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MARCADOR LIPÍDICO POR MÉTODO ESI-MS PARA QUANTIFICAÇÃO DE ADULTERAÇÃO EM BEBIDA VEGETAL À BASE DE AMÊNDOA

Zeinab el Hajj Hussein

Maringá
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Dissertação apresentada ao programa de Pós-graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de mestre em Ciência de Alimentos.

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ZEINAB EL HAJJ HUSSEIN

"QUANTIFICAÇÃO DE ADULTERAÇÃO EM BEBIDA VEGETAL A BASE DE AMÊNDOA, POR ADIÇÃO DE AMENDOIM POR ESPECTROMETRIA DE MASSA".

Dissertação apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós-graduação em Ciência de Alimentos, para obtenção do grau de Mestre em Ciência de Alimentos.

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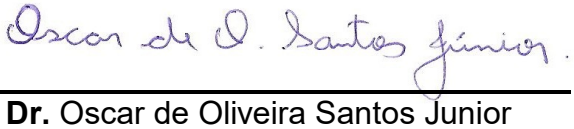

Prof. Dr. Oscar de Oliveira Santos Junior
Orientador

Maringá – 2022

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BIOGRAFIA

Zeinab el Hajj Hussein, nascida no sul do Líbano e naturalizada brasileira a partir do ano 2017, graduada em nutrição pela faculdade UNICESUMAR no ano 2020 e atualmente pós-graduanda em Nutrição Clínica na UNICESUMAR. Logo que me formei já me ingressei no mestrado por isso não tive muita experiência e não consegui atuar na área da nutrição ainda. Atualmente como mestranda na área de Ciências de Alimentos adquiri conhecimentos nos seguintes temas: adulteração de alimentos; alergias alimentares; produção de novos produtos; fortificação de alimentos.

Dedico

A meu marido em especial e a minha mãe, por todo suporte concedido as minhas escolhas e sonhos. E com muito carinho aos meus amigos de pesquisa pelo apoio e pela ajuda. Também ao meu orientador por todos os ensinamentos.

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APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de um artigo científico.

AUTORES: Zeinab El Hajj Hussein, Patricia Daniele da Silva Santos, Eloize da Silva Alves, Carlos Eduardo Rubio Senes, Jesuí Vergílio Visentainer, Oscar Oliveira Santos.

REVISTA: Food Analytical Methods.

TÍTULO: Lipid marker ESI-MS method for quantification of adulteration in almond-based vegetable beverage.

INTRODUÇÃO. Nos últimos anos, houve um aumento no interesse por produtos que possam substituir o leite de origem animal na dieta, como as bebidas de origem vegetal, isto ocorre devido à grande quantidade de pessoas com intolerância à lactose, além das alergias alimentares proveniente de algumas proteínas presentes no leite, além de também poder satisfazer parte do público vegano, que optam pelo consumo de produtos de origem vegetal (Aydar et al., 2020). A bebida vegetal não contém lactose e colesterol, possibilitando seu emprego na substituição do leite de origem animal, podendo assim atender as restrições nutricionais dos consumidores (Silva et al., 2020). Bebidas vegetais são extratos hidrossolúveis originados de sementes e oleaginosas como soja, caju, arroz, aveia, amendoim e amêndoa (Cordova, 2019) com adição de água, gerando um produto de aspecto pastoso, coloração esbranquiçada e características sensoriais acentuadas (Kehinde, 2020), devido ao alto valor de mercado, as bebidas vegetais se tornaram alvo de fraudes, principalmente aquelas à base de amêndoa, podendo ser misturada de maneira intencional com outro tipo de oleaginosas diminuindo o custo da produção e aumentando o lucro (Campmajó et al., 2020). Dentre os adulterantes empregados nos diferentes tipos de fraudes destaque-se o amendoim, devido a sua composição química muito semelhante (Walker et al., 2016). A ingestão de uma pequena quantidade (0.2 mg) ou apenas uma exposição indesejada e acidental a resíduos do amendoim já e o suficiente para causar reação alérgica em indivíduos sensíveis, podendo gerar reação anafilática fatal quando ingerido (Houben et al., 2020; Remington et al., 2020). Diversos estudos já foram realizados para avaliar adulteração de amêndoa por amendoim, e na busca desta detecção se fez necessário a utilização de técnicas analíticas, entre elas, a espectroscopia de refletância no infravermelho próximo (NIRS) (Ghoshet al., 2016; Firmani et al., 2019; López-calleja et al., 2013), cromatografia gasosa com espectrometria de massa (CG-MS) (Troya et al., 2015), cromatografia líquida com espectrometria de massa (CL-MS) (Jiang et al., 2015; Kim et al., 2014; Viana et al., 2016), ressonância nuclear magnética (RMN) (Arana et al., 2015), ultravioleta (UV) (Alamprese et al., 2013). Porém, em nenhuma delas detectou marcadores que por si só possam ser utilizados para quantificar a fraude em bebidas à base de amêndoa por amendoim.

OBJETIVOS. Mapear os triacilgliceróis presentes em amostra de bebida vegetal a base de amêndoa e bebida vegetal a base de amendoim para encontrar marcadores lipídicos, e que possibilitem avaliar amostras comerciais de bebida vegetal de amêndoa, verificando sua autenticidade e quantificando o percentual de amendoim adicionado**.**

MATERIAL E METODOS. Foram preparadas duas bebidas padrão, uma de amêndoa e outra de amendoim segundo Oliveira (2017) seguindo 4 etapas: amostragem, hidratação previa, trituração, filtração, e depois as bebidas foram pasteurizadas segundo Aamir et al. (2013), após foram adquiridas sete marcas de bebida vegetal de amêndoa em mercados locais de Maringá, Paraná, Brasil. As análises foram realizadas com as bebidas comercializadas e nas bebidas padrões consequentemente onde avaliou se o perfil lipídico para verificar a adulteração. As extrações de lipídios das amostras foram realizadas em triplicata de acordo com a metodologia desenvolvida por Bligh & Dyer (1959), os perfis lipídicos das bebidas vegetais foram avaliados envolvendo a razão massa/carga (*m/z*) 800 a 1000. Para isso seguiu a

metodologia da Silveira et al. (2017), os ésteres metílicos de ácidos graxos (EMAGs) foram preparados por metilação dos lipídios totais de acordo com a metodologia ISO 5509 (2000). A análise cromatográfica foi realizada em um cromatógrafo a gás da ThermoScientific equipado com detector de ionização em chama (DIC), coluna capilar de sílica fundida CP-7420 (Select EMAG, 100.0 m de comprimento, 0.25 mm de diâmetro interno e 0.25 µm de espessura filme de cianopropil como fase estacionária). Após análises, o padrão (LA) foi intencionalmente adulterado com adição de amendoim (LP) em sete níveis diferentes (0,1, 5, 10, 20, 50, 70, 90, 100% (v / v) com cinco repetições cada, para avaliar a adulteração. Os resultados da composição de ácidos graxos por CG-DIC foram submetidos à análise estatística de variância (ANOVA) e teste de Tukey ($p>0.05$), utilizando o software Assistat (Silva & Azevedo, 2016).

RESULTADOS E DISCUSSÃO. Através da análise das composições em ácidos graxos das amostras tanto padrão e os comerciais por GC-FID**,** foram identificados entre onze e dezenove ácidos graxos nas amostras, esta diferença pode estar relacionada com condições climáticas durante a estação de crescimento, juntamente com o tipo de solo predominante na área do cultivo. Dentre os principais ácidos graxos podemos destacar o ácido oleico (O, 18:1n-9) com 62.74% seguido pelo ácido linoleico (L, 18:2n-6) com 27.48%. Dentre os principais ácidos graxos podemos destacar o ácido oleico (O, 18:1n-9) com 62.74% seguido pelo ácido linoleico (L, 18:2n-6) com 27.48%. Os dados estão coerentes com a literatura, segundo Kodadet al. (2014) o ácido oleico em suas amostras está na faixa 47 - 77% da composição total; e segundo Čolićet al. (2017) e Zhu et al. (2015) para o ácido graxo linoléico de 3 - 27% da composição total. Já para o amendoim apesar do ácido oléico ser o majoritário com 78.26% a uma redução do percentual de ácido linoléico significativamente e estatisticamente com valor de 2.78%, no entanto, esta diferença do ácido linoléico não pode ser utilizada como marcador para fraude já que foram relatados em Colic et al. (2017) e Zhu et al. (2015), valores de ácido linoléico próximo de 3%. Para as amostras comerciais todas elas se assemelharam a amostra padrão de amêndoa pelo perfil de ácido graxo.

Com a intenção de investigar a adição de (LP/ bebida vegetal de amendoim) no (LA/ bebida vegetal de amêndoa), foi obtida a *fingerprint* de ambas as bebidas a partir da amostra padrão preparada. Avaliando os *fingerprint* pode-se observar que existe a presença dos íons *m/z* 919, 961 e 989 presentes na amostra (LP), podendo ser utilizados como marcadores lipídicos, pois, não estão presentes na amostra (LA). No entanto, o marcador *m/z* 919 foi escolhido neste trabalho devido a ingredientes comerciais (creme de coco, castanha de caju e soja) apresentarem a razão *m/z* 961 e 989. Deste modo, o marcador lipídico *m/z* 919 foi determinado para avaliar a adulteração, devido à presença dele na amostra LP em maior intensidade e sua ausência na amostrá-la LA. De acordo com as análises realizadas das amostras comercializadas, houve a presença do marcador lipídico, nas amostras B, C e F confirmando assim a adulteração destas marcas com bebida vegetal a base de amendoim, onde os espectros, destas se encontram no material suplementar. Na amostra C encontra-se no rótulo que poderia se encontrar amendoim, indicativo que a técnica aplicada pode ser utilizada para detecção de fraude com amendoim em bebidas à base de amêndoa. As amostras A, D, E e G, não apresentaram o marcador *m/z* 919,

que possuem o espectro similar da bebida vegetal à base de amêndoa, onde os mesmos se encontram no material suplementar. Para a identificação de TAGs, utiliza-se a técnica de espectrometria de massas (MS), sendo uma das principais técnicas de separação e caracterização utilizada para analisar misturas químicas complexas devidas sua capacidade de separar por massa e carga (*m/z*), detectar e caracterizar moléculas de diversos tipos, composição e tamanho. Além disso, a técnica possui a combinação de velocidade, sensibilidade e seletividade, o que a torna uma ferramenta poderosa para caracterizar óleos vegetais com marcadores lipídicos (Santos et al., 2006; da Silveira et al., 2017). A quantificação da adulteração através do percentual de LP adicionado em LA, obteve-se uma curva analítica avaliando o aumento da intensidade de *m/z* 919 com um aumento de LP em LA, esta curva obteve um R^2: 0,990, e uma equação da reta y= 1.10^7x+ 4.10^7. Utilizando esta equação da reta, substituindo as intensidades da razão *m/z* 919 em x obtém-se as concentrações das adulterações em y, utilizando a equação linear foi possível obter a concentração das amostras comerciais que estavam adulteradas. Sete amostras de bebida vegetal de amêndoas foram analisadas para quantificar as amostras de LP em LA com o método proposto. Através da metodologia proposta foi possível verificar a adulteração na faixa de concentração de 1.47- 9.82%. Um baixo desvio padrão relativo alcançado demonstra que a técnica proposta confirma a precisão ideal (resultados expostos em material complementar). Da mesma forma, a técnica é eficaz para verificar a adição de LP em LA, uma vez que é rápida para ser utilizada como análise de rotina para verificação de fraudes além de não requerer análise complementar para confirmar seus resultados, sendo por si conclusiva.

CONCLUSÕES. Através da análise de triacilglicerois (TAG) foi possível detectar um marcador lipídico específico na amostra LP e ausente em LA- *m/z* 919 $[TAG+NH_4]^+$ presente em seu TAG os ácidos graxos palmítico (16:0), araquidico (20:4n-6) e mirístico (14:0). Das amostras comerciais a base de amêndoa, três delas (B, C e F) continham o marcador (*m/z* 919) indicando adulteração nestas amostras por amendoim. Portanto, está metodologia aplicada foi capaz de quantificar pequenas quantidades (maiores que 1.0%) do adulterante nas amostras que são comercializadas, que pode acarretar malefícios na saúde do consumidor alérgico a amendoim.

Palavras-chave: Bebida vegetal; adulteração; alergia alimentar; amêndoa; amendoim; ESI-MS.

GENERAL ABSTRACT

INTRODUCTION. In recent years, there has been an increase in interest in products that can replace milk of animal origin in the diet, such as beverages of plant origin, this is due to the large number of people with lactose intolerance, in addition to food allergies from some proteins. present in milk, in addition to being able to satisfy part of the vegan public, who choose to consume products of plant origin (Aydar et al., 2020). The vegetable drink does not contain lactose and cholesterol, allowing its use in the replacement of milk of animal origin, thus being able to meet the nutritional restrictions of consumers (Silva et al., 2020). Vegetable drinks are water-soluble extracts from seeds and oilseeds such as soybeans, cashews, rice, oats, peanuts and almonds (Cordova, 2019) with the addition of water, generating a product with a pasty appearance, whitish color and accentuated sensory characteristics (Kehinde, 2020). , due to their high market value, vegetable drinks have become the target of fraud, especially those based on almonds, which can be intentionally mixed with other types of oilseeds, reducing the cost of production and increasing profit (Campmajó et al., 2020). Peanut stands out among the adulterants used in different types of fraud, due to its very similar chemical composition (Walker et al., 2016). Ingestion of a small amount (0.2 mg) or just an unwanted and accidental exposure to peanut residues is enough to cause an allergic reaction in sensitive individuals, which can generate a fatal anaphylactic reaction when ingested (Houben et al., 2020; Remington et al., 2020; Remington et al., 2020; Remington et al. al., 2020). Several studies have already been carried out to evaluate almond adulteration by peanuts, and in the search for this detection it was necessary to use analytical techniques, including near-infrared reflectance spectroscopy (NIRS) (Ghoshet al., 2016; Firmani et al. ., 2019; López-calleja et al., 2013), gas chromatography with mass spectrometry (GC-MS) (Troya et al., 2015), liquid chromatography with mass spectrometry (CL-MS) (Jiang et al. , 2015; Kim et al., 2014; Viana et al., 2016), magnetic nuclear resonance (NMR) (Arana et al., 2015), ultraviolet (UV) (Alamprese et al., 2013). However, in none of them detected markers that by themselves can be used to quantify fraud in almond-based peanut drinks. **AIMS.** To map the triacylglycerols present in samples of almond-based vegetable drink and peanut-based vegetable drink to find lipid markers, and that make it possible to evaluate commercial samples of almond-based vegetable drink, verifying their authenticity and quantifying the percentage of peanuts added. **MATERIAL AND METHODS.** Two standard drinks were prepared, one with almond and one with peanut according to Oliveira (2017) following 4 steps: sampling, previous hydration, grinding, filtration, and then the drinks were pasteurized according to Aamir et al. (2013), after seven brands of almond vegetable drink were acquired in local markets in Maringá, Paraná, Brazil. The analyzes were carried out with the commercialized drinks and in the standard drinks consequently where the lipid profile was evaluated to verify the adulteration. The lipid extractions of the samples were carried out in triplicate according to the methodology developed by Bligh & Dyer (1959), the lipid profiles of the vegetable drinks were evaluated involving the mass/charge ratio (m/z) from 800 to 1000. the methodology by Silveira et al. (2017), fatty acid methyl esters (FMEs) were prepared by methylation of total lipids according to the ISO 5509 (2000) methodology. Chromatographic analysis was performed on a ThermoScientific gas chromatograph equipped with a flame ionization detector (DIC), CP-7420 fused silica capillary column (Select EMAG, 100.0 m long, 0.25 mm internal diameter and 0.25 μm thick). cyanopropyl film as stationary phase).

After analysis, the standard (LA) was intentionally adulterated with the addition of peanuts (LP) at seven different levels (0.1, 5, 10, 20, 50, 70, 90, 100% (v/v) with five replicates each, to assess adulteration.The results of fatty acid composition by GC-DIC were subjected to statistical analysis of variance (ANOVA) and Tukey's test ($p>0.05$), using the Assistet software (Silva & Azevedo, 2016).

RESULTS AND DISCUSSION. By analyzing the fatty acid composition of both standard and commercial samples by GC-FID, between eleven and nineteen fatty acids were identified in the samples, this difference may be related to climatic conditions during the growing season, along with the type of predominant soil in the cultivation area. Among the main fatty acids we can highlight oleic acid (O, 18:1n-9) with 62.74% followed by linoleic acid (L, 18:2n-6) with 27.48%. Among the main fatty acids we can highlight oleic acid (O, 18:1n-9) with 62.74% followed by linoleic acid (L, 18:2n-6) with 27.48%. The data are consistent with the literature, according to Kodadet al. (2014) the oleic acid in their samples is in the range 47 - 77% of the total composition; and according to Čolićet al. (2017) and Zhu et al. (2015) for linoleic fatty acid from 3 - 27% of the total composition. As for peanuts, despite oleic acid being the majority with 78.26%, a reduction in the percentage of linoleic acid significantly and statistically with a value of 2.78%, however, this difference in linoleic acid cannot be used as a marker for fraud since they were reported in Colic et al. (2017) and Zhu et al. (2015), linoleic acid values close to 3%. For the commercial samples, all of them resembled the standard sample of almond by the fatty acid profile.

In order to investigate the addition of (LP/peanut vegetable drink) in (LA/almond vegetable drink), fingerprints of both beverages were obtained from the prepared standard sample. Evaluating the fingerprints, it can be observed that there is the presence of ions m/z 919, 961 and 989 present in the sample (LP), which can be used as lipid markers, since they are not present in the sample (LA). However, the marker m/z 919 was chosen in this work because commercial ingredients (coconut cream, cashew nut and soy) present the ratio m/z 961 and 989. In this way, the lipid marker m/z 919 was determined to evaluate adulteration, due to its presence in the LP sample in greater intensity and its absence in the LA sample. According to the analyzes carried out on the commercialized samples, there was the presence of the lipid marker in samples B, C and F, thus confirming the adulteration of these brands with peanut-based vegetable drink, where the spectra of these are found in the supplementary material. In sample C, peanuts could be found on the label, indicating that the technique applied can be used to detect fraud with peanuts in almond-based beverages. Samples A, D, E and G did not show the marker m/z 919, which have the similar spectrum of the almond-based vegetable drink, where they are found in the supplementary material. Mass spectrometry (MS) is used to identify TAGs, being one of the main separation and characterization techniques used to analyze complex chemical mixtures due to its ability to separate by mass and charge (m/z), detect and characterize molecules of different types, composition and size. In addition, the technique has a combination of speed, sensitivity and selectivity, which makes it a powerful tool for characterizing vegetable oils with lipid markers (Santos et al., 2006; da Silveira et al., 2017). The quantification of adulteration through the percentage of LP added in LA, an analytical curve was obtained evaluating the increase in the intensity of m/z 919 with an increase in LP in LA, this curve obtained an R2: 0.990, and an equation of the straight line $y= 1.107x+ 4.107$. Using this straight line equation, substituting the intensities of the m/z 919 ratio in x, the concentrations of adulterations in y are obtained, using the linear equation it was possible to obtain the concentration of the commercial samples

that were adulterated. Seven samples of almond vegetable drink were analyzed to quantify the LP samples in LA with the proposed method. Through the proposed methodology, it was possible to verify adulteration in the concentration range of 1.47-9.82%. A low relative standard deviation achieved demonstrates that the proposed technique confirms the ideal precision (results shown in complementary material). Likewise, the technique is effective to verify the addition of LP in LA, since it is quick to be used as a routine analysis for fraud verification, in addition to not requiring additional analysis to confirm its results, being conclusive in itself.

CONCLUSIONS. Through the analysis of triacylglycerols (TAG) it was possible to detect a specific lipid marker in the LP sample and absent in LA-m/z 919 [TAG+NH4]+ present in its TAG the palmitic (16:0), arachidic (20 :4n-6) and mystical (14:0). Of the commercial almond-based samples, three of them (B, C and F) contained the marker (m/z 919) indicating adulteration in these samples by peanuts. Therefore, this applied methodology was able to quantify small amounts (greater than 1.0%) of the adulterant in the samples that are commercialized, which can cause harm to the health of the peanut allergic consumer.

Keywords: Vegetable drink; adulteration; food allergy; almond; peanut; ESI-MS.

ARTICLE

Lipid marker ESI-MS method for quantification of adulteration in almond-based vegetable beverage

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Abstract

Food allergy is a frequent problem all over the world, and it often happens because allergens consume items whose labels do not explicitly list all of the substances used in their production. Peanuts are one of the foods that most cause food allergies, generating a severe and even fatal anaphylactic reaction with just 2mg or even traces. Since peanuts have a chemical composition similar to almonds and much lower prices, they can be used to adulterate almond-based drinks or may be present unintentionally without a precautionary statement on the label (PAL) and both forms are considered fraud for putting the health of the allergic consumer at risk. There are publications in the literature that conduct investigations to determine this type of fraud, however, none that is quick, sensitive and conclusive. Thus, in this work samples of almond and peanut vegetable beverages were formulated separately and analyzed by electrospray ionization mass spectrometry (ESI-MS) with direct infusion. A triacylglycerol (TAG) was found as a lipid marker in the peanut beverage and absent in the almond drink (m/z 919 $[TAG+NH4\ ]^{+}$). Intentional adulterations of the almond beverage were carried out, with the addition of a peanut beverage (0, 1, 5, 10, 20, 50, 70 and 90 and 100%), following the increase in intensity of the ion m/z 919 to obtain a analytical curve where it was possible to determine the concentration of the adulterant in the samples of almond vegetable beverages. Following, seven commercial samples of almond vegetable beverages were analyzed to find possible fraud. Peanuts were found in three commercial almond beverages, with concentrations ranging from 1.47-9.82 %. Therefore, the technique used was efficient to detect fraud carried out both qualitatively and quantitatively, due to its sensitivity and detectability.

Keywords: Vegetable beverage; adulteration; food allergy; almond; peanuts; GC-FID; ESI-MS.

Introduction

In recent years, there has been an increase in interest in products that can replace animal milk in the diet, such as vegetable beverages, due to the large number of people with lactose intolerance, others for having food allergies from some proteins present in milk , serve the vegan public who choose to consume products of plant origin (Aydar et al., 2020), and who are concerned with environmental sustainability, taking into account the greater environmental impact related to milk production, including emissions of effect gases greenhouse, water use and land resources (Henchion et al., 2021).

The vegetable beverage does not contain lactose or cholesterol, enabling its use as a substitute for animal milk, which allows consumers to comply with nutritional restrictions. (Silva et al., 2020). In this way, the North American vegetable beverage the market was not designed to have a compound annual growth rate rather it has a annual growth rate of 14.1% between 2019 and 2024 (Statista, 2020).

Vegetable beverages are water-soluble extracts originating from seeds and oilseeds such as soy, cashew, rice, oats, peanuts, and almonds (Cordova, 2019), producing a pasty product, whitish coloration, and accentuated sensory characteristics (Kehinde, 2020), containing relevant concentrations of proteins, lipids, vitamins, and minerals, being considered advantageous from the nutritional point of view (Chavan et al., 2018; Cordova, 2019). In this way, a wide variety of vegetable beverages are available to the consumers (The Cornucopia Institute, 2019).

The chemical and nutritional composition of the vegetable beverage differs significantly from animal milk. Thus, the vegetable beverage is produced, homogenized, pasteurized, and fortified by the industry, with the addition of essential vitamins, minerals, and proteins to prevent nutritional insufficiency when consumed by consumers. (Instituto Cornucópia, 2019).

However, due to the high market value, some vegetable beverages, especially those based on almonds, have become a target of fraudulent practices, and could purposely mixed with other types of oilseeds, reducing the cost of production and increasing profit. (Campmajó et al., 2020). Among the adulterants used in the different types of fraud, peanuts stand out, because of their very similar chemical composition (Walker et al., 2016).

However, according to Anvisa (2018) peanuts are identified as allergenic food, and fall within the eight foods that cause about 90% of food allergies. The ingestion of a small amount (0.2 mg) or simply accidental and unintentional exposure to residues of this oilseed is sufficient to cause an allergic reaction in sensitive individuals, which may produce a fatal anaphylactic reaction (Houben et al., 2020; Remington et al., 2020).

Several studies have already been carried out to evaluate adulteration of almonds by peanuts, and in the search for this detection, it was necessary to use analytical techniques, among them, near-infrared reflectance spectroscopy (NIRS) (Ghoshet al., 2016; Firmani et al ., 2019; López-calleja et al., 2013), gas chromatography with mass spectrometry (GC-MS) (Troya et al., 2015), liquid chromatography with mass spectrometry (LC-MS) (Jiang et al., 2015; Kim et al., 2014; Viana et al. , 2016), nuclear magnetic resonance (NMR) (Arana et al., 2015), ultraviolet (UV) (Alamprese et al., 2013). However, none of them detected markers that by themselves can be used to quantify the fraud in almond-based beverages using peanuts.

Thus, the present work aims to map the triacylglycerols (TAG) present in samples of almond-based beverages and peanut-based beverages to find lipid markers, allowing commercial samples of almond-based beverages evaluation, authenticity verification, and quantification of the percentage of peanuts added.

Materials and methods

Sampling

300 g of almonds (Prunus spp.) and 300 g of peanuts (Arachis spp.) were purchased in natura from a local market in Maringá-PR, Brazil. The seeds were used for the formulation of standard vegetable beverages based on almond (LA) and peanut (LP). The preparation of the beverages was carried out according to Oliveira (2017), and described in the following steps. To assess the authentication of commercial almond vegetable beverages, seven different brands were purchased from local market in the city of Maringá, Paraná State, Brazil.

Prior hydration

50.0 g of each seed (almond, peanut) were hydrated separately with 800 mL of water filtered in a plastic container where they were stored for 6 hours at ambient temperature (25 °C) (Oliveira, 2017).

Crushing

The liquid was filtered and the hydrated seeds were placed separately in a blender (Philips, São Paulo, Brazil) under rotation speed 1 until a homogeneous mixture was obtained. Then 250 mL of filtered, returned to the stirring at speed 1 for 3 min to obtain an uniform liquid mixture of white color. Between the almond and peanut samples, the blender was washed and sanitized to avoid cross contamination between the samples.

Filtration

The samples were filtered through a properly cleaned and sanitized fabric filter and the aqueous extract obtained was collected in a clean and sterilized flask.

Pasteurization

The samples were pasteurized according to Aamir et al. (2013), reaching 72 ºC in the center of the sample and maintained for 15 seconds. After pasteurization, the samples were stored in a previously sterilized glass bottle to be used in the analysis as a standard sample, and placed under refrigeration at 4 °C until lipid extraction.

Industrialized beverages

The analyzes were carried out on the commercialized beverages and consequently compared to the standard almond sample. Table 1 presents information from labels regarding the additional lipid composition of the seven marketed brands.

Lipid extraction

The lipid extractions of the samples were performed in triplicate according to the methodology developed by Bligh & Dyer (1959), where in the first stage approximately 100.0 ± 0,001 g of sample were weighed and 100.0 ± 0,1 mL of chloroform and 200.0 ± 0,1 mL of methanol were added. Then, the mixture was homogenized for 2 min on a 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 magnetic stirrer (Fisatommod: 653, Brasil). In the sequence 100.0 ± 0,1 mL of chloroform were added and the mixture was stirred again, for 30 seconds, an additional 100.0 ± 0,1 mL of water was added to the mixture in order to form the two phase separation. The mixture was stirred for another 30 seconds, the sample was filtered through a Büchner funnel using a paper filter (Whatman Nº 1) under vacuum and the filtrate was transferred to a separating funnel. After separation, the organic phase (bottom) was collected in a 250 mL flat-bottomed flask (LaborGlas Brasil, Schott Duran, Germany) and the solvent was evaporated on a rotary evaporator (Fisatommod: 802, Brazil). Lipid samples were collected, kept at -18 ºC in a freezer until analysis.

Triacylglycerol (TAG) determination by electrospray ionization mass spectrometry (ESI-MS)

The lipid profiles of vegetable beverages were evaluated involving the mass/load ratio (m/z) 800 to 1000. For that, the methodology of Silveira et al. (2017) was followed, where 50.0 µL of lipids from each sample were diluted in 950.0 µL of chloroform (Synth, São Paulo, Brazil). Subsequently, 5.0 µL of this solution was diluted with the addition of 1.0 mL of methanol/chloroform 9: 1 (high performance liquid chromatography (HPLC), grade JTBaker®, Radnor, USA). Afterward, 20.0 uL of 0.10 mol L-1 ammonium formate, prepared in methanol (97%, Sigma-Aldrich, Darmstadt, Germany) were added to the samples for adduct formation, and consequently favoring ionization via [TAG + NH4] + , without affecting the reproducibility of the lipid profile.

A triple quadrupole mass spectrometer (XEVO TQ-D, Waters, Massachusetts, USA) with electrospray ionization source (ESI) was used. The samples were introduced into the system by direct infusion, being ionized by electrospray, operating in the positive ion mode [ESI (+)]. The conditions of the mass spectrometer were: infusion flow (50.0 µL.min − 1), desolvation gas flow (500 Lh-1), source temperature (150 ºC), desolvation temperature (250 ºC), capillary tension (3.00 kV), cone voltage (35.00 V) and flow of desolvation gas (nitrogen, 450 Lh-1). The samples were analyzed and the data were processed using the MassLynx ™ software.

Methylation of total lipids

Fatty acid methyl esters (FAMEs) were prepared by methylation of total lipids according to the ISO 5509 (2000) methodology. 100.0 ± 0,001 mg of lipids were weighed in a tube, 2.0 mL of heptane were added and stirred for 2 min on a magnetic stirrer (Fisatommod: 653, Brazil). Then, 2 mL of KOH esterifying reagent (2 mol L- 1 in methanol) was added, the solution was vortexed for 3 min (Phoenix, São Paulo, Brazil) and taken to the refrigerator for 24 hours for phase separation. The upper phase was collected using a Pasteur pipette, transferred to a vial, and analyzed by gas chromatography.

Gas chromatography analysis

Chromatographic analysis was performed on a Thermo Scientific gas chromatograph equipped with a flame ionization detector (FID), fused silica capillary column CP-7420 (Select FAME, 100.0 m long, 0.25 mm internal diameter, and 0.25 µm thick cyanopropyl film as stationary phase). The injector and detector temperatures were 235 ºC. The column temperature was increased to 65 ºC for 4 min, followed by a heating ramp from 16 ºC min -1 to 185 ºC, which was maintained for 12 min. Subsequently, a new ramp of 20 ºC.min-1 was applied up to 235 ºC and kept for 9 min, totaling an analysis time of 35 min. Gas flows were 1.2 mL.min-1 for carrier gas (H2), 30.0 mL.min-1 for replacement gas (N2) and in FID 30.0 and 300.0 mL.min-1 for gas (H2)

and synthetic air, respectively. The samples were injected in split mode, with a 1:40 ratio. The injection volume was 1.0 µL. FAMEs were identified by comparing the retention time of the sample constituents with those of analytical standards (standard mixture FAME, C4-C24, Saint Louis, United States, Sigma-Aldrich). Peak areas were determined using the ChromQuestTM 5.0 software and the fatty acid compositions were expressed as a relative percentage of total fatty acid. All samples were analyzed in triplicate.

Addition of peanuts in almond-based vegetable beverage

To assess tampering, the (LA) standard was intentionally adulterated with the addition of (LP) at seven different levels (0.1, 5, 10, 20, 50, 70, 90, 100% (v/v)), with five repetitions each, according to Table 2.

Statistical analysis

The results of the fatty acid composition by GC-FID were subjected to statistical analysis of variance (ANOVA) and Tukey's test ($p > 0.05$), using the Assistat software (Silva & Azevedo, 2016).

Results and Discussions

Fatty acid composition by GC-FID

The fatty acid compositions of the samples are shown in Table 3. According to Table 3, between eleven and nineteen fatty acids were identified in the samples, this difference may be related to the climatic conditions during the growing season, along with the predominant soil type in the growing area. Among the main fatty acids, we can highlight oleic acid (O, 18: 1n-9) with 62.74%, followed by linoleic acid (L, 18: 2n-6) with 27.48%. Data were consistent with the literature, according to Kodad et al. (2014), the oleic acid in their samples are in the range 47 - 77% of the total composition; and according to Čolićet al. (2017) and Zhu et al. (2015), for linoleic fatty acid of 3 - 27% of

the total composition. For peanuts, although oleic acid is the main with 78.26% at a statistically significant reduction in the percentage of linoleic acid with a value of 2.78%, however, this difference in linoleic acid cannot be used as a marker of fraud because they were reported in Colic et al. (2017) and Zhu et al. (2015), linoleic acid values close to 3%. For commercial samples, they all resembled the standard almond sample by the fatty acid profile. According to resolution RDC Nº 482/1999 of ANVISA (Anvisa, 1999) and the similar results obtained in the existing fatty acids, as well as their concentrations, gas chromatography can not differentiate product based on almond that contains peanuts. Therefore, determining the profile of TAG is of paramount importance.

Triacylglycerol (TAG) analysis

To investigate the addition of (LP) to (LA), the fingerprint of both beverages was obtained from the prepared standard sample, as shown in Fig. 1. The evaluation of Fig. 1A and 1B shows that the m/z 919, 961, and 989 ions are present in sample B, and can be used as lipid markers, as they are not present in sample A. However, the marker m/z 919 was chosen in this work since commercial ingredients (coconut cream, cashew nuts, and soybeans) present the ratio m/z 961 and 989.

Thus, the lipid marker m/z 919 was determined to assess adulteration, due to its presence in the LP sample in greater intensity and its absence in the LA sample, as shown in Fig. 1 and 2. TAGs from this marker are formed by fatty acids: palmitic (P, 16: 0), arachidic (AA, 20: 4n-6), myristic (M, 14: 0), and they can be considered specific lipid markers for adulteration.

According to the analyzes carried out on the commercialized samples, there was the presence of the lipid marker in samples B, C, and F, which confirms the adulteration of these marks with the peanut-based vegetable beverage. These spectra are showed in the supplementary material. On the label of sample C, it is informed that peanuts could be found, this indicates that the applied technique can be used to detect peanut fraud in almond-based beverages. The samples A, D, E and G, did not present the marker m/z

919, which have a similar spectrum of the vegetable drink based on almonds. These spectra can be verified in the supplementary material.

for the identification of TAGs, the mass spectrometry (MS) technique is used, being one of the main separation and characterization techniques used to analyze complex chemical mixtures due to their ability to separate by mass and charge (m/z), detect and characterize molecules of different types, composition, and size. In addition, the technique has a combination of speed, sensitivity, and selectivity, which makes it a powerful tool to characterize vegetable oils with lipid markers (Santos et al., 2006; da Silveira et al., 2017).

Quantification of the adulteration

Through the percentage of LP added in LA (Table 2), an analytical curve was obtained to estimate the increase in the intensity of m/z 919 with an increase in LP in LA. This curve achieved R 2 = 0.990, and linear equation y = 1.107 x + 4.107. Replacing the intensities of the m/z ratio 919 in x in the equation of the line, the adulteration concentrations in y can be obtained. As shown in Fig. 2, using the linear equation it was possible to obtain the concentration of the commercial samples that were adulterated according to Table 2.

Analysis of commercial samples

Seven samples of almond-based vegetable beverages were analyzed to quantify the LP samples in LA with the proposed method and the results are shown in Table 4. Through the proposed methodology, it was verified the adulteration in the concentration range of 1.47-9.82%. According to Table 1, sample C informed on the label that it could contain peanuts, and this fact was confirmed by the result. The low relative standard deviation (RSD) achieved shows that the proposed technique confirms the ideal precision (results exposed in supplementary material). Likewise, the technique is effective for verifying the addition of LP in LA, since it is fast to use as a routine

analysis for fraud verification and does not require further analysis to confirm its results, being conclusive in itself.

Conclusion

A lipid marker of TAG, from a characteristic m/z (919 [TAG + NH4] +), was quickly and practically identified, proving to be an efficient method. Peanut adulterations were found in three commercial samples based on almonds. Therefore, this method was found to be suitable for quantifying modest levels (> 1.0%) of adulterants in commercial samples, which can harm the health of consumers allergic to peanuts. Finally, the conventional physicochemical and chromatographic methods are not effective, as they are only capable of insinuating a possible gross fraud, but not confirming it as the proposed new method.

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Data availability statement

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Compliance with Ethical

Standards Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human or animal subjects.

Informed Consent

Not applicable.

References

Aamir M, Ovissipour M, Sablani SS, Rasco B (2013) Predicting the Quality of Pasteurized Vegetables Using Kinetic Models: a review. International Journal of Food Science. 2013: 1-29, 2013. http://dx.doi.org/10.1155/2013/271271.

Alamprese C, Casale M, Sinelli N, Lanteri S, Casiraghi E (2013) Detection of minced beef adulteration with turkey meat by UV–vis, NIR and MIR spectroscopy. Lwt - Food Science And Technology. 53: 225-232. http://dx.doi.org/10.1016/j.lwt.2013.01.027.

Arana VA, Medina J, Alarcon R, Moreno E, Heintz L, Schäfer H, Wist J (2015) Coffee's country of origin determined by NMR: the colombian case. Food Chemistry. 175: 500-506. http://dx.doi.org/10.1016/j.foodchem.2014.11.160.

Aydar E, Tutuncu S, Ozcelik B (2020) Plant-based milk substitutes: bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. Journal Of Functional Foods. 70: 103975-103986. http://dx.doi.org/10.1016/j.jff.2020.103975.

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Canadian Journal Of Biochemistry And Physiology. 37: 911-917 http://dx.doi.org/10.1139/o59-099.

Campmajó G, Saez-Vigo R, Saurina J, Nðñez O (2020) High-performance liquid chromatography with fluorescence detection fingerprinting combined with chemometrics for nut classification and the detection and quantitation of almond-based product adulterations. Food Control. 114: 107265-107280, http://dx.doi.org/10.1016/j.foodcont.2020.107265.

Chavan M, Gat Y, Harmalkar M, Waghmare R (2018) Development of nondairy fermented probiotic drink based on germinated and ungerminated cereals and legume. Lwt. 91: 339-344. http://dx.doi.org/10.1016/j.lwt.2018.01.070.

Čolić SD, Akłić MMF, Lazarević KB, Zec GN, Gałić UM, Zagorac DČD, Natić, MM (2017) Fatty acid and phenolic profiles of almond grown in Serbia. Food Chemistry. 234: 455-463. http://dx.doi.org/10.1016/j.foodchem.2017.05.006.

Cordova AG (2019) Consumo de bebidas vegetais no brasil: análise da percepção do consumidor, pelo uso de word association. 2019. 44 f. tcc (Graduação) - Curso de Engenharia de Alimentos, Engenharia Química e Engenharia de Alimentos, Universidade Federal de Santa Catarina, Florianópolis, 2019. Cap. 14.

Silveira, R et al. (2017) Rapid methodology via masss pectrometry to quantify addition of soy bean oil in extra virgin olive oil: a comparison with traditional methods adopted by food industry to identify fraud. Food Research International. 102: 43-50. https://doi.org/10.1016/j.foodres.2017.09.076.

Firmani P, Bucci R, Marini F, Biancolillo A (2019) Authentication of "Avola almonds" by near infrared (NIR) spectroscopy and chemometrics. Journal Of Food Composition And Analysis. 82: 103235-103275 http://dx.doi.org/10.1016/j.jfca.2019.103235.

Ghosh S, Mishra P, Mohamad S, Santos RM, Iglesias BD, Elorza, PB. (2016) Discrimination of peanuts from bulk cereals and nuts by near infrared reflectance spectroscopy. Biosystems Engineering. 151:178-186 http://dx.doi.org/10.1016/j.biosystemseng.2016.09.008.

Henchion M, Moloney AP, Hyland J, Zimmermann J, Mccarthy S. (2021) Review: trends for meat, milk and egg consumption for the next decades and de role played by livestock systems in the global production of proteins. Animal, [S.L.], 100287. http://dx.doi.org/10.1016/j.animal.2021.100287.

Houben GF, Baumert JL, Blom WM, Kruizinga AG, Meima MY, Remington BC, Wheeler MW, Westerhout J, Taylor SL (2020) Full range of population Eliciting Dose values for 14 priority allergenic foods and recommendations for use in risk characterization. Food And Chemical Toxicology. 146: 111831-111839. http://dx.doi.org/10.1016/j.fct.2020.111831.

http://portal.anvisa.gov.br/.Guia sobre Programa de Controle de Alergênicos, 2018.

Jiang S, Tan H, Guo C, Gong L, Shi F (2015) Development of an ultra-highperformance liquid chromatography coupled to high-resolution quadrupole-Orbitrap mass spectrometry method for the rapid detection and confirmation of illegal adulterated sedative-hypnotics in dietary supplements. Food Additives & Contaminants: Part A, 32: 1749-1759 http://dx.doi.org/10.1080/19440049.2015.1041427.

Kehinde BA et al (org.). Advances in Food and Nutrition Research: vegetable milk as probiotic and prebiotic foods. In: Kehinde, BA et al. Advances in Food and Nutrition Research: vegetable milk as probiotic and prebiotic foods. 94. ed. India/ United States: All Rights Reserved, 2020. Cap. 4. p. 1043-4526. Kim HJ, Lee JH, Park HJ, Kim JY, Cho S, Kim WS (2014) Determination of non-opioid analgesics in adulterated food and dietary supplements by LC-MS/MS. Food Additives & Contaminants: Part A, 31: 973-978. http://dx.doi.org/10.1080/19440049.2014.908262.

Kodad O, Estopañán G, Juan T, Alonso JM, Espiau MT, Company RSI (2014) Oil content, fatty acid composition and tocopherol concentration in the Spanish almond genebank collection. Scientia Horticulturae. 177: 99-107. http://dx.doi.org/10.1016/j.scienta.2014.07.045.

López-calleja IM, Lacruz S, Pegels N, González I, García T, Martín R (2013) Development of a real time PCR assay for detection of allergenic trace amounts of peanut (Arachishypogaea) in processed foods. Food Control. 30: 480-490. http://dx.doi.org/10.1016/j.foodcont.2012.09.017.

Oliveira, TAG (2017) Desenvolvimento de produtos à base de amêndoa. 89 f. Dissertação (Mestrado) - Curso de Engenharia Alimentar, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa- Portugal, 2017. Cap. 3.

Remington BC, Westerhout J, Meima MY, Blom WM, Kruizinga AG, Wheeler, Matthew W, Taylor SL, Houben GF, Baumert JL (2020) Updated population minimal eliciting dose distributions for use in risk assessment of 14 priority food allergens. Food And Chemical Toxicology. 139: 111259-111270. http://dx.doi.org/10.1016/j.fct.2020.111259.

Santos LS, et al. (2006) Chemotaxonomic markers of organic, natural, and genetically modified soy beans detected by direct infusion nelectrospray ionization mass

spectrometry. Journalofradioanalyticaland nuclear chemistry 269: 505-509. https://doi.org/10.1007/s10967-006-0415-z.

Silva ARA, Silva MMN, Ribeiro BD (2020) Health issues and technological aspects of plant-based alternative milk. Food Research International. 131: 108972- 108990. http://dx.doi.org/10.1016/j.

foodres.2019.108972 Silva FDAS, Azevedo CAV. (2016). The Assistat Software Version 7.7 and its use in the analysis of experimental data. Afr. J. Agric. Res. 11, 39, 3733-3740. 10.5897/AJAR2016.11522

Statistica: O Portal de Estatística. 2020. "US Planta-Based Leites: Statistics&fatos".Acessado em 03/03/2021.https://www.statista.com/statistics/1092536/global-plant-based-foodand-beverage-market-value-growth-by-region/#statisticContainer.

The cornucopia institute. Viroqua: Report Design And Layout: Draft Horse Studio, v. 54665, n. 826, 01 jun. 2019.https://www.cornucopia.org/wpcontent/uploads/2019/06/PlantBasedBeverageReport.pdf.

Troya F, Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso EF (2015) Classification of vegetable oils according to their botanical origin using n-alkane profiles established by GC–MS. Food Chemistry. 167: 36-39. http://dx.doi.org/10.1016/j.foodchem.2014.06.116.

Viana C, Zemolin GM, Müller LS, Molin TR, Seiffert H, Carvalho LM (2018) Liquid chromatographic determination of caffeine and adrenergic stimulants in food supplements sold in Brazilian e-commerce for weight loss and physical fitness. Food Additives & Contaminants: Part A. [S.L.], p. 1-9. http://dx.doi.org/10.1080/19440049.2015.1112040.

Walker MJ, Burns DT, Elliott CT, Gowland MH, Mills EN (2016) Clare. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. 141:24-35, 2016. http://dx.doi.org/10.1039/c5an01457c.

Zhu Y, Wilkinson KL, Wirthensohn MG (2015) Lipophilic antioxidant content of almonds (Prunus dulcis): a regional and varietal study. Journal Of Food Composition And Analysis. 39: 120-127. http://dx.doi.org/10.1016/j.jfca.2014.12.003.

Table list

Table 1 Composition provided by the label of the almond-based vegetable beverage according to the brands analyzed

Sample	**Country of origin**	**Source of vegetable lipid**	**Additional lipid source in the composition**
a	Brazil	Almond	Coconut cream, Sunflower lecithin
b	Brazil	Almond	Soy
c	Brazil	Almond	Soy, peanuts, cashews
d	Italy	Almond	Soy
e	Brazil	Almond	Sunflower Lecithin
f	Brazil	Almond	Sunflower Lecithin
g	Portugal	Almond	Sunflower Lecithin

Table 2 Almond milk intentionally adulterated with the addition of peanut milk

Percentage of tampering (%)	LA	LP
0	100	0
1	99	1
5	95	5
10	90	10
20	80	20
50	50	50
70	30	70
90	10	90
100	0	100

LA: almond milk; LP: peanut milk. Analysis repeated five times.

Table 3 Fatty Acid Composition (%) of vegetable beverages

Fatty Acid Composition (%)	LA	LP	A	B	C	D	E	F	G
6:0	$0.55^{b}\pm0.06$	$3.80^{a}\pm0.08$	ND	ND	ND	$0.75^{b}\pm0.11$	$0.15^{c}\pm0.01$	$0.20^{c}\pm0.12$	$0.13^{c}\pm0.01$
8:0	$0.01^{bc}\pm0.01$	$0.04^{ab}\pm0.0$	ND	ND	ND	$0.03^{abc}\pm0.00$	$0.05^{a}\pm0.02$	$0.03^{abc}\pm0.1$	$0.05^{abc}\pm0.02$
10:0	ND	$0.01^{c}\pm0.00$	$1.55^{a}\pm0.05$	$0.02^{bc}\pm0.00$	$0.08^{b}\pm0.00$	ND	$0.03^{bc}\pm0.00$	$0.03^{bc}\pm0.01$	$0.03^{bc}\pm0.00$
11:0	ND	$0.15^{b}\pm0.02$	$1.60^{a}\pm0.04$	$0.01^{c}\pm0.00$	$0.06^{bc}\pm0.00$	ND	$0.02^{c}\pm0.00$	$0.06^{bc}\pm0.06$	$0.02^{c}\pm0.00$
12:0	$0.06^{de}\pm0.02$	$0.04^{e}\pm0.01$	$15.35^{a}\pm0.26$	$0.12^{cde}\pm0.00$	$0.60^{b}\pm0.01$	$0.03^{e}\pm0.00$	$0.25^{cd}\pm0.00$	$0.03^{e}\pm0.01$	$0.25^{c}\pm0.00$
14:0	$0.10^{cd}\pm0.06$	$0.15^{bcd}\pm0.01$	$5.30^{a}\pm0.03$	$0.09^{cd}\pm0.00$	$0.25^{b}\pm0.00$	$0.05^{d}\pm0.01$	$0.20^{bc}\pm0.05$	$0.15^{bcd}\pm0.04$	$0.20^{cd}\pm0.05$
16:0	$6.75^{b}\pm0.09$	$7.65^{a}\pm0.08$	$8.15^{a}\pm0.01$	$6.00^{cd}\pm0.07$	$6.40^{bc}\pm0.02$	$5.80^{d}\pm0.08$	$6.60^{b}\pm0.06$	$6.55^{bc}\pm0.41$	$6.60^{b}\pm0.06$
16:1n-7	$0.50^{ab}\pm0.14$	$0.30^{b}\pm0.03$	$0.40^{ab}\pm0.00$	$0.30^{ab}\pm0.00$	$0.40^{ab}\pm0.00$	$0.40^{ab}\pm0.02$	$0.50^{ab}\pm0.07$	$0.55^{a}\pm0.09$	$0.50^{ab}\pm0.07$
17:0	$0.07^{a}\pm0.02$	$0.07^{a}\pm0.01$	ND	$0.04^{ab}\pm0.00$	$0.05^{ab}\pm0.00$	$0.05^{a}\pm0.00$	$0.07^{a}\pm0.02$	$0.06^{a}\pm0.01$	$0.07^{a}\pm0.02$
17:1	$0.20^{ab}\pm0.06$	$0.30^{a}\pm0.05$	ND	$0.08^{b}\pm0.00$	$0.10^{ab}\pm0.00$	$0.07^{b}\pm0.00$	$0.08^{ab}\pm0.02$	$0.15^{ab}\pm0.06$	$0.08^{ab}\pm0.02$
18:0	$1.20^{e}\pm0.09$	$2.15^{b}\pm0.00$	$2.05^{bc}\pm0.01$	$1.35^{e}\pm0.00$	$1.35^{e}\pm0.00$	$3.05^{a}\pm0.07$	$1.75^{d}\pm0.09$	$1.35^{e}\pm0.01$	$1.75^{cd}\pm0.09$
18:1n-9	$62.75^{e}\pm0.60$	$78.25^{a}\pm0.11$	$47.90^{f}\pm0.30$	$71.30^{b}\pm0.10$	$68.00^{c}\pm0.04$	$71.85^{b}\pm0.14$	$64.45^{d}\pm0.03$	$68.80^{c}\pm0.56$	$64.45^{d}\pm0.03$
18:2n-6	$27.50^{a}\pm0.02$	$2.80^{h}\pm0.03$	$17.50^{g}\pm0.08$	$20.20^{f}\pm0.03$	$22.40^{d}\pm0.02$	$17.75^{g}\pm0.00$	$25.65^{b}\pm0.05$	$21.50^{e}\pm0.32$	$25.65^{c}\pm0.05$
18:3n-3	$0.03^{c}\pm0.00$	$0.80^{a}\pm0.03$	$0.25^{b}\pm0.01$	$0.02^{c}\pm0.00$	$0.03^{c}\pm0.00$	$0.02^{c}\pm0.00$	$0.03^{c}\pm0.00$	$0.22^{b}\pm0.00$	$0.03^{c}\pm0.00$
18:3n-6	$0.04^{c}\pm0.01$	ND	$0.08^{ab}\pm0.00$	$0.09^{ab}\pm0.01$	$0.09^{a}\pm0.00$	$0.10^{a}\pm0.00$	$0.06^{bc}\pm0.01$	$0.06^{bc}\pm0.01$	$0.06^{ab}\pm0.01$
20:0	$0.06^{cd}\pm0.01$	$1.30^{a}\pm0.04$	ND	$0.15^{b}\pm0.00$	$0.10^{bc}\pm0.00$	$0.08^{bc}\pm0.01$	$0.06^{bcd}\pm0.01$	$0.10^{bc}\pm0.03$	$0.06^{bcd}\pm0.01$
22:0	$0.18^{b}\pm0.08$	$1.42^{a}\pm0.07$	ND	$0.09^{b}\pm0.00$	$0.08^{b}\pm0.00$	$0.02^{b}\pm0.00$	$0.03^{b}\pm0.00$	$0.04^{b}\pm0.00$	$0.03^{b}\pm0.00$

24:0	0.02^{b}±0.00	0.73^{a}±0.06	ND	0.08^{b}±0.00	0.07^{b}±0.00	0.02^{b}±0.00	0.02^{b}±0.01	0.07^{b}±0.05	0.02^{b}±0.01
24:1n-9	0.15^{ab}±0.05	0.20^{a}±0.01	ND	0.06^{ab}±0.00	0.05^{ab}±0.00	0.02^{ab}±0.00	0.06^{ab}±0.01	0.08^{ab}±0.03	0.06^{ab}±0.01
Σ SFA	8.90^{cde}±0.24	17.40^{b}±0.05	33.95^{a}±0.58	7.95^{e}±0.11	9.00^{cde}±0.03	9.85^{c}±0.18	9.20^{cd}±0.07	8.65^{de}±0.92	9.65^{cd}±0.10
Σ MUFA	63.55^{f}±0.19	79.05^{a}±0.05	48.25^{g}±0.44	71.80^{b}±0.14	68.50^{d}±0.06	72.30^{b}±0.18	65.08^{e}±0.02	69.60^{c}±0.49	64.90^{e}±0.04
Σ PUFA	27.55^{a}±0.05	3.60^{h}±0.00	17.82^{g}±0.13	20.30^{f}±0.03	22.55^{d}±0.03	17.90^{g}±0.01	25.75^{b}±0.09	21.80^{e}±0.43	24.75^{c}±0.03

*Results expressed as mean ± standard deviation (SD) of triplicate; Values with different letters on the same line are significantly different ($p < 0.05$) by the Tukey test; LA: Almond vegetable beverage; LP: Peanut vegetable beverage; A - B - C - D - E - F – G: Commercial vegetable almond beverages; ND: Not detected.

Table 4 Adulteration analysis in commercial samples.

Sample	%
A	-
B	9.82±0.98
C	7.94±0.43
D	-
E	-
F	1.47±0.51
G	-

Average of three repetitions with the respective coefficients of variation (%).

Figure captions

Fig. 1. TAG profile obtained by direct infusion in ESI (+) - MS in the region of m/z 800 - 1100 for A) LA: Almond vegetable beverage; B) LP: Peanut vegetable beverage.

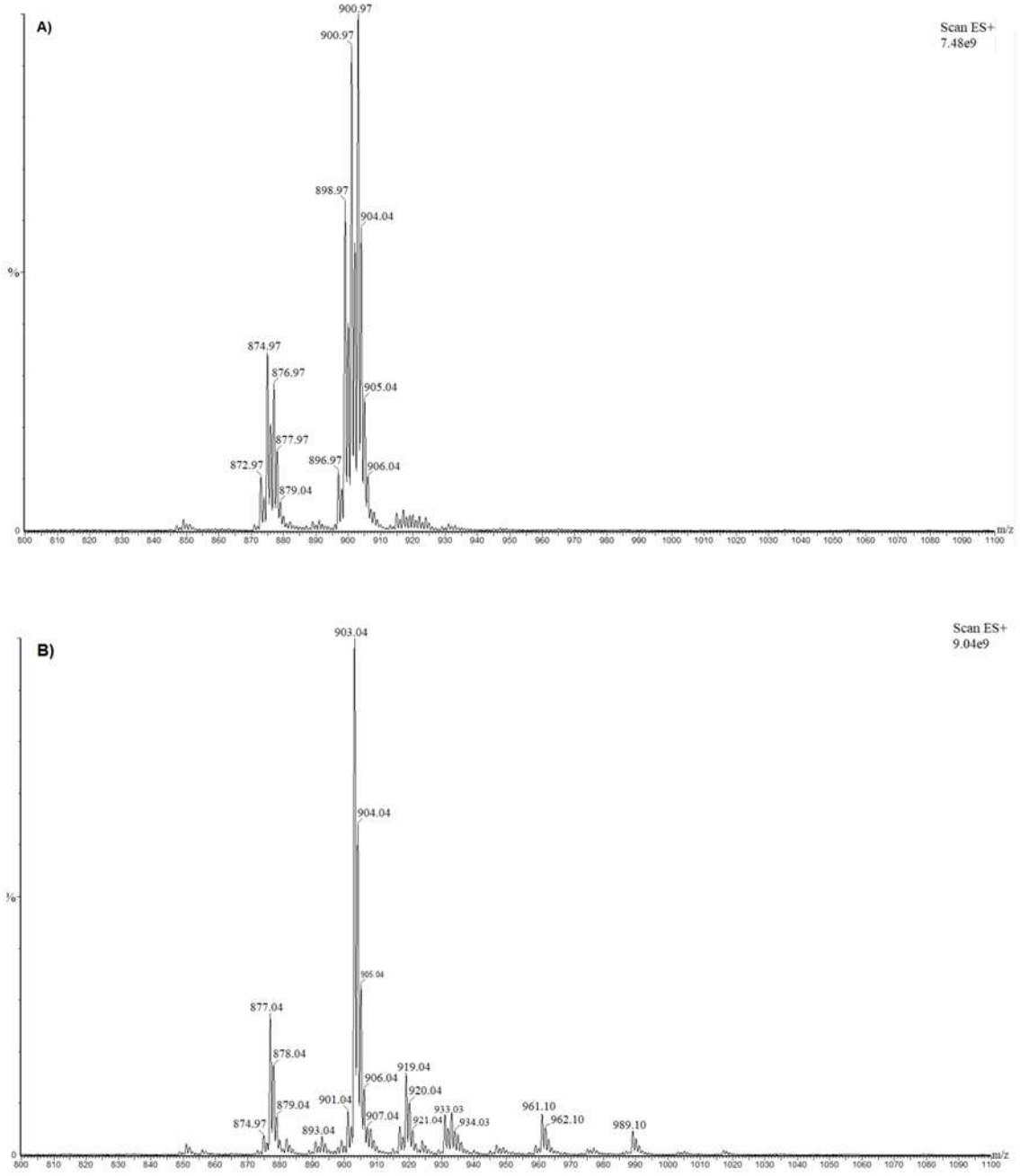


Fig. 2. Ion calibration curve m/z 919 [TAG+NH4] +.

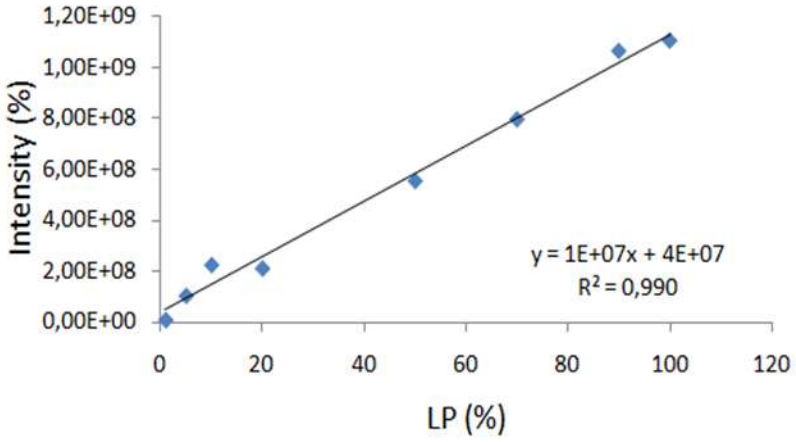
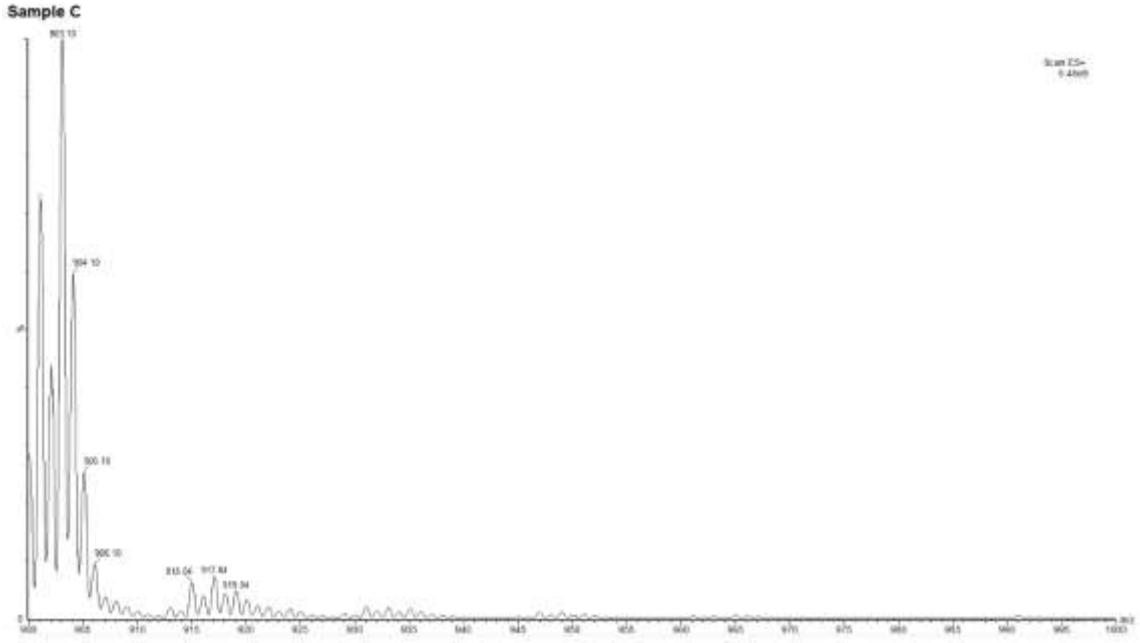


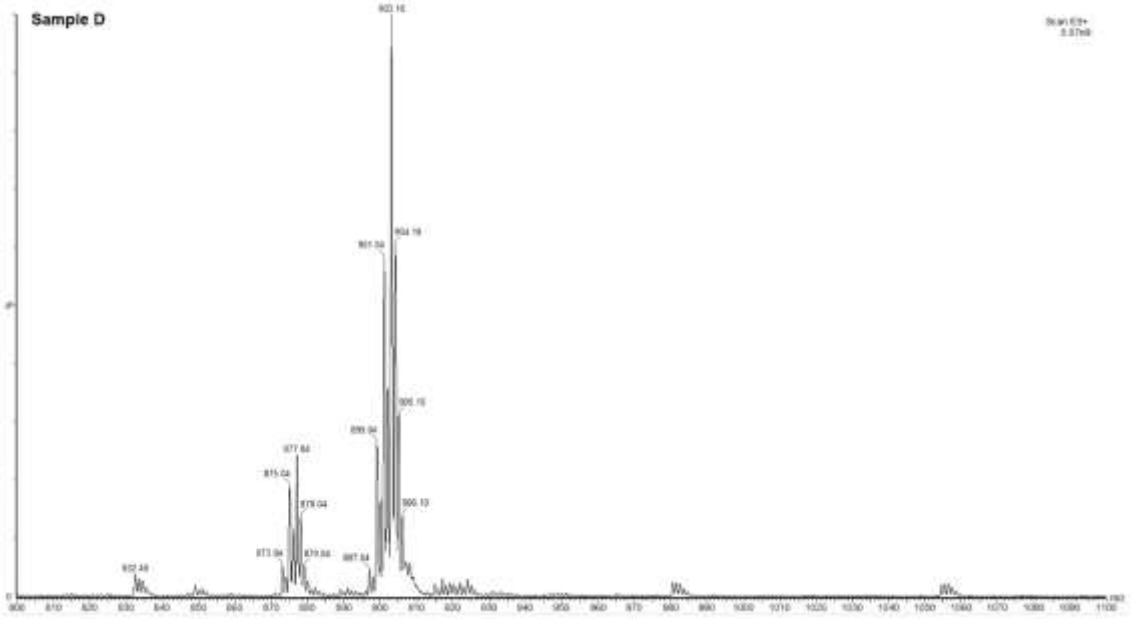
Fig. 3. Complementary material

Sample B

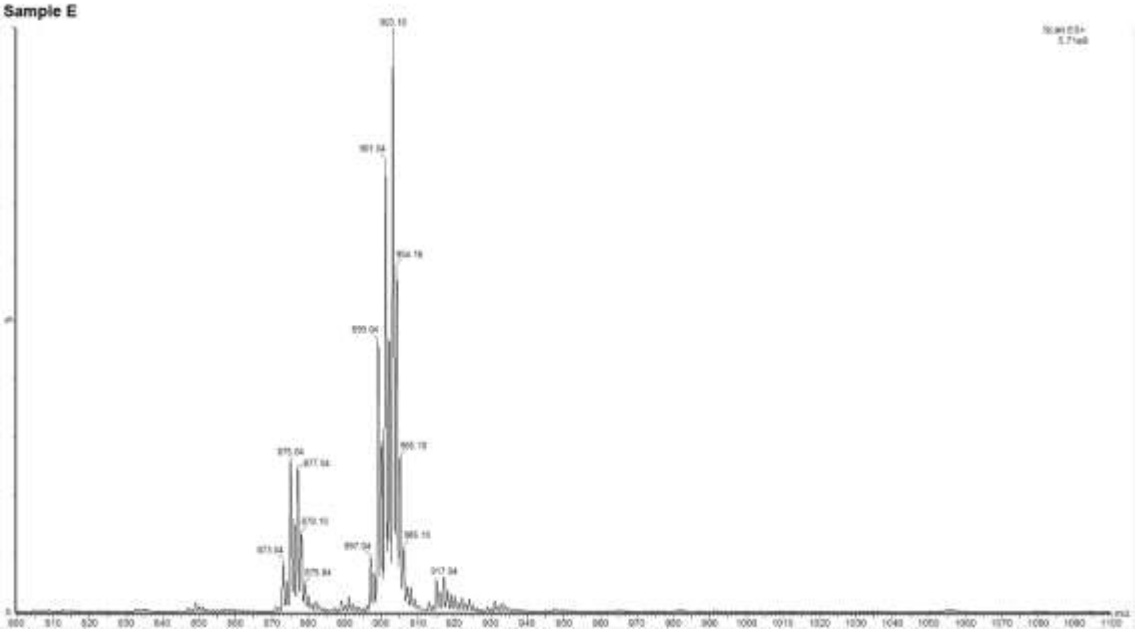
Sample C



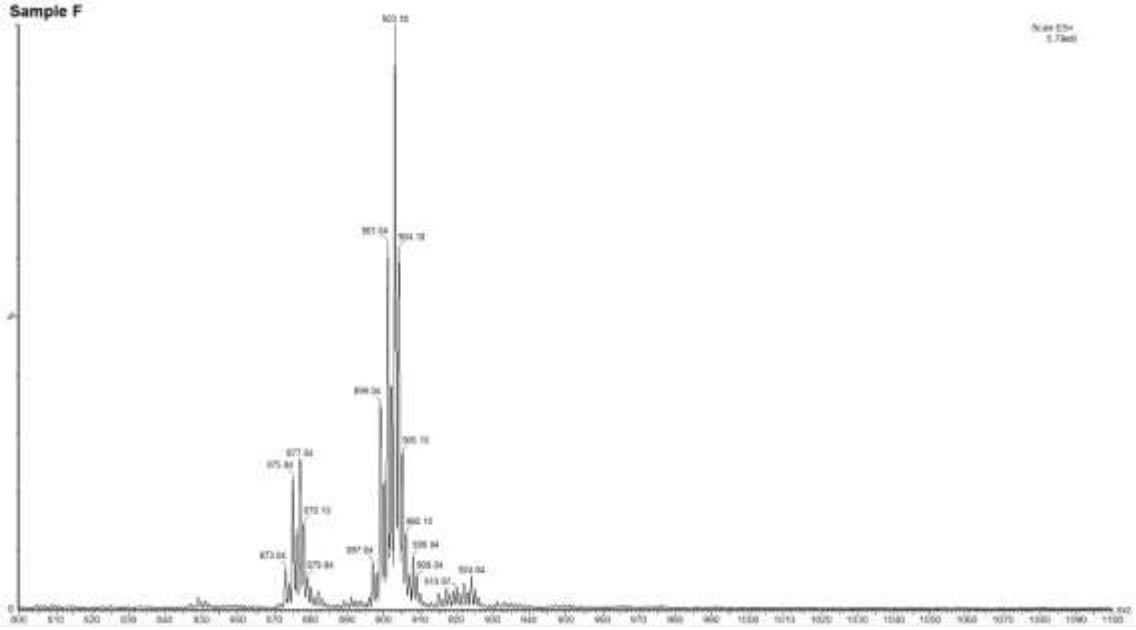
Sample D



Sample E



Sample F



Sample G