^{c}	19.29^{b}	20.08^{a}	19.53^{ab}
Δ E	-	0.89^{c}	2.99^{ab}	3.11^{ab}	3.68^{a}	3.31^{ab}

$^{a–f}$ Means with different letters within the same row indicate significant differences ($P<0.05$).

EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.

3.2. Melting behavior

The meltdown of ice cream can be quantified by determining the mass that drips from the product through a mesh screen as a function of time when the product is allowed to melt while being held at a selected temperature (Bollinger et al., 2000). Shape retention results from the interactions between fat globules with each other and between fat globules and air, as the ice crystals melt away (Sung & Goff, 2010). According to Goff and Hartel (2013) adjusting the formula to produce slow melt can cause slow release of delicate flavors. Products containing a high amount of air (high overrun) or of fat tend to melt slowly. In the present study, the melting time can be seeing in figure 1, note that sample F1 (control) with 100% palm fat and sample F6 with 100% EVOO showed similar behaviors, as well as sample F2, suggesting that the fat type did not interfere in the melting process. But, on the other hand, sample F4 that is a mix of fats, presented the most different behavior, it melted slowly and presenting lower melt volume throughout the time, 65 min were required to melt 50 mL of the sample. In samples F3, F4 and F5, a steeper slope was observed for the curves corresponding to products containing fat mixture, indicating higher melt drainage velocity. Concerning consumers, meltdown has significant importance, because a fast melt product may be a messy situation and disagreeable when ice cream is eaten in a cone or stick (Goff & Hartel, 2013). Concerning this statement and melting data described, the results suggest that samples F1, F2 and F6 did not show difference between them, and we can conclude that the type of fat did not affected the melting behavior. On the other hand, formulations with mixes of fats might be more pleasant for consumers, being sample F4 followed by F5 and F3.



EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.

Fig. 1. Effect of EVOO incorporation on the melting time of ice creams

3.3. Color measurements

Color may produce emotional link leading to product differentiation, strengthened customer loyalty, competitiveness, and reduced perception time for the brand, increased sales, enhanced positive emotions and customer relationship (Aghdaie & Honari, 2014). In addition, olive oil obtained from olive fruit (Olea europaea) by mechanical pressing has a color ranging from green-yellow to gold depending on the degree of the fruit's ripeness. Pheophytin "a" and lutein are the major components of the chlorophyll and carotenoid fractions, respectively (Minguez-Mosquera et al., 1991). In the current study, the values of luminosity showed a tendency of whiter color of samples F1 and F2 (Table 2), with more amount of palm fat, in comparison with the others. Besides that, the color intensity ranged from -3.36 (F1) to -1.90 (F6) ($P<0.05$), indicating that the control sample tended more to the green color intensity than

F6, with 100% EVOO, however, for a vanilla ice cream the greenish color may not be what consumers expect, on the other hand, may increase the perception of the presence of chlorophyll compounds present in olive oil. Samples F1 and F2 were considered responsible for the highest whiteness. Chroma showed a tendency to increase as the concentration of EVOO increased in the samples, presenting lower values for F1 (17.45) and F2 (17.56) ($P<0.05$) and higher for F5 (20.20) and F6 (19.62). Chroma is considered a quantitative attribute of colorfulness and determines the degree of difference of a hue in comparison to a grey color with the same lightness. A sample with higher chroma is related to higher color intensity perceived by humans (Pathare et al., 2013). The result of ΔE obtained from the mathematical calculation, where greater values indicate larger color change during storage, showed significant difference among the sample F2 and all others ($P>0.05$), with F2 being the sample that had the least impact on color change during storage.

3.4. Textural analyses

Hardness of the product at typical serving temperature is an important consideration, especially for retail dipping operations (Goff & Hartel, 2013). Data concerning hardness (Table 2) presented significance difference ($P<0.05$) between control sample (F1: 29.05 N) and sample with 100% EVOO (F6: 92.18 N), especially, because shows that the type of fat interfered in the hardness of the ice creams. A higher amount of EVOO (100%) increased the force needed for the probe to penetrate the ice cream, probably due to the possible increase in serum phase viscosity. It is also possible to observe in the results a tendency of hardness to grow as the percentage of EVOO in the samples is increased. Hardness is affected by several factors: principally initial freezing point (sugar content), total solids, overrun and amount and type of stabilizer (Goff & Hartel, 2013). Matsakidou, Blekas, and Paraskevopoulou (2010) evaluated the effect of margarine substitution by EVOO on the texture of Madeira-type cakes and this replacement showed significant changes in all texture attributes except adhesiveness and springiness. Cakes prepared with EVOO or EVOO/margarine mixture showed higher hardness and cohesiveness values ($P<0.05$).

It is noteworthy to discuss about the profile of EVOO, Goff and Hartel (2013) presented the variation of the liquid fat content with the temperature for fats suitable for use in ice cream, showing that at 0°C, freezer freezing temperature with a scraped surface and where fat builds up, palm fat has approximately 40% liquid fat which makes it "softer", but other vegetables fats sources such as corn, sunflower, safflower, peanuts and others, is different because at this temperature they have around 80% of liquid fat, so they do not crystallize. Considering that EVOO and peanut oil have similar lipid profiles due to the high content of oleic acid, it can be suggested that they will have similar behaviors, so it does not crystallize at 0°C in ice cream. The liquid fat content is important for ideal partial coalescence during freezing of ice cream, which is the process of fat grouping and agglutination, because if there is excess fat during dynamic freezing it spreads on the air surface leading to collapse of air bubbles and unwanted texture.

Thus, since EVOO is liquid at freezing temperature (0°C) which may cause partial or even complete coalescence to occur, further studies are needed to characterize and evaluate the behavior and impact of liquid oils and/or oil mixture in the frozen desserts.

3.5. Size of ice crystals, air bubbles and fat globules

According to Goff and Hartel (2013) after ice cream freezing the fat globule clusters are formed as a result of partial coalescence of the individual globules. Wildmoser, Scheiwiller, and Windhab (2004) mentioned when fat particles size are larger than 2 µm, fat influences the overrun due to aggregation induced by the presence of air bubbles. In this study the mean size of fat particles of samples ranged to 4.62 (F2) to 8.08 (F4) µm. Marshall, Goff, and Hartel (2003) cited that the size, number and physical condition of fat globules in the ice cream mix determine the beat rate and stability of the whipped product. Small fat globules and limited lump formation improve the beating. According to the authors, the main reason for the homogenization step is to prepare a stable and uniform fat suspension by reducing the size of the fat globules to less than approximately 2µm, a fact that may help to explain the result obtained, since the homemade equipment used for homogenization of the mixture and the smaller scale may justify the results obtained.

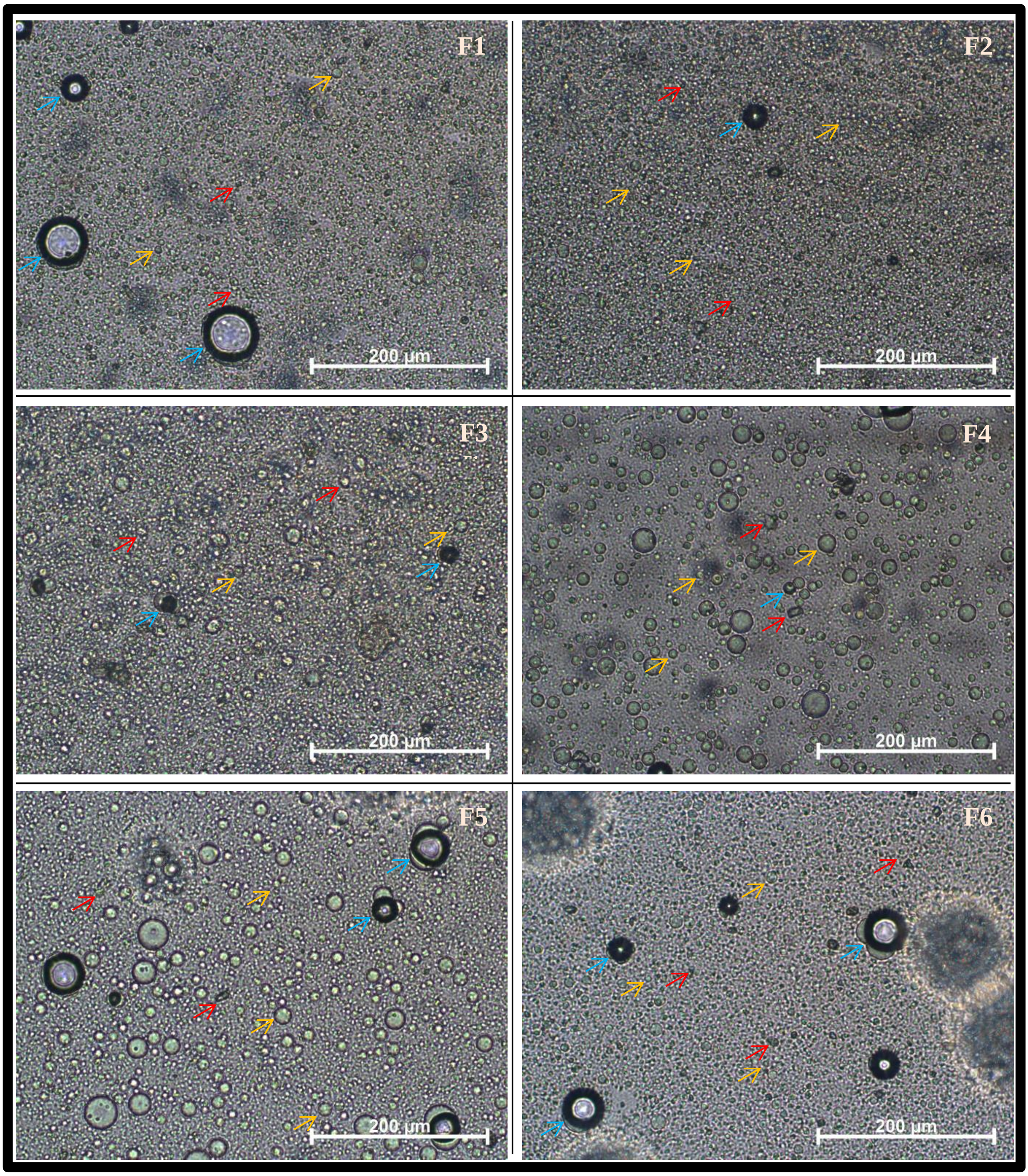
The results of the sizes of ice crystals, air bubbles and fat globules are presented on Table 3, and Figure 2 represents the structure of ice creams samples.

Table 3

Average sizes of ice crystals, air bubbles and fat globules

Sample	F1	F2	F3	F4	F5	F6
Air bubbles (µm)	19.91	9.02	8.73	7.02	16.32	16.61
Fat globules (µm)	5.31	4.62	5.06	8.08	7.87	6.10
Ice crystals (µm)	72.31	78.34	66.91	131.28	84.40	55.80

EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.



EVOO: extra virgin olive oil; F1: 100% palm fat; F2: 80% palm fat + 20% EVOO; F3: 60% palm fat + 40% EVOO; F4: 40% palm fat + 60% EVOO; F5: 20% palm fat + 80% EVOO; F6: 100% EVOO.

Fig. 2. Structure of ice creams containing: 100% palm fat (F1), 80% palm fat + 20% EVOO (F2), 60% palm fat + 40% EVOO (F3), 40% palm fat + 60% EVOO (F4), 20% palm fat + 80% EVOO (F5), 100% EVOO (F6), observed by bright field microscopy method (50×). Air bubble (→), ice crystal (→), fat particles (→).

According to Goff (2017) the ice crystals should be 30-50 µm in diameter - the larger they are from manufacture or become due to temperature fluctuations in storage, the more coarse/icy the ice cream will taste. Cook and Hartel (2010) mentioned that the fat might reduce the ice crystal size by taking the place of water. So, there will be less water to freeze when fat replaces some of this water, for a given number of nuclei formed in ice cream. The samples of this study should large sizes of ice crystals ranging from 55.80 (F6) to 131.28 (F4) µm. The whipping process helps keep the ice crystals small and discrete (Goff, 2017), so these results can suggest that the homemade equipment and process used for whipping may have affected the size of crystals, because if hardening is slow, ripening processes cause some crystals to melt away as the others grow. These big ice crystals can affect the sensorial quality of ice cream, reflecting on the smoothness of the ice cream and can be reject by consumers (Goff & Hartel, 2013).

According to Warren and Hartel (2014) air bubbles are important to help in provide scoopability and preventing shrinkage to ice cream. Goff and Hartel (2013) mentioned that the whipping process also helps to incorporate air in the form of tiny bubbles what is often found between 20 and 25 µm. Without air, ice cream could not be scooped or chewed in the mouth, but it is dispersed in tiny bubbles means that the ice cream tastes smooth and the air is not noticeable. The mean air cell diameters for all of the ice creams samples ranged between 7.02 (F4) and 19.91 (F1) µm. The result suggested that the fat type did not promote larger than expected air bubbles, which may have been influenced by the type of freezer used, beyond the freezing time.

4. Conclusion

Samples F1, F2 and F6 showed similar behavior during the melting test, and the same happened with samples F3, F4 and F5, what suggest that the samples with mixture of fats can be more pleasant for consumers, because the melting was slower. Besides, was possible to observe that EVOO increases hardness and improves the color quality in the ice cream.

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Author contributions

PDC, LZ and RSC drafted the manuscript. PDC, ISQ, GMS and JPS prepared all samples and conducted overrun analyze and melting behavior. DKAR, CACJ, JPS and PDC realized the color measurements and textural analyses, ACPD, MN and PDC the size of ice crystals, air bubbles and fat globules. RSC and PDC made extensive restructuring and revisions of the manuscript.

CONCLUSÃO GERAL

O AOEV tem potencial para ser usado como ingrediente de valor agregado na indústria de sorvetes para melhorar o perfil de ácido graxo monoinsaturado (ácido oleico), os fenólicos totais e a atividade antioxidante. Além disso, a adição de AOEV pode contribuir na prevenção de doenças cardiovasculares, como verificado nos índices aterogênico e trombogênico. Ainda, a adição do AOEV indicou menor tempo de derretimento, aumentou a dureza e alterou a cor.

Os resultados do *Check-All-That-Apply* (CATA) mostraram que 11 dos 16 atributos avaliados apresentaram diferença significativa ($P<0,05$). Logo, conclui-se que os consumidores foram capazes de discriminar as amostras e caracterizá-las sensorialmente de acordo com as diferentes proporções de gordura de palma e AOEV.

Quanto à aceitação, as amostras apresentaram boa aceitação e a substituição da gordura de palma pelo AOEV não ocasionou alteração no produto. E o mapa de preferência interno definiu dois grupos, um com as amostras com menos azeite e outro com as amostras com mais azeite.

Contudo, avaliando todos os resultados e pensando em desenvolvimento de produto, a amostra F5, com 80% de azeite de oliva extravirgem e 20% de óleo de palma, seria a mais indicada para ser desenvolvida, uma vez que apresentou resultados favoráveis nas análises físicas, físico-químicas e com os consumidores foi tão bem aceita quando a amostra controle.

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