UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
Programa de Pós-Graduação em Ciência de Alimentos

Avaliação de adulteração em fontes lipídicas com emprego de infusão direta ESI-MS

ROBERTA DA SILVEIRA

Maringá
2021

ROBERTA DA SILVEIRA

Avaliação de adulteração em fontes lipídicas com emprego de infusão direta ESI-MS

Tese apresentada ao programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de doutor em Ciência de Alimentos

Maringá

2021

ROBERTA DA SILVEIRA

"AVALIAÇÃO DE ADULTERAÇÃO EM FONTES LIPÍDICAS COM EMPREGO DE INFUSÃO DIRETA ESI-MS"

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

Prof. Dr. Oscar de Oliveira Santos Júnior

Profa. Dra. Adriela Albino Rydlewski Ito

Profa. Dra. Marília Bellanda Galuch

Prof. Dr. Thiago Ferreira dos Santos Magon

Prof. Dr. Jesuí Vergílio Visentainer
Orientador

Maringá – 2021

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

S587a

Silveira, Roberta da
Avaliação de adulteração em fontes lipídicas com emprego de infusão direta ESI-MS / Roberta da Silveira. -- Maringá, PR, 2021.
73 f.: il., tabs.

Orientador: Prof. Dr. Jesuí V. Visentainer.
Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência de Alimentos, 2021.

1. Rações caninas - Composição. 2. Rações caninas - Perfil lipídico. 3. Ácidos graxos. 4. Qualidade lipídica. I. Visentainer, Jesuí V., orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência de Alimentos. III. Título.

CDD 23.ed. 664.66

Síntique Raquel Eleutério - CRB 9/1641

Orientador

Jesuí V. Visentainer

BIOGRAFIA

Roberta da Silveira nasceu em Santa Catarina, na cidade de Guaraciaba. Possui graduação em Ciência e Tecnologia de Alimentos pela Universidade Federal de Santa Catarina (UFSC), com graduação sanduíche na *University of Manitoba* (UofM), no Canadá, e mestrado em Ciência de Alimentos pela Universidade Estadual de Maringá (UEM). Tem experiência nas áreas de lipídios atuando principalmente nos seguintes temas: avaliação de fraude lipídica com avaliação do perfil lipídico pelo emprego de infusão direta em espectrometria de massas com fonte de ionização por eletrospray (DI – ESI – MS) e composição de ácidos graxos por cromatografia gasosa com detector de ionização de chama (CG – DIC).

Dedico

À minha família.

AGRADECIMENTOS

Com satisfação, agradeço:

Meu orientador Prof. Dr. Jesuí Vergílio Visentainer, pela valiosa orientação;

Prof. Dr. Oscar O. Santos Jr., pela disposição em ajudar sempre que possível;

O PPC;

A CAPES, pelo incentivo financeiro;

A todos os colegas e amigos do grupo APLE-A, que sem eles muito disto não seria possível, em especial:

À Patrícia D. S. Santos, Luciana P. Manin, Jessica S. Pizzo, Adriela A. Rydlewski e Eloize S. Alves, pela enorme ajuda dentro e fora do laboratório.

Agradeço a minha amiga desde o primeiro dia de laboratório: Marília B. Galuch, por todos os momentos;

Agradeço minha psicóloga Amanda Alves, a qual me auxiliou incontáveis vezes;

Agradeço também meus pais, pela paciência e incentivo;

Agradeço minha eterna companheira de todas as horas, Magnólia;

E, por fim, sinceramente agradeço todos àqueles que me acompanharam e de alguma maneira contribuíram para o meu crescimento profissional e pessoal ao longo do curso de Doutorado.

APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de dois artigos científicos:

1. Roberta da Silveira, Patrícia Daniele Silva dos Santos, Jessica Santos Pizzo, Nayane Mattos, Matheus Campos de Castro, Oscar Oliveira Santos, Jesuí Vergilio Visentainer. ***Evaluation of Dog Food Authenticity Through Lipid Profile Using GC-FID and ESI-MS***. *Journal of the Brazilian Chemical Society*. vol. 31, n. 12, 2511-2517, 2020.

2. Roberta Silveira, Jessica Santos Pizzo, Luciana Pelissari Manin, Adriela A. Rydlewski, Marília B. Galuch, Patrícia Daniele Silva dos Santos, Oscar Oliveira Santos, Jesuí Vergilio Visentainer. ***Assessment of grape seed oils lipid quality by GC-FID and ESI-MS: rapid method to detect adulteration.*** *Journal of Food Composition and Analysis*. Submetido dia 19 de Abril de 2021.

GENERAL ABSTRACT

INTRODUCTION. The pet food industry is continuously developing and seeking new ingredients for greater animal welfare and health. The feed must be balanced in order to meet all the animal nutritional requirements (NRC, 2006). Dog food is classified according to the ingredients quality and its cost, being divided in *standard*, *premium* and *super-premium* (Carciofi *et al*., 2009). Generally, in feed composition are present flours of meat, bone and chicken viscera, as well as phosphates, cereal flours and bran, and food additives such as acidulants, antioxidants and flavorings (Goes *et al*., 2013). The lipid sources used in feed are usually chicken fat, bovine tallow, swine lard, fish and vegetable oils (ABINPET, 2016). Aiming the animal health, the nutritional supplementation with omega-3 (n-3) and omega-6 (n-6) is widely used. The incorporation of essential fatty acids (FAs), such as linoleic (18:2n-6) and α-linolenic (18:3n-3) acids, is related to lower rate of behavioral changes, as well as to increase learning capacity and visual acuity in pups (Heinemann *et al*., 2005). Eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) FAs are part of the n-3 fatty acid family. EPA is involved in the eicosanoids synthesis, particularly prostaglandins, leukotrienes, and thromboxanes, competing with arachidonic acid (20:4n-6, AA) for cyclooxygenase and 5-lipoxygenase enzymes, leading to increased production of anti-inflammatory eicosanoids, rather than pro-inflammatory eicosanoids derived from AA (Vaughn *et al*., 1994; Bispo *et al*., 2014). DHA is essential for the neurological system development and it is present in the retinal membrane. Furthermore, EPA and DHA have beneficial effects on the immune and inflammatory systems, assist in the protection of cardiac and renal functions (anti-inflammatory and antihypertensive actions) and stimulate learning ability (Zeng *et al*., 2011; Kralovec *et al*. 2012; Bispo *et al*., 2014).

AIMS. Observing the crescent number of adulterated products with the addition of low cost vegetable oils is crucial to evaluate the authenticity of the lipid source used in the manufacture of dog food. Therefore, diverse feed brands and classifications were analyzed in order to verify fraud existence regarding the FA composition, mainly EPA and DHA. Consequently, fatty acid composition and lipid profile of each feed were obtained by gas chromatographic techniques with flame ionization detector (GC-FID) and mass spectrometry with electrospray ionization (ESI-MS), respectively.

MATERIAL AND METHODS. Dog feed considered n-3 and n-6 sources (composition on the label) from different classifications and brands available in the Brazilian market were purchased in the city of Maringá, Paraná - Brazil (23°25'31"S51°56.18°C).

For the FAs analysis by GC-FID, Figueiredo's (2016) direct methylation method was employed in order to extract and prepare the fatty acid methyl esters (FAMEs). FAMEs were separated on a Thermo GC, trace ultra 3300 model, equipped with FID, automatic injector and fused silica capillary column CP-7420 (Select FAME, 100 m long, 0.25 mm internal diameter and 0.25 μm cyanopropyl). Gas flow as follows: 1.2 mL $min^{-1}$of H_2, 30 mL min^{-1} of N_2, 35 and 300 mL min^{-1} of H_2 and synthetic air, respectively, to the detector flame. The injected volume was 1.0 μL, using a sample split of 40:1, with injector and detector temperatures being 250 and 230 °C, respectively. Heating ramp was applied in the column, initiating the temperature at 165 °C for 18 min and raised to 235 °C with heating rate of 4 °C min^{-1}, remaining for 20 min (Silveira *et al*., 2017). FAMEs were identified by comparing its retention times with standards (FAME Mix, C4-C24, Sigma-Aldrich) and the results were expressed as mg g^{-1} of total lipids, determined

automatically by integration of peak areas through Chromquest™ 5.0 software.

For the lipid profile analysis by ESI-MS, the lipid fraction was extracted according to Figueiredo (2016). Then, 50 μL of the extracted oil was diluted in chloroform (950 μL). 1.0 mL of methanol/chloroform (9:1, v/v) was added in 5.0 μL of this solution and then 20 μL of ammonium formate (0.10 mol L^{-1} in methanol) were added (Youzbachi *et al.*, 2015). The final solution was infused directly into a triple-quadrupole Xevo-TQD MS equipped with ESI Z spray™ ionization source (Waters, Milford, MA, USA). The lipid profiles were obtained in the ratio range of 100 to 1200 *m/z* and extracted in positive (ESI+) mode. ESI source parameters were as follows: source temperature of 150 °C, desolvation temperature of 200 °C, capillary voltage of 3.0 kV and cone voltage of 20.0 V. High purity nitrogen was produced by nitrogen generator (NM32LA, Peak Scientific®, Renfrewshire, Scotland) and it was used as desolvation gas with flow rate of 500 L h^{-1}. The sample solutions were injected with a continuous flow of 10.0 μL min^{-1}. Data were processed using MassLynx™ software.

The results obtained from the FA composition analysis by GC-FID were submitted to analysis of variance (ANOVA), the means were compared using the Tukey test, with significance level of 95%, and the results obtained by ESI-MS were analyzed by principal component analysis (PCA), through the R Studio software (R Studio Team, 2015).

RESULTS AND DISCUSSION. Feed manufacturers must present the guarantee levels on its products labels (MAPA, 2007). These levels establish the product nutritional quality offered to the consumer, demonstrating a quality standard that is dependent on the adequate quality control during and after the production process, as well as the raw material used. Among the information presented in the guarantee levels are: FAs levels such as n-3, n-6, EPA and DHA. The FA composition analysis was carried out with the objective of comparing the FAs amount of the samples with the respective guarantee levels declared by the manufacturers. Oleic acid (18:1n-9) was the major FA in all samples analyzed, followed by linoleic (18:2n-6) and palmitic (16:0) acids. Myristic acid (14:0) was found in the range of 0.19 to 0.51%, 16:0 acid in the range of 0.61 to 1.23%, stearic acid (18:0) in the range of 5.83 to 9.85%, 18:1n-9 acid in the range of 31.98 to 39.48%, 18:2n-6 acid in the range of 23.62 to 28.96 % and α-linolenic acid (18:3n-3) in the range of 1.10 to 2.65%.

In all samples analyzed, there is the addition of chicken oil. This information is presented in the labels of each sample and was confirmed with FA composition analysis by GC-FID comparing the feed and the chicken oil samples (Figueiredo *et al.*, 2016), becoming clear that all analyzed samples have the same FA composition, predominating the FAs 18:1n-9, 18:2n-6 and 16:0. On the labels of samples 1, 2, 4, 7 and 9, there is the information that fish oil is added, these being the main sources of EPA and DHA (Lowe *et al.*, 2008; Bispo *et al.*, 2014). However, the information on the amount of EPA+DHA, presented on the feed sample labels, does not agree with the analysis results.

According to the lipid profiles Figures, it can be observed that all samples presented its individual characteristic and also some similarity among it, also the FAs present in the feeds are in the (triacylglycerol) TAG form, due to the most abundant region of ions, predominantly in *m/z* 800 to 1000.

The lipid profiles in the TAG region of all feed samples were similar to the profile presented by Cajka *et al.* (2013) for chicken oil. Hence, to confirm this similarity, chicken oil extraction was carried out by Bligh and Dyer (1959) and ESI(+)-MS direct infusion analysis was performed. Thus, it is possible to observe the similarity between the profiles of all the feed samples (1-10) with the chicken oil profile, proving the presence of it in all feed samples (1-10).

The major TAG encountered were 52:3 (*m/z* 874), followed by 52:2, 54:4, 54:5, 52:4, and 54:3. These results are according to Porcari *et a*l. (2016) and Cajka *et al.* (2013) for chicken oil lipid profile. One of the possible TAGs found for *m/z* 874 is PLO, consisting of the FAs 16:0/18:2n-6/18:1n-9. These FAs were also found in greater amounts in the analysis performed by GC-FID.
The feed manufactures of samples 1, 2, 4, 7, and 9 reported the presence of EPA and DHA at the guarantee levels on its labels. These two FAs come predominantly from fish oil, such as sardines and salmon (Bispo *et al.*, 2014). Therefore, to verify and compare the lipid profile of the sardine and salmon oils with the lipid profiles presented by the feed, both oils were extracted by Bligh and Dyer (1959) and the direct infusion analysis by ESI(+)-MS was carried out. It can be observed that none of the feed samples presented lipid profiles similar to the profiles analyzed for sardine or salmon. Moreover, the profiles obtained for fish oils were also compared in this work with the lipid profiles of fish oils present in omega-3 capsules analyzed by direct infusion using ESI(+)-MS with ionization $[TAG+NH4]^+$, and it was observed that the capsules have profiles similar to those obtained for sardine oil (Galuch *et al*., 2018).
The results obtained by ESI(+)-MS confirm the data obtained by GC-FID, showing the presence of chicken oil in the feed and absence of EPA and DHA, since no lipid profiles were found similar to fish oils or TAGs containing these two FAs.
PCA was performed to clarify the results contribution obtained by ESI-MS. PC1 (65.1%) and PC2 (21.2%) explained 86.3% of the total variance. A separation was observed in two distinct groups, in PC1 negative quadrant one group was formed by samples 1, 2, 3 and 10 due to the closer signal strength *m/z*, and the samples 1 and 2 contributed positively to PC2 and samples 3 and 10 contributed negatively to this separation. In PC1 positive quadrant another group was formed by samples 5,6,7 and 8 due to the fact previously exposed. For this group only sample 6 contributed positively to PC2, while samples 5,7 and 8 contributed negatively to PC2.
CONCLUSIONS. The FA composition obtained was compared to the dog food labels samples, it was observed that the omega 3 and omega 6 amounts are within the limit determined by each manufacturer, however, the labeling information on the EPA and DHA concentrations are not in accordance with the results obtained by GC-FID nor by ESI-MS. PCA analysis revealed that PC1 and PC2 explained 86.3% of the total variance.
Consequently, the information displayed on the labels are in disagreement with the results obtained for the fatty acid composition analysis by GC-FID and for the lipid profile analysis by ESI(+)-MS.

Keywords: dog feed; fatty acid composition; product quality; GC-FID; ESI-MS; lipid profile.

RESUMO GERAL

INTRODUÇÃO. A indústria de alimentos pet está continuamente se desenvolvendo e buscando novos ingredientes visando maior bem-estar e saúde animal. Sendo assim, a ração deve ser balanceada para atender todas as necessidades nutricionais caninas (NRC, 2006). Ração é classificada de acordo com a qualidade e custo de seus ingredientes, sendo dividida em *standard, premium* e *super-premium* (Carciofi *et al*., 2009). Geralmente, na composição da ração estão presentes: farinhas de carne, ossos e vísceras de frango, bem como fosfatos, farinhas e farelo de cereais e aditivos alimentares como acidulantes, antioxidantes e aromatizantes (Goes *et al*., 2013). As fontes lipídicas utilizadas na ração são usualmente gordura de frango, sebo bovino, banha de porco, óleos vegetais e de peixes (ABINPET, 2016). Visando a saúde animal, a suplementação nutricional com ômega-3 (n-3) e ômega-6 (n-6) é amplamente utilizada; a incorporação de ácidos graxos essenciais, como os ácidos linolêico (18:2n-6) e α-linolênico (18:3n-3), está relacionada à menor taxa de alterações comportamentais (Heinemann *et al*., 2005). Os ácidos graxos eicosapentaenóico (20:5n-3, EPA) e docosahexaenóico (22:6n-3, DHA) fazem parte da família dos ácidos graxos n-3. Onde EPA e DHA têm efeitos benéficos nos sistemas imunológico e inflamatório, auxiliando na proteção das funções cardíacas e renais (ações anti-inflamatórias e anti-hipertensivas) e estimulam a capacidade de aprendizagem (Zeng *et al*., 2011; Kralovec *et al*., 2012; Bispo *et al*., 2014).

OBJETIVOS. Observando o crescente número de produtos adulterados com adição de óleos vegetais de baixo custo, faz-se fundamental a avaliação da autenticidade da fonte lipídica utilizada na fabricação de ração canina. Portanto, diversas marcas e classificações de rações foram analisadas a fim de verificar a existência de fraudes quanto à composição de ácidos graxos, principalmente EPA e DHA. Consequentemente, a composição de ácidos graxos e o perfil lipídico de cada ração foram obtidos por técnicas de cromatografia gasosa com detector de ionização de chama (CG-DIC) e espectrometria de massa com ionização por eletrospray (ESI-MS), respectivamente.

MATERIAL E METODOS. Rações para cães consideradas fontes n-3 e n-6 de diferentes classificações e marcas disponíveis no mercado brasileiro foram adquiridas na cidade de Maringá, Paraná - Brasil (23°25'31"S51°56,18°C).

Para a análise de ácidos graxos (AGs) por CG-DIC, o método de metilação direta de Figueiredo (2016) foi empregado para extrair e preparar os ésteres metílicos de ácidos graxos (EMAGs). Os EMAGs foram separados em um Thermo CG, modelo trace ultra 3300, equipado com DIC, injetor automático e coluna capilar de sílica fundida CP-7420 (Select EMAG, 100 m de comprimento, 0,25 mm de diâmetro interno e 0,25 μm de cianopropil). Fluxo de gás sendo: 1,2 mL min^{-1} de H_2, 30 mL min^{-1} de N_2, 35 e 300 mL min^{-1} de H_2 e ar sintético, respectivamente, para a chama do detector. O volume injetado foi de 1,0 μL, usando divisão de amostra de 40:1, com temperaturas do injetor e do detector de 250 e 230 °C, respectivamente. Foi aplicada rampa de aquecimento na coluna, iniciando a temperatura em 165 °C por 18 min e elevando para 235 °C com taxa de aquecimento de 4 °C min^{-1}, permanecendo por 20 min (Silveira *et al*., 2017). Os EMAGs foram identificados comparando seus tempos de retenção com padrões (EMAG Mix, C4-C24, Sigma-Aldrich) e os resultados foram expressos em mg g^{-1} de lipídios totais, determinados automaticamente pela integração das áreas de pico por meio do software Chromquest™ 5.0.

Para a análise do perfil lipídico por ESI-MS, a fração lipídica foi extraída de acordo com Figueiredo (2016). 50 μL do óleo extraído foram diluídos em clorofórmio (950 μL). 1,0 mL de metanol/clorofórmio (9:1, v/v) foi adicionado em 5,0 μL desta solução e

20 µL de formato de amônio (0,10 mol L^{-1} em metanol) foram adicionados (Youzbachi *et al.*, 2015). A solução final foi infundida diretamente em um Xevo-TQD MS triplo-quadrupolo equipado com fonte de ionização ESI Z spray™ (Waters, Milford, MA, EUA). Os perfis lipídicos foram obtidos na faixa de 100 a 1200 *m/z* e extraídos no modo positivo (ESI+). Os parâmetros da fonte ESI foram: temperatura da fonte de 150 °C, temperatura de dessolvatação de 200 °C, voltagem capilar de 3,0 kV e voltagem do cone de 20,0 V. O nitrogênio de alta pureza foi produzido pelo gerador de nitrogênio (NM32LA, Peak Scientific®, Renfrewshire, Escócia) e foi utilizado como gás de dessolvatação com vazão de 500 L h^{-1}. As soluções de amostra foram injetadas com fluxo contínuo de 10,0 µL min^{-1}. Os dados foram processados usando o software MassLynx™.

Os resultados obtidos na análise de composição de AG por CG-DIC foram submetidos à análise de variância (ANOVA), as médias foram comparadas pelo teste de Tukey, com nível de significância de 95%, e os resultados obtidos pelo ESI-MS foram analisados por análise de componentes principais (PCA), por meio do software R Studio (R Studio Team, 2015).

RESULTADOS E DISCUSSÃO. Os fabricantes de rações devem apresentar os níveis de garantia nos rótulos de seus produtos (MAPA, 2007). Esses níveis estabelecem a qualidade nutricional do produto oferecido ao consumidor, demonstrando um padrão de qualidade que depende do adequado controle de qualidade durante e após o processo de produção, bem como da matéria-prima utilizada. Entre as informações apresentadas nos níveis de garantia estão: níveis de AG como n-3, n-6, EPA e DHA. A análise da composição dos AGs foi realizada com o objetivo de comparar a quantidade de AGs das amostras com os níveis de garantia declarados pelos fabricantes.

O ácido oleico (18:1n-9) foi o principal AG em todas as amostras analisadas, seguido pelos ácidos linoléico (18:2n-6) e palmítico (16:0). O ácido mirístico (14:0) foi encontrado no intervalo de 0,19 a 0,51%, o ácido 16:0 no intervalo de 0,61 a 1,23%, o ácido esteárico (18:0) no intervalo de 5,83 a 9,85%, o ácido 18:1n-9 no intervalo de 31,98 a 39,48%, o ácido 18:2n-6 no intervalo de 23,62 a 28,96% e o ácido α-linolênico (18:3n-3) no intervalo de 1,10 a 2,65%. Além disso, não foi possível identificar EPA e DHA em nenhuma das amostras analisadas.

Em todas as amostras analisadas, há adição de óleo de frango. Informação apresentada nos rótulos de cada amostra e confirmada com a análise de CG-DIC comparando as amostras de ração e de óleo de frango (Figueiredo *et al.*, 2016), ficando claro que todas as amostras analisadas possuem a mesma composição de AG, predominando os AGs 18:1n-9, 18:2n-6 e 16:0. Nos rótulos das amostras 1, 2, 4, 7 e 9, consta a informação de que o óleo de peixe é adicionado, sendo este a principal fonte de EPA e DHA (Lowe *et al.*, 2008; Bispo *et al.*, 2014). No entanto, as informações sobre a quantidade de EPA e DHA, apresentadas nos rótulos das amostras, não estão de acordo com os resultados da análise.

De acordo com as Figuras do perfil lipídico, pode-se observar que todas as amostras apresentam características individuais e também alguma semelhança entre elas. Além disso, os AGs presentes nas rações estão na forma de (triacilglicerol) TAG, devido à região mais abundante de íons ser predominantemente em *m/z* 800 a 1000.

Os perfis lipídicos na região de TAG de todas as amostras de ração foram semelhantes ao perfil apresentado por Cajka *et al.* (2013) para óleo de frango. Portanto, para confirmar essa semelhança, a extração do óleo de frango foi realizada por Bligh e Dyer (1959) e a análise de infusão direta ESI(+)-MS foi realizada. Assim, é possível observar a semelhança entre os perfis de todas as amostras de ração (1-10) com o perfil do óleo de frango, comprovando sua presença em todas as amostras de ração (1-10).

Os principais TAGs encontrados foram: 52:3 (*m/z* 874), seguido por 52:2, 54:4, 54:5, 52:4

e 54:3. Esses resultados estão de acordo com Porcari *et al*. (2016) e Cajka *et al*. (2013) para perfil lipídico de óleo de frango. Um dos possíveis TAGs encontrados para *m/z* 874 é PLO, sendo os AGs 16:0/18:2n-6/18:1n-9. Esses AGs também foram encontrados em maiores quantidades nas análises de CG-DIC.

Os fabricantes de rações das amostras 1, 2, 4, 7 e 9 relataram a presença de EPA e DHA nos níveis de garantia em seus rótulos. Esses dois AGs vêm predominantemente de óleo de peixe, como sardinha e salmão (Bispo *et a*l., 2014). Portanto, para verificar e comparar o perfil lipídico dos óleos de sardinha e salmão com os perfis lipídicos apresentados pela ração, os óleos desses dois peixes foram extraídos por Bligh e Dyer (1959) e a análise de infusão direta por ESI(+)-MS foi realizada. Pode-se observar que nenhuma das amostras de ração apresentou perfil lipídico semelhante aos perfis analisados para sardinha ou salmão. Além disso, os perfis obtidos para os óleos de peixe foram comparados com os perfis lipídicos de óleos de peixe presentes em cápsulas de ômega-3 analisados por infusão direta usando ESI(+)-MS com ionização $[TAG+NH_4]^+$, e foi observado que as cápsulas apresentam perfis semelhantes aos obtidos para o óleo de sardinha (Galuch *et al*., 2018).

O PCA foi realizado para esclarecer a contribuição dos resultados obtidos pelo ESI-MS. PC1 (65,1%) e PC2 (21,2%) explicaram 86,3% da variância total. Foi observada uma separação em dois grupos, no quadrante negativo de PC1 um grupo foi formado pelas amostras 1, 2, 3 e 10 devido à intensidade do sinal mais próxima *m/z*, e as amostras 1 e 2 contribuíram positivamente para PC2 e as amostras 3 e 10 contribuíram negativamente para essa separação. No quadrante positivo do PC1 outro grupo foi formado pelas amostras 5, 6, 7 e 8 devido ao fato previamente exposto. Para este grupo, apenas a amostra 6 contribuiu positivamente para PC2, enquanto as amostras 5, 7 e 8 contribuíram negativamente para PC2.

CONCLUSÕES. A composição de AG foi comparada com os rótulos das amostras de ração, observou-se que as quantidades de ômega 3 e ômega 6 estão dentro do limite determinado por cada fabricante, entretanto, as informações de rotulagem das concentrações de EPA e DHA não estão de acordo com os resultados obtidos por CG-DIC nem por ESI-MS. A análise de PCA revelou que PC1 e PC2 explicaram 86,3% da variância total.

Consequentemente, as informações apresentadas nos rótulos estão em desacordo com os resultados obtidos para a análise da composição de ácidos graxos por CG-DIC e para a análise do perfil lipídico por ESI(+)-MS.

Palavras chaves: ração canina; composição em ácido graxo; qualidade de produto; CG-DIC; ESI-FID; perfil lipídico.

GENERAL ABSTRACT

INTRODUCTION. Vegetable oils are complex mixtures, mostly composed of triacylglycerols (TAG), fatty acids (FA) and minor components (Indelicato *et al*., 2017). Among the FAs, there are: saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FAs. Considering the PUFAs, some cannot be synthesized by the human body and are considered essential FAs, as is the case of linoleic (18:2) and α-linolenic (18:3n-3) acids, respectively considered FAs of n-3 and n-6 PUFA families. These FAs possess antiatherogenic and antithrombotic properties and affect the lipoprotein concentration, membrane fluidity, membrane enzyme function and modulation of other compounds (Yang *et al*., 2018).

Vegetable oils account for more than 75% of the total lipids consumed in the world. Moreover, it is constantly applied in the manufacturing process as ingredient for several foods and products (Garavaglia *et al*., 2016). This manufacturing process produce undesirable by-products. Grape (*Vitis vinifera*) is one of the most consumed fruit worldwide, predominantly as juice (Martin *et al*., 2020), producing expressive amount of grape pomace. The dry pomace includes approximately 20–26% of seeds (Yalcin *et al*., 2016), which is rich in phytochemicals, bioactive compounds and unsaturated FAs (Crew *et al*. 2006; Villani *et al*. 2015; Shinagawa *et al.* 2017; Ma & Zhang, 2017).

The grape seeds oil main characteristic is its elevated content of unsaturated FAs, particularly 18:2 and oleic (18:1) acids (Yalcin *et al*., 2016). Plus, it also contains high vitamin E content, which is important for human health (Al Juhaimi *et al*., 2017). The unsaturated FAs makes this oil popular for culinary, pharmaceutical, cosmetic and medical industries (Yalcin *et al*., 2016). Due to its beneficial properties, as it is rich in PUFAs and MUFAs, grape seed oil assists in the body's energy deposit, with high antioxidant influence, being considered a product with high added value (Oikonomou *et al*. 2018). Nevertheless, it is extensively used on human skin, as it assists in healing, smoothing, calming, antibacterial, antioxidant and normalizing effects (Michalaka & Kiełtyka-Dadasiewicz, 2018).

AIMS. Considering the growing market of products containing grape seed oil (Martin *et al*., 2020), this research focused on assessing the quality of it, once the substitution of vegetable oils with higher prices for oils of lesser value is alarming in Brazil (Silveira *et al.* 2017; Galuch *et al*. 2018; Pizzo *et al.* 2018). It was achieved through direct infusion by electrospray ionization mass spectrometry (ESI-MS) and gas chromatography with flame ionization detection (GC-FID), both techniques can easily determine adulteration in vegetable oil due to the TAG profile differentiation and the FA composition characterization, respectively (Silveira *et al.* 2017; Galuch *et al*. 2018; Pizzo *et al.* 2018).

MATERIAL AND METHODS. Ten samples of grape seed oils (GSO) were acquired from local market of Maringa – PR, Brasil. In order to obtain the pure grape seed oils (PGSO), a bunch of grapes were purchased at the local market in Maringa – PR, Brasil, the grape seeds were separated from the fruit, placed in a bowl and washed with running water. Three lots containing three samples each of refined soybean oils (RSO) was obtained from local market of Maringa – PR, Brasil. All samples from RSO and GSO were preserved in its original container under refrigeration (6 to 10 °C) and sheltered from light. For the PGSO obtaining, Ribeiro *et al*. (2016) was followed.

For the FA composition by GC-FID, fatty acid methyl esters (FAMEs) were prior prepared according to Hartman & Lago (1973) and modified by Maia & Rodriguez-Amaya (1993). A Thermo Scientific Trace Ultra 3300 gas chromatograph (GC), equipped with flame ionization detector (FID) and split/splitless injector, was employed. Separations were made on a fused silica capillary column CP-7420 (Select FAME, 100 m x 0.25 mm i.d. x 0.25 µm

cyanopropyl film thickness). The H_2, as carrier gas, with flow rate of 1.2 mL min^{-1}, and the N_2, as make-up gas, with flow rate of 30 mL min^{-1} were employed. The flow rates of 30 and 300 mL min^{-1} for H_2 and synthetic air, respectively, were employed to compose the detector flame. The injector and the detector temperatures were maintained at 230 and 250 ºC, respectively. The initial oven temperature of 165 ºC was maintained for 18 min, raised to 235 ºC at a rate of 4 ºC min^{-1}, and it was continued for 20 min. Samples were injected in split mode, with 1:40 ratio and injection volume of 1.0 µL. FAMEs were identified by comparing the peaks retention time present in the samples with those present in the standard mixture of 37 FAMEs (C4:0-C24:0, Sigma–Aldrich, USA). The results were expressed as relative percentage of total FAs for each peak identified, automatically determined by Chromquest™ 5.0 software.

For the TAG lipid profile by direct infusion ESI(+)-MS, samples were prepared according to Youzbachi *et al.* (2015) and modified by Silveira *et al*. (2017). TAG profile was obtained by direct infusion of the final solution into a Xevo TQD™ triple quadrupole MS (Waters, USA), equipped with Z spray™ ESI source operating in positive mode (ESI(+)-MS), with continuous flow rate of 50.0 µL min^{-1}, comprising the mass/charge (*m/z*) range of 100–1200. Desolvation temperature was 250 ºC. Capillary and cone voltage were 3.00 kV and 35.0 V, respectively. Mass spectrometry/mass spectrometry (MS/MS) analysis was set at collision energy of 19 V, in order to fragment the main TAGs identified of the GSO, RSO and PGSO. Data were processed using MassLynx™ software.

All analyzes were performed in triplicate and the results were expressed as mean values ± standard deviation (SD). Besides, the results of FA composition were submitted to analysis of variance (ANOVA) test followed by Tukey's test with 95% significance level. Data were processed using PAST3 software (Silva & Azevedo, 2016).

RESULTS AND DISCUSSION. In the FA composition of the GSO samples fourteen FAs were identified. Linoleic acid (L, 41.08 – 61.57%), an omega-6 FA, predominated, followed by oleic (O, 21.42 – 37.42%), palmitic (P, 16:0; 5.81 – 11.38%) and stearic (S, 18:0; 3.03 – 4.00%) acids. Due to the high content of omega-6, resveratrol, vitamin E and phenolic, GSO is established as an important antioxidant and antimicrobial activity, and anti-aging effects (Lin *et al*., 2017; Michalaka & Kiełtyka-Dadasiewicz, 2018). According to the Codex Standard for Named Vegetable Oils (*Codex Alimentarius*, 1999), the content of L acid in GSO varies between 58.0 and 78.0%; the content of O acid varies between 12.0 and 28.0%; the content of P acid varies between 5.5 and 11.0%; and the content of S acid varies between 3.0 and 6.5%. The maximum content allowed by the Codex Standard for linolenic acid (Ln, 18:3n-3) is 1%. In this study, only the PGSO sample presented all FAs according to *Codex Alimentarius*. The highest L acid content was found in PGSO (61.57%) followed by U9 (54.35%) > U3 (53.95%) > U5 (53.46%) > U10 (53.42%) > U7 (52.67%) > U6 (52.35%) > U1 (50.65%) > U8 (50.40%) > U4 (48.14%) > U2 (41.08%). Results obtained in this study indicated that the L acid content was outside of the range established by the *Codex Alimentarius* for samples U1, U2, U3, U4, U5, U6, U7, U8, U9 and U10. Samples U2, U4 and U9 exhibited O acid content above the allowed by *Codex Alimentarius*. Ln acid content in all samples varied between 0.13 and 6.33%. The highest Ln acid content was found in the U3 (6.33%) sample followed by U10 (6.62%) > U2 (5.98%) > U7 (5.90%) > U1 (5.66%) > U6 (5.60%) > U8 (5.46%) > U4 (5.31%) > U5 (5.13%) > U9 (0.26%) > PGSO (0.13%). Therefore, samples U1, U2, U3, U4, U5, U6, U7, U8, and U10 revealed Ln acid content above the allowed by *Codex Alimentarius*. Usually, Ln acid contents in GSOs are lower than in RSOs. Furthermore, low Ln acid levels are desired in GSOs for better oxidative stability, which is important for health protection and economic reasons (Göktürk Baydar *et al*., 2007). In this study, it can be observed that the Ln acid composition of samples U1, U2, U3, U4, U5, U6, U7, U8, and U10 were similar to the Ln acid composition of RSOs samples, indicating

possible adulteration. Besides, the FA composition results obtained by PGSO sample are close to data reported by other authors. For the other samples (U1, U2, U3, U4, U5, U6, U7, U8, U9 and U10), the results are distinct to data reported by other authors (Beveridge *et al.*, 2005; Crew *et al*., 2006; Lachman *et al*., 2015; Shinagawa *et al.*, 2017).

In the TAG lipid profile, is well-stablished that vegetable oils have a characteristic TAG profile, and adulterations result in significant modifications on these profiles (Silveira *et al.*, 2017; Pizzo *et al.*, 2018). Therefore, it is possible to observe in the lipid profiles that the PGSO sample presented higher intensities of the TAG LLL (896 *m/z*), followed by LLO (898 *m/z*) and LLP (872 *m/z*). These results are agreeing with those found in the literature. Other TAGs, as LLLn (894 *m/z*), LnLP (870 *m/z*), OOL (900 *m/z*), PLO (874 *m/z*), PLP (848 *m/z*), OOO (902 *m/z*), POO (876 *m/z*), POP (850 *m/z*) and SOO (904 *m/z*), varied from 0.44 to 7.23% (Jakab *et al*. 2002).

According to the supplementary information (S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10) the samples U1, U3, U5, U6, U7, U8 and U10 presented higher intensities of TAG LLL followed by LLO and OOL, while for sample U9, it was found that the major component was LLO followed by OOL and LLL. The U4 sample presented the TAG sequence: LLO, LLL, and OOL, while the U2 sample presented as the major component the LLL followed by LLO and OOO. However, for the lipid profiles (Figures S1-S10), it is evident that the samples U1, U3, U5, U6, U7, U8 and U10 are similar to each other, although distinct from the PGSO sample. Furthermore, samples U2, U4 and U9 are distinct from all other samples and from each other. It could be observed in the region 898 - 904 *m/z*, 896 – 904 *m/z* and 800 - 900*m/z*, respectively. PGSO was distinct from all other samples (848 - 950 *m/z*). All GSOs samples presented higher intensities in the region 910 - 1000 *m/z*, being different from the PGSO sample, which present lower intensities in this region. These differences are in accordance with the results obtained in the FA analyzes by GC-FID. Therefore, it is observed that the TAG profile of samples U1, U3, U5, U6, U7, U8 and U10 are similar to RSO and, consequently, adulterated with RSO, while U2, U4 and U9 are adulterated with another vegetable oil, which modify its TAG profile. It is observed that samples U1, U3, U5, U6, U7, U8 and U10 present relative percentage similar to RSO, which is in accordance with the TAG profile and the FA composition analysis. Samples U2, U4 and U9 are distinct from all other samples, which confirms the GC-FID analysis results.

CONCLUSIONS. The FA composition by GC-FID and the TAG profile by direct infusion ESI(+)-MS provide a valuable assessment of the grape seed oils lipid composition. In this study, ten samples of GSO, RSO and PGSO were analyzed and an adulteration was verified by the addition of RSO in seven GSO samples, and by the addition of another vegetable oil in three grape seed oils. The results obtained in this study demonstrate the quality control importance of GSO, which are susceptible to adulteration and, therefore, have reduced benefits to human consumption, since it is a high cost product. In addition, ESI(+)-MS analysis revealed that it is a very attractive candidate in rapid and routine analyzes in industries to verify the quality of grape seed oil in industries.

Keywords: TAG profile; FA composition; lipid profile; lipid quality; mass spectrometry.

RESUMO GERAL

INTRODUÇÃO. Óleos vegetais são misturas complexas, compostas principalmente por triacilgliceróis (TAG), ácidos graxos (AG) e componentes menores (Indelicato *et al*., 2017). Entre os AGs, encontram-se: AGs saturados (AGS), monoinsaturados (AGMI) e poliinsaturados (AGPI). Considerando os AGPIs, alguns não podem ser sintetizados pelo corpo humano e são considerados essenciais, é o caso dos ácidos linoléico (18:2) e α-linolênico (18:3n-3), respectivamente considerados AGs das famílias AGPI n-3 e n-6. Estes possuem propriedades antiaterogênicas e antitrombóticas e afetam a concentração de lipoproteínas, a fluidez e função enzimática da membrana, e a modulação de outros compostos (Yang *et al*., 2018).

Óleos vegetais representam mais de 75% do total de lipídios consumidos no mundo, e são constantemente aplicados no processo de fabricação como ingrediente para diversos alimentos e produtos (Garavaglia *et al*., 2016), gerando subprodutos indesejáveis. A uva (*Vitis vinifera*); uma das frutas mais consumidas mundialmente, principalmente como suco (Martin *et al*., 2020), produz quantidade expressiva de bagaço de uva. O bagaço seco inclui aproximadamente 20-26% de sementes (Yalcin *et al*., 2016), rica em fitoquímicos, compostos bioativos e AGs insaturados (Crew *et al*. 2006; Villani *et al*. 2015; Shinagawa *et al*. 2017; Ma & Zhang, 2017).

O óleo de semente de uva apresenta como principal característica elevado teor de AGs insaturados, principalmente ácidos 18:2 e oleico (18:1) (Yalcin *et al*., 2016). Além disso, também contém alto teor de vitamina E; importante para a saúde humana (Al Juhaimi *et al*., 2017). Os AGs insaturados tornam este óleo popular para as indústrias culinária, farmacêutica, cosmética e médica (Yalcin *et al*., 2016). Devido às suas propriedades benéficas para o consumo e por ser rico em AGMIs e AGPIs, o óleo de semente de uva auxilia no depósito de energia do corpo, com alta capacidade antioxidante, sendo considerado um produto de alto valor agregado (Oikonomou *et al*. 2018). Sendo amplamente utilizado na pele humana, auxiliando na cicatrização, suavização, além de ter efeitos calmantes, antibacterianos, antioxidantes e normalizadores (Michalaka & Kiełtyka-Dadasiewicz, 2018).

OBJETIVOS. Considerando o crescente mercado de produtos contendo óleo de semente de uva (Martin *et al*., 2020), esta pesquisa teve como foco avaliar a qualidade do mesmo, uma vez que a substituição de óleos vegetais com preços mais elevados por óleos de menor valor é alarmante no Brasil (Silveira *et al*. 2017; Galuch *et al*. 2018; Pizzo *et al*. 2018). Isto foi alcançado através de infusão direta por espectrometria de massa de ionização por eletrospray (ESI-MS) e cromatografia gasosa com detecção de ionização de chama (CG-DIC), ambas técnicas podem facilmente determinar adulteração em óleo vegetal devido à diferenciação do perfil de TAG e caracterização da composição de AG, respectivamente (Silveira *et al*. 2017; Galuch *et al*. 2018; Pizzo *et al*. 2018).

MATERIAL E METODOS. Dez amostras de óleos de semente de uva (OSU) foram adquiridas no mercado local de Maringá - PR, Brasil. Para a obtenção dos óleos puros de semente de uva (OSUP), foi adquirido um cacho de uvas no mercado local Maringá - PR, Brasil, as sementes foram separadas da fruta, colocadas em um recipiente e lavadas com água corrente. Três lotes contendo amostras de óleos refinados de soja (ORS) foram obtidos no mercado local de Maringá - PR, Brasil. Todas as amostras de ORS e OSU foram preservadas em seu recipiente original sob refrigeração (6 a 10 °C) e ao abrigo da luz. Para a obtenção do OSUP, Ribeiro *et al*. (2016) foi seguido.

Para a composição de AG por CG-DIC, os ésteres metílicos de ácidos graxos (EMAGs) foram previamente preparados de acordo com Hartman & Lago (1973) e modificados por Maia & Rodriguez-Amaya (1993). Foi utilizado cromatógrafo gasoso (CG) Thermo Scientific Trace Ultra 3300, equipado com detector de ionização de chama (DIC) e

injetor split/splitless. As separações foram feitas em coluna capilar de sílica fundida CP-7420 (Select FAME, 100 m x 0,25 mm d.i. x 0,25 µm de espessura de filme de cianopropil). Foram utilizados: H_2, como gás carreador, com vazão de 1,2 mL min^{-1}, e N_2, como gás de reposição, com vazão de 30 mL min^{-1}. As vazões de 30 e 300 mL min^{-1} para H_2 e ar sintético, respectivamente, foram empregadas para compor a chama do detector. As temperaturas do injetor e do detector foram mantidas em 230 e 250 ºC, respectivamente. A temperatura inicial do forno de 165 ºC foi mantida por 18 min, elevada para 235 ºC a uma taxa de 4 ºC min^{-1} e continuada por 20 min. As amostras foram injetadas em modo *split*, com proporção de 1:40 e volume de injeção de 1,0 µL. Os EMAGs foram identificados pela comparação do tempo de retenção dos picos presentes nas amostras com picos presentes na mistura padrão de 37 FAMEs (C4:0-C24:0, Sigma – Aldrich, EUA). Os resultados foram expressos como porcentagem relativa do total de AGs para cada pico identificado, determinado automaticamente pelo software Chromquest™ 5.0.

Para o perfil lipídico TAG por infusão direta ESI(+)-MS, as amostras foram preparadas de acordo com Youzbachi *et al*. (2015) e modificado por Silveira *et al*. (2017). O perfil de TAG foi obtido por infusão direta da solução final em um Xevo TQD™ triplo quadrupolo MS (Waters, EUA), equipado com fonte Z spray™ ESI operando em modo positivo (ESI(+)-MS), com vazão contínua de 50,0 µL min^{-1}, compreendendo a faixa de massa/carga (*m/z*) de 100-1200. A temperatura de dessolvatação foi 250 ºC. As tensões capilar e cônica foram 3,00 kV e 35,0 V, respectivamente. A análise por espectrometria de massa/espectrometria de massa (MS/MS) foi fixada em energia de colisão de 19 V, a fim de fragmentar os principais TAGs identificados do OSU, ORS e OSUP. Os dados foram processados usando o software MassLynx™.

Todas as análises foram realizadas em triplicata e os resultados expressos como valores médios ± desvio padrão (DP). Além disso, os resultados da composição do AG foram submetidos ao teste de análise de variância (ANOVA) seguido do teste de Tukey com nível de significância de 95%. Os dados foram processados no software PAST3 (Silva & Azevedo, 2016).

RESULTADOS E DISCUSSÃO. Na composição de AG das amostras de OSU, 14 AGs foram identificados. Ácido linoléico (L, 41,08 - 61,57%), um AG ômega-6, predominou, seguido pelos ácidos oleico (O, 21,42 - 37,42%), palmítico (P, 16:0; 5,81 - 11,38%) e esteárico (S, 18:0; 3,03 - 4,00%). OSU foi reconhecido com importante atividade antioxidante e antimicrobiana e efeitos anti-envelhecimento (Lin *et al.*, 2017; Michalaka & Kiełtyka-Dadasiewicz, 2018). De acordo com o *Codex Standard for Named Vegetable Oils* (*Codex Alimentarius*, 1999), o conteúdo de ácido L no OSU varia entre 58,0 e 78,0%; o teor de ~~ácido~~ O varia entre 12,0 e 28,0%; o conteúdo de ácido P varia entre 5,5 e 11,0%; e o teor de ácido S varia entre 3,0 e 6,5%. O conteúdo máximo permitido pelo *Codex Standard* para ácido linolênico (Ln, 18:3n-3) é 1%. Neste estudo, apenas a amostra de OSUP apresentou todos os AGs de acordo com o *Codex Alimentarius*. O maior teor de ácido L foi encontrado em OSUP (61,57%) seguido por U9 (54,35%) > U3 (53,95%) > U5 (53,46%) > U10 (53,42%) > U7 (52,67%) > U6 (52,35%) > U1 (50,65%) > U8 (50,40%) > U4 (48,14%) > U2 (41,08%). Os resultados obtidos neste estudo indicaram que o teor de ácido L estava fora da faixa estabelecida pelo *Codex Alimentarius* para as amostras U1, U2, U3, U4, U5, U6, U7, U8, U9 e U10. As amostras U2, U4 e U9 exibiram teor de ácido O acima do permitido pelo *Codex Alimentarius*. O conteúdo de ácido Ln em todas as amostras variou entre 0,13 e 6,33%. O maior teor de ácido Ln foi encontrado na amostra U3 (6,33%), seguido por U10 (6,62%) > U2 (5,98%) > U7 (5,90%) > U1 (5,66%) > U6 (5,60%) > U8 (5,46 %) > U4 (5,31%) > U5 (5,13%) > U9 (0,26%) > OSUP (0,13%). Portanto, as amostras U1, U2, U3, U4, U5, U6, U7, U8 e U10 revelaram teor de

ácido Ln acima do permitido pelo *Codex Alimentarius*. Normalmente, os conteúdos de ácido Ln em OSUs são mais baixos do que em ORSs. Além disso, níveis baixos de ácido Ln são desejados em OSUs para melhor estabilidade oxidativa, o que é importante para a proteção da saúde e por razões econômicas (Göktürk Baydar *et al*., 2007). Neste estudo, pode-se observar que a composição do ácido Ln das amostras U1, U2, U3, U4, U5, U6, U7, U8 e U10 foram semelhantes à das amostras ORSs, indicando possível adulteração.

No perfil lipídico de TAG, está bem estabelecido que os óleos vegetais têm um perfil característico, e as adulterações resultam em modificações significativas nesses perfis (Silveira *et al*., 2017; Pizzo *et al*., 2018). Portanto, é possível observar nos perfis lipídicos que a amostra de OSUP apresentou maiores intensidades do TAG LLL (896 *m/z*), seguida do LLO (898 *m/z*) e LLP (872 *m/z*). Esses resultados estão de acordo com os encontrados na literatura. Outros TAGs, como LLLn (894 *m/z*), LnLP (870 *m/z*), OOL (900 *m/z*), PLO (874 *m/z*), PLP (848 *m/z*), OOO (902 *m/z*), POO (876 *m/z*), POP (850 *m/z*) e SOO (904 *m/z*), variou de 0,44 a 7,23% (Jakab *et al*. 2002).

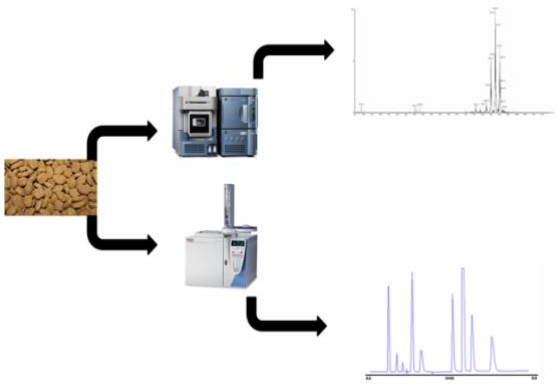
De acordo com as informações suplementares (S1, S2, S3, S4, S5, S6, S7, S8, S9 e S10), as amostras U1, U3, U5, U6, U7, U8 e U10 apresentaram maiores intensidades de TAG LLL seguidas de LLO e OOL, enquanto para a amostra U9, verificou-se que o componente majoritário foi LLO seguido de OOL e LLL. A amostra U4 apresentou a sequência TAG: LLO, LLL e OOL, enquanto a amostra U2 apresentou como componente principal o LLL seguido por LLO e OOO. Já para os perfis lipídicos (Figuras S1-S10), é evidente que as amostras U1, U3, U5, U6, U7, U8 e U10 são semelhantes entre si, embora distintas da amostra OSUP. Além disso, as amostras U2, U4 e U9 são distintas de todas as outras amostras e umas das outras. Pode ser observado na região 898 - 904 *m/z*, 896 - 904 *m/z* e 800 - 900 *m/z*, respectivamente. OSUP foi distinto de todas as outras amostras (848 - 950 *m/z*). Todas as amostras de OSUs apresentaram intensidades maiores na região 910 - 1000 *m/z*, sendo diferente da amostra de OSUP, que apresenta intensidades mais baixas nesta região. Essas diferenças estão de acordo com os resultados obtidos nas análises de AG por CG-DIC. Portanto, observa-se que o perfil de TAG das amostras U1, U3, U5, U6, U7, U8 e U10 são semelhantes ao ORS e, consequentemente, adulterados com ORS, enquanto U2, U4 e U9 são adulterados com outro óleo vegetal. Observa-se que as amostras U1, U3, U5, U6, U7, U8 e U10 apresentam percentuais relativos semelhantes ao ORS, o que está de acordo com o perfil de TAG e a análise de composição de AG. As amostras U2, U4 e U9 são distintas de todas as outras amostras, o que confirma os resultados da análise CG-DIC.

CONCLUSÕES. A composição de AG por CG-DIC e o perfil de TAG por infusão direta ESI(+)-MS fornecem valiosa avaliação da composição lipídica dos óleos de semente de uva. Neste estudo, foram analisadas dez amostras de OSU, ORS e OSUP e verificada adulteração pela adição de ORS em sete amostras de OSU e pela adição de outro óleo vegetal em três óleos de semente de uva. Os resultados obtidos neste estudo demonstram a importância do controle de qualidade dos OSU, que são suscetíveis à adulteração e, portanto, apresentam benefícios reduzidos para o consumo humano. Além disso, a análise ESI(+)-MS revelou ser um candidato muito atraente em análises rápidas e de rotina em indústrias para verificar a qualidade do óleo de semente de uva.

Palavras chaves: Perfil TAG; Composição de AG; perfil lipídico; qualidade lipídica; espectrometria de massa.

Graphical Abstract (GA)

GA Figure:



GA Text: Regarding the adulteration increase in numerous products, it is essential to perform an assessment to verify the guarantee levels declared by the manufacturers in the packaging of its products using analytical techniques.

Evaluation of dog food authenticity through lipid profile using GC-FID and ESI-MS

Roberta da Silveira[a], Patrícia Daniele Silva dos Santos[b], Jessica Santos Pizzo[b], Nayane Mattos[b], Matheus Campos de Castro[b], Oscar Oliveira Santos[b*], Jesuí Vergilio Visentainer[a]

[a]Departamento de Ciência de Alimentos, Universidade Estadual de Maringá (UEM), Maringá-PR, Brasil.

[b]Departamento de Química, Universidade Estadual de Maringá (UEM), Maringá-PR, Brasil.

*dasilveira.roberta@gmail.com
ORCID ID: 0000-0002-9631-8480

Abstract

Considering the increasing number of low cost vegetable oil product adulterations, it is necessary to assess the authenticity of the lipid sources used in the dog food manufacture, consequently different brands and classifications of feed were analyzed to verify the product authenticity by gas chromatography with flame ionization detector (GC-FID) and by electrospray ionization mass spectrometry (ESI-MS). Fatty acid (FA) composition analysis was performed to compare the FAs amount in samples with the respective guarantee levels stated by the manufacturers on its product label. The omega 3 and 6 percentages GC-FID results are within the limits presented by each manufacturer, while the timnodonic + cervonic acids amount information presented on feed sample labels is not in accordance with GC-FID results. Consequently, the samples were analyzed by ESI-MS, the results exposed the chicken oil presence in the feed and eicosapentaenoic (EPA) + docosahexaenoic (DHA) absence, since no lipid profiles similar to fish oils or triacylglycerols (TAGs) containing these two FAs were found. Thus, the information presented on labels are in disagreement with the results obtained both by GC-FID FA composition analysis and by ESI-MS lipid profile analysis. Principal component analysis (PCA) confirmed that the main contribution was from FAs found by GC-FID.

Keywords: dog food, fatty acid composition, product quality, GC-FID, ESI-MS, lipid profile.

1. Introduction

The pet food industry is continuously developing and seeking new ingredients for greater animal welfare and health. The feed must be balanced in order to meet all the animal nutritional requirements,[1] as well as it should contain sources of protein, fiber, carbohydrates, fats and minerals.[2-3] Dog food is classified according to the ingredients quality and its cost, being divided in *standard*, *premium* and *super-premium*. The *standard* has low-quality and low-cost ingredients; *premium* has intermediary ingredients and cost; and *super-premium* are formulated with high-quality and high-cost ingredients.[4] Generally, in feed composition are present flours of meat, bone and chicken viscera, in addition to phosphates, cereal flours and bran, and food additives such as acidulants, antioxidants and flavorings.[5] The lipid sources used in feed are usually chicken fat, bovine tallow, swine lard, fish oil and vegetable oils.[6]

Aiming the animal health, the nutritional supplementation with omega-3 (n-3) and omega-6 (n-6) is widely used. Studies suggest that the incorporation of essential fatty acids, such as linoleic (18: 2n-6) and α-linolenic acids (18: 3n-3), is related to lower rate of behavioral changes, as well as to increase learning capacity and visual acuity in pups.[7] In addition, the use of n-3 and n-6 enriched dog food is also considered a secure option to assist in the treatment of canine atopic dermatitis.[8]

Eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) FAs are part of the n-3 FAs family, which is important in animal health. EPA is involved in the eicosanoids synthesis, particularly prostaglandins, leukotrienes, and thromboxanes, competing with arachidonic acid (20:4n-6, AA) for cyclooxygenase and 5-lipoxygenase enzymes, leading to increased production of anti-inflammatory eicosanoids, rather than pro-inflammatory eicosanoids derived from AA.[9] DHA is essential for the neurological system development and it is present in the retinal membrane. Furthermore, EPA and DHA have beneficial effects

on the immune and inflammatory systems, assist in the protection of cardiac and renal functions (anti-inflammatory and antihypertensive actions) and stimulate learning ability.[10-11-12]

Observing the crescent adulterated products number with the addition of low cost vegetable oils, it is crucial to evaluate the authenticity of the lipid source used in the manufacture of dog food. Therefore, diverse feed brands and classifications were analyzed in order to verify fraud existence regarding the FAs composition, mainly EPA and DHA. Consequently, fatty acid composition and lipid profile of each feed were obtained by gas chromatographic techniques with flame ionization detector and mass spectrometry with electrospray ionization, respectively.

2. Experimental

A fatty acid methyl esters standard mixture (FAMEs 189-19) and methyl tricosanoate (23:0me) were purchased from Millipore Sigma (St. Louis, MO, USA). The other reagents; chloroform, methanol, n-heptane, sulfuric acid, and hydrochloric acid, were also purchased from Millipore Sigma (Darmstadt, Germany) and used without further purification.[13]

Dog feed considered n-3 and n-6 sources (composition on the label) from different classifications and brands available in the Brazilian market were purchased at Pet Shops in the city of Maringá, Paraná - Brazil (23°25'31"S51°56.18°C). Table 1 shows the classification and minimum levels of n-3, n-6 and EPA+DHA presented on the label.

Table 1 - Classification and levels of omega 6, omega 3 and EPA+DHA presented on the label.

Samples	Classification	Omega 6	Omega 3	EPA+DHA
1	Premium	20 g/kg (2%)	1500 mg/kg (0.15%)	600 mg/kg (0.06%)
2	Premium	20 g/kg (2%)	3000 mg/kg (0.3%)	420 mg/kg (0.042%)
3	Premium	20 g/kg (2%)	3000 mg/kg (0.3%)	ND
4	Premium	20 g/kg (2%)	3000 mg/kg (0.3%)	420 mg/kg (0.042%)
5	Premium	25 g/kg (2.5%)	2500 mg/kg (0.25%)	ND
6	Premium	24 g/kg (2%)	3000 mg/kg (0.3%)	ND
7	Super premium	20 g/kg (2%)	3000 mg/kg (0.3%)	700 mg/kg (0.07%)
8	Standard	12 g/kg (1.2%)	2400 mg/kg (0.24%)	ND
9	Super premium	15 g/kg (2%)	ND	3100 mg/kg (0.31%)
10	Standard	10g/kg (1.0%)	1800 mg/kg (0.18%)	ND

ND: not available

Comparar com o mesmo mg/kg

Quantificar a fraude

Passar a injeção na coluna e selecionar alguns lipídios pra quantificar

2.1 Fatty acid composition by GC-FID

Figueiredo's (2016)[13] direct methylation method was employed in order to extract and prepare the fatty acid methyl esters (FAMEs). 100.0 mg of sample was weighed in a 10 cm test tube, 2.0 mL of NaOH (1.5 mol L^{-1} in methanol) was added. The sample was macerated with a glass rod in order to form a thin film and increase the contact surface. Then, the test tubes were placed in ultrasound bath for 8 min. After the alkaline reaction was over, 2.0 mL of H_2SO_4 or HCl (1.5 mol L^{-1} in methanol) was added, and the test tube was again placed in the ultrasound bath for 8 min. Then, 1.0 mL of n-heptane was added, the tubes were shaken for 30 s and then centrifuged at 2000 rpm for 1.00 min. After that, 500 µL of internal standard (23:0me) was added, and the upper phase was collected and injected into the GC. The procedure was performed in an ultrasonic bath model Eco-Sonics Q 5.9/25 (Unique, São Paulo, Brazil) with 165 W of power and 25 kHz. FAMEs were separated on a Thermo gas chromatograph, trace ultra 3300 model, equipped with flame ionization detector (FID), with automatic injector and fused silica capillary column CP-7420 (Select FAME, 100 m long, 0.25 mm internal diameter and 0.25 µm cyanopropyl). Gas flow as follows: 1.2 mL $min^{-1}$of H_2, 30 mL min-1 of N_2, 35 and 300 mL min^{-1} of H_2 and synthetic air, respectively, to the detector flame. The injected volume was 1.0 µL, using a sample split of 40:1, with injector and detector temperatures being 250 and 230 °C, respectively. Heating ramp was applied in the column, initiating the temperature at 165 °C for 18 min and raised to 235 °C with heating rate of 4 °C/min, remaining for 20 min.[3] FAMEs were identified by comparing their retention

times with standards (FAME Mix, C4-C24, Sigma-Aldrich) and the results were expressed as mg g^{-1} of total lipids, determined automatically by integration of peak areas through Chromquest™ 5.0 software.

2.2 Lipid profile by direct infusion with ESI(+)-MS

The feed lipid fraction was extracted according to the methodology described by Figueiredo (2016).[13] Then, 50 μL of the extracted oil was diluted in chloroform (950 μL). 1.0 mL of methanol/chloroform (9:1, v/v) was added in 5.0 μL of this solution and then 20 μL of ammonium formate (0.10 mol L^{-1} in methanol) were added.[14]

The final solution was infused directly into a triple-quadrupole Xevo-TQD mass spectrometer equipped with electrospray Z spray™ ionization source (Waters, Milford, MA, USA). The lipid profiles were obtained in the ratio range of 100 to 1200 *m/z* and extracted in positive mode (ESI(+)). ESI(+) source parameters were as follows: source temperature of 150 °C, desolvation temperature of 200 °C, capillary voltage of 3.0 kV and cone voltage of 20.0 V. High purity nitrogen was produced by nitrogen generator (NM32LA, Peak Scientific®, Renfrewshire, Scotland) and it was used as desolvation gas with flow rate of 500 L h^{-1}. The sample solutions were injected with a continuous flow of 10.0 μL min^{-1}. Data were processed using MassLynx™ software.

2.3 Statistical analysis

The results obtained from the FA composition analysis by GC-FID were submitted to analysis of variance (ANOVA), the means were compared using the Tukey test, with significance level of 95%, and the results obtained by ESI(+)-MS were analyzed by principal component analysis, through the RStudio software.[15]

3. Results and Discussion

3.1 Fatty acid composition by GC-FID

Feed manufacturers must present the guarantee levels on its products labels.[16] These levels establish the product nutritional quality offered to the consumer, demonstrating the quality standard that is dependent on the adequate quality control during and after the production process, plus the raw material used. Among the information presented in the guarantee levels are: FAs levels such as n-3, n-6, EPA and DHA.

FA composition analysis was carried out with the objective of comparing the FAs amount of the samples with the respective guarantee levels declared by the manufacturers. The results are presented in Table 2.

Table 2: Fatty acid composition of pet food samples (1-10) obtained by GC-FID

Fatty acid	Pet food samples (%)									
	1	2	3	4	5	6	7	8	9	10
14:00	0.77±0.010^{bc}	0.93±0.02^{bcd}	0.79±0.20^{ab}	0.83±0.10^{de}	0.82±0.05^{a}	0.61±0.010^{ab}	0.78±0.050^{bc}	0.91±0.15^{e}	0.76±0.020^{ce}	1.23±0.030^{bcd}
16:00	22.40±0.70^{bc}	22.88±0.54^{bc}	21.93±0.55^{ab}	24.81±0.90^{f}	21.90±0.70^{a}	22.60±0.65^{c}	24.41±0.50^{d}	24.87±0.45^{d}	21.78±0.20^{e}	22.41±0.55^{a}
16:1n-7	3.12±0.10^{abc}	2.99±0.10^{abc}	2.81±0.10^{ab}	3.70±0.55^{e}	3.18±0.10^{ab}	4.25±0.07^{bd}	3.89±0.10^{cd}	3.92±0.60^{ad}	3.29±0.10^{de}	2.21±0.050^{a}
18:00	7.39±0.32^{b}	8.30±0.17^{bd}	7.43±0.20^{ab}	5.83±0.50^{d}	6.840±0.20^{a}	6.88±0.20^{bc}	6.99±0.10^{bc}	8.14±0.10^{cd}	7.71±0.40^{e}	9.85±0.20^{b}
18:1n-9	35.11±0.82^{c}	34.63±0.65^{c}	35.40±0.75^{bc}	38.80±0.54^{f}	39.48±0.80^{b}	35.78±0.90^{d}	36.51±0.65^{d}	35.50±0.70^{d}	33.78±0.75^{e}	31.98±0.30^{a}
18:1n-7	1.38±0.030^{bd}	1.39±0.003^{bc}	1.37±0.05^{ab}	1.31±0.15^{e}	1.71±0.10^{bc}	1.78±0.010^{d}	1.44±0.020^{cd}	1.26±0.050^{bc}	1.79±0.10^{f}	1.28±0.050^{a}
18:2n-6	28.31±0.82^{c}	27.28±0.56^{bc}	28.31±0.65^{b}	23.62±0.75^{d}	24.33±0.90^{a}	25.45±0.75^{bc}	24.65±0.65^{bc}	24.23±0.50^{bc}	28.89±0.85^{e}	28.96±0.55^{a}
18:3n-3	1.55±0.030^{ab}	1.58±0.070^{a}	1.96±0.050^{a}	1.10±0.20^{b}	1.71±0.15^{a}	2.65±0.05^{c}	1.33±0.060^{ab}	1.17±0.15^{a}	2.00±0.05^{c}	2.08±0.10^{a}
AGS	30.53±1.05^{d}	32.11±0.73^{e}	30.15±0.90^{c}	31.47±1.45^{h}	29.59±0.95^{a}	30.09±0.85^{e}	32.18±0.65^{f}	33.92±0.65^{g}	30.25±0.60^{i}	33.49±0.80^{b}
AGMI	39.61±0.95^{e}	39.01±0.74^{d}	39.58±0.90^{c}	43.81±1.30^{j}	44.37±0.95^{b}	41.48±0.95^{f}	41.84±0.80^{h}	40.68±1.35^{g}	38.86±0.910^{i}	35.47±0.40^{a}
AGPI	29.86±0.85^{g}	28.86±0.65^{cd}	30.27±0.70^{c}	24.72±0.90^{h}	26.04±1.05^{a}	28.43±0.80^{f}	25.98±0.70^{e}	25.40±0.65^{de}	30.89±0.90^{i}	31.04±0.65^{b}

Average of three repetitions with their coefficients of variation (%). Values accompanied by different letters in the same line indicate significant difference ($p < 0.05$) by Tukey test. AGS = Saturated Fatty Acid, AGMI = Monounsaturated Acid, PUFA = Polyunsaturated Fatty Acid

As shown in Table 2, oleic acid (18:1n-9) was the major FA in all samples analyzed, followed by linoleic (18:2n-6) and palmitic acid (16:0). Myristic acid (14:0) was found in the range of 0.19 to 0.51%, 16:0 acid in the range of 0.61 to 1.23%, stearic acid (18:0) was found in the range of 5.83 to 9.85%, 18:1n-9 acid was found in the range of 31.98 to 39.48%, 18:2n-6 acid was found in the range of 23.62 to 28.96 % and α-linolenic acid (18:3n-3) had a concentration of 1.10 to 2.65%. Furthermore, it was not possible to identify EPA and DHA FAs in any of the samples analyzed.

Among the polyunsaturated (PUFA) found, 18:2n-6 and 18:3n-3 are essential FAs not synthesized by the body, being considered extremely important in animal feed, once it is required to consume it exclusively through diet. Plus, these FAs are precursors of n-3 and n-6 and its ingestion is essential to combat health problems, such as inflammations, behavioral changes, and it acts in cancer prevention.[17]

In all samples analyzed, there is the addition of chicken oil. This information is presented in the labels of each sample and was confirmed with FA composition analysis by GC-FID comparing the feed samples and the chicken oil analyzes[13] becoming clear that all analyzed samples have the same FA composition, predominating the FAs 18:1n-9, 18:2n-6 and 16:0.

Besides the chicken oil, on the labels of samples 1, 2, 4, 7 and 9, there is the information that fish oil is added in its composition, these being the main sources of EPA and DHA.[18-12] However, the information on the amount of EPA+DHA, presented on the feed sample labels (Table 1), does not agree with the analysis results (Table 2). But, the results obtained by GC-FID on the percentages of omega 3 and omega 6 are within the limits presented by each manufacturer.

The extrusion applied in the processing of dry feed utilizes high temperatures, humidity and pressure, and assists to sterilize the food, reducing anti-nutritional factors, and

improving digestibility. Nevertheless, the conditions modify physically and chemically numerous nutrients and the final product quality,[19] causing, for example, lipid oxidation.[20]

In the study carried out by Ribeiro (2018), which verified the oxidative changes occurring in extruded foods for cats containing poultry fat as lipid source, was observed the reduction of fatty acid during the extrusion process step of processed foods.

Thus, in this study, through the FA composition analysis by GC-FID, it can be seen that there are two possible conclusions: a) the manufacturer did not add DHA and EPA FAs in the analyzed feed or, b) the extraction process decreased the amount of DHA and EPA FAs at values lower than the guarantee levels displayed on feed labels. However, these guarantee levels should demonstrate the minimum amount of ingredients, such as DHA and EPA, present in the final product offered to the consumer.

3.2 Lipid profile by direct infusion with ESI(+)-MS

Oils and fats have an unique lipid profile.[21] In order to verify the lipid profile of the oils extracted from the samples, ESI(+)-MS direct infusion technique was employed, once this technique has been used to characterize oils and fats rapidly and with little sample preparation, as well as it allows the observation of a characteristic pattern of the samples.[22-25]

Figure 1 shows the lipid profiles of all feed samples (1 to 10) obtained by ESI(+)-MS, in the ratio *m/z* 100-1200.

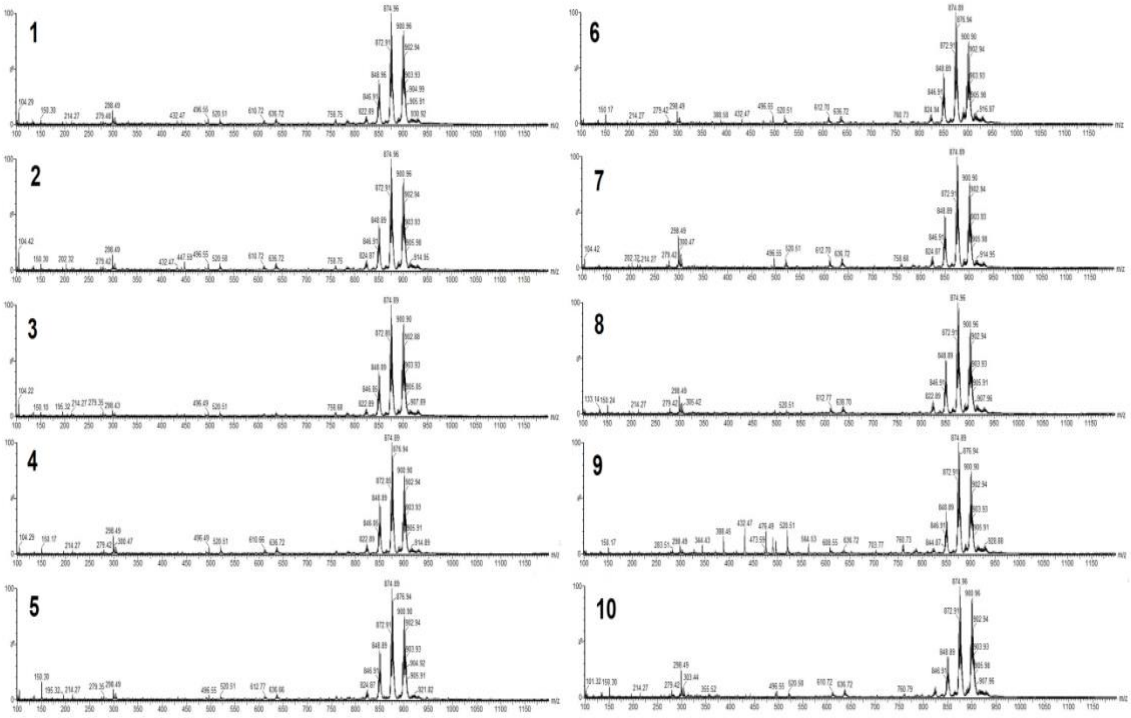


Figure 1 – Lipid profile of pet food obtained by ESI(+)-MS, in the region of *m/z* 100-1200.

The lipid profile was expanded in the TAG region, between *m/z* 700-1000, and it is exposed in figure 2.

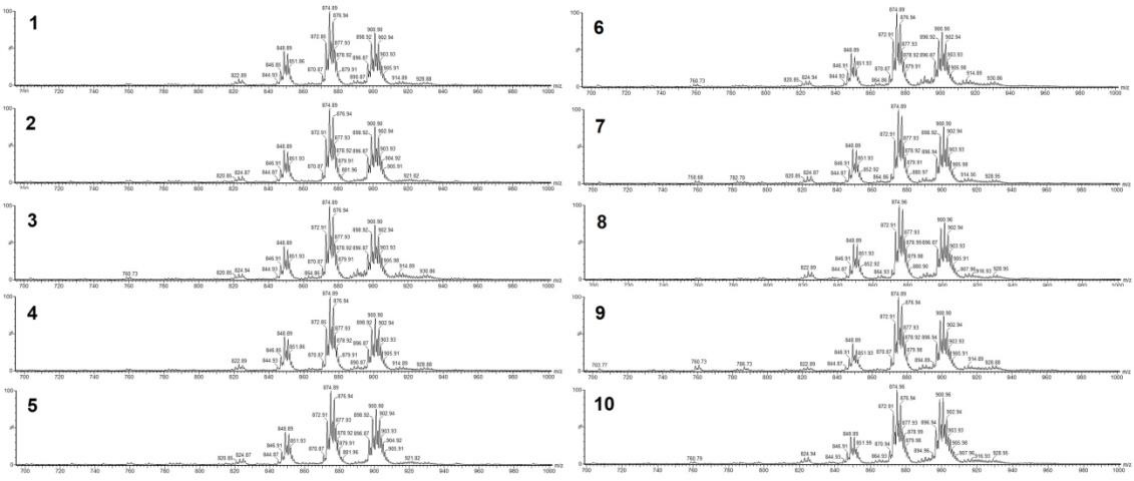


Figure 2 – Lipid profile of pet food obtained by ESI(+)-MS, in the region of *m/z* 700-1000.

According to Figures 1 and 2, it can be observed that all samples presented characteristic lipid profiles and similarity among it, also the FAs present in the feeds are in the TAG form, due to the most abundant region of ions, predominantly in *m/z* 800 to 1000.

The lipid profiles in the TAG region of all feed samples were similar to the profile presented by Cajka *et al.*[23] for chicken oil. Hence, to confirm this similarity, chicken oil extraction was carried out by Bligh and Dyer[9] and ESI(+)-MS direct infusion analysis was performed. Figure 3 shows the lipid profile of the chicken oil, in the ratio *m/z* 50 - 1200.

Thus, it is possible to observe the similarity between the profiles of all the feed samples (1-10) (Figure 1) with the chicken oil profile (Figure 3), proving the presence of it in all feed samples (1-10).

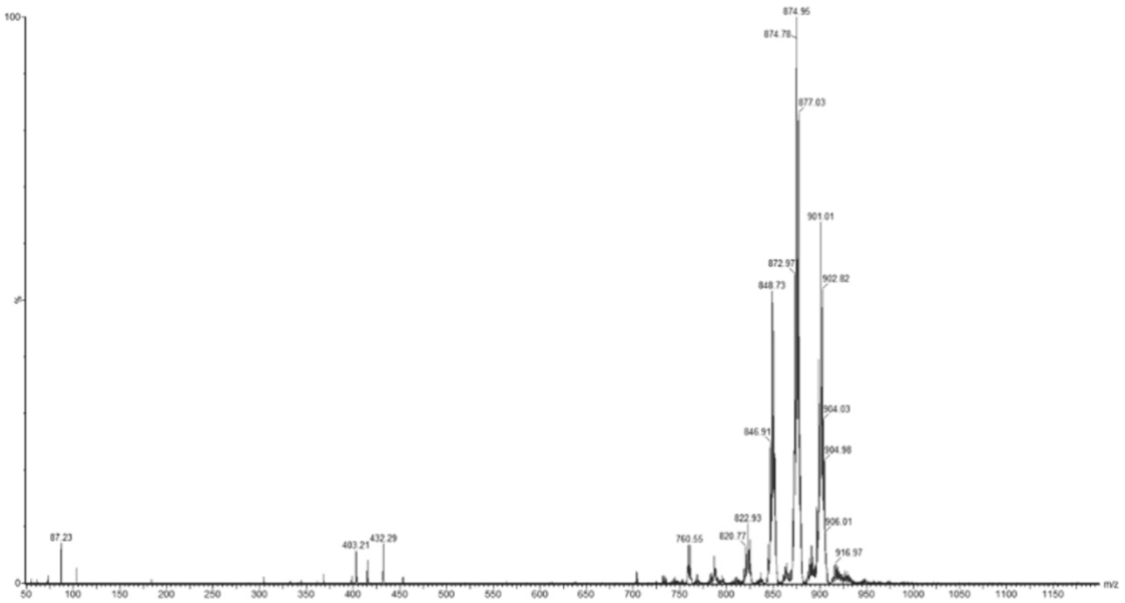


Figure 3: Lipid profile of chicken oil obtained by ESI(+)-MS, in the region of m/z 50-1200

Table 3 presents possible assignment of TAGs identified from ESI(+)-MS profiles, and the ions peaks were described in relative percentages. The most intense ion peak of all pet food samples, *m/z* 874 (TAG 52:3), was assigned as 100%.

Table 3- $[TAG + NH_4]^+$ ions and relative abundances (%) determined by ESI(+)-MS for pet food samples

$[TAG+NH_4]^+$ m/z	TAG[a]	Composition	CN/DB[b]	Samples (%)[c]									
				1	2	3	4	5	6	7	8	9	10
820	MPoO - MPoV - MPL – PpoPo	$C_{51}H_{94}O_6$	48:2	4.9	5.2	4.4	5.4	4.9	5.6	6.1	6.7	4.0	4.8
822	MPO - MPV – PPPo	$C_{51}H_{96}O_6$	48:1	7.5	8.4	6.8	8.9	8.1	8.1	9.8	11.7	5.3	8.3
824	PPP	$C_{51}H_{98}O_6$	48:0	6.9	8.8	6.6	7.6	8.9	8.3	10.7	10.3	4.3	9.4
844	PPoLn - MLL - MVLn - MOLn – PoPoL	$C_{53}H_{94}O_6$	50:4	5.3	5.5	4.9	5.5	5.2	6.0	5.6	5.3	5.8	5.4
846	OML - PPLn - PPoL - PoPoO - MSL - PoPoV - MVL	$C_{53}H_{96}O_6$	50:3	16.4	17.3	15.6	19.2	17.7	20.2	19.3	19.5	16.7	15.5
848	MVO - MVV - SPoPo - PLP - SLM - MOO - PPoV - PPoO	$C_{53}H_{98}O_6$	50:2	40.0	41.0	39.8	46.2	44.6	45.3	47.3	47.9	37.3	36.4
850	OPP – PpoS	$C_{53}H_{100}O_6$	50:1	36.7	38.3	36.3	42.9	41.9	41.1	45.7	47.6	29.2	33.6
852	PPS – MSS	$C_{53}H_{102}O_6$	50:0	14.4	19.6	17.8	21.5	20.9	20.9	23.1	24.2	14.4	18.6
866	PoLnLn	$C_{55}H_{92}O_6$	52:7	2.5	3.4	2.7	2.2	2.1	3.1	2.8	3.7	2.0	5.0
868	SLnLn – PoLLn	$C_{55}H_{94}O_6$	52:6	2.8	3.9	3.0	2.6	3.0	4.9	3.5	3.0	3.9	4.7
870	LLPo-PoVLn-PoOLn	$C_{55}H_{96}O_6$	52:5	3.2	15.8	14.6	13.0	13.1	15.9	13.1	13.2	17.5	18.4

872	LLP - PoOL - SLnPo - PLnV - VLPo – PlnO	$C_{55}H_{98}O_6$	52:4	65.3	65.5	66.3	57.1	58.6	61.7	58.4	64.4	65.1	69.4
874	PLO - SLnP - OPoV - VPoV - OPoO - PLV – SLPo	$C_{55}H_{100}O_6$	52:3	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
876	POO - POV -SPoV - PVV - SOPo - SLP	$C_{55}H_{102}O_6$	52:2	79.7	82.3	82.1	85.8	88.2	85.2	91.5	94.8	88.3	80.6
878	SPoS – SOP	$C_{55}H_{104}O_6$	52:1	43.9	45.6	46.1	47.2	49.7	48.0	51.3	52.3	48.5	46.3
880	SSP	$C_{55}H_{106}O_6$	52:0	16.4	17.1	17.2	15.5	18.7	17.7	18.4	22.1	18.9	21.8
886	OLM	$C_{56}H_{100}O_6$	53:4	2.7	3.9	3.5	3.3	2.5	6.0	4.5	3.9	5.4	4.9
888	OOM	$C_{56}H_{102}O_6$	53:3	2.9	7.3	6.2	6.1	4.2	10.6	8.2	8.1	8.7	7.5
894	LLLn - OLnLn - VLnLn-	$C_{57}H_{96}O_6$	54:7	4.6	9.5	9.7	6.4	6.9	12.5	7.8	9.0	10.5	13.6
896	LLL - OLLn - LVLn – SLnLn	$C_{57}H_{98}O_6$	54:6	42.8	42.7	41.6	28.7	31.7	34.5	34.3	41.1	36.9	42.3
898	OLL - SLnL - OLnO - VLL - VLnV - OLnV	$C_{57}H_{100}O_6$	54:5	79.2	75.7	74.9	56.5	62.9	62.8	64.4	69.4	68.6	87.1
900	SLnO - SLnV - OLV - SLL - VLV - OLO -	$C_{57}H_{102}O_6$	54:4	84.2	82.6	83.2	71.2	75.3	74.1	76.6	76.3	74.2	89.7
902	OVO - VVV - OOO - SLV -	$C_{57}H_{104}O_6$	54:3	60.7	59.7	61.6	56.1	60.9	58.5	62.3	61.2	54.1	61.8

	OVV - SLO - SLnS												
904	OOS - SSL – SVV	$C_{57}H_{106}O_6$	54:2	33.9	33.9	35.6	31.9	35.5	33.4	36.0	35.2	30.5	35.4
906	SVV - SOO - SVO – SLS	$C_{57}H_{108}O_6$	54:1	15.1	15.8	16.6	13.9	16.1	16.5	17.3	18.4	14.1	19.0
908	SSS	$C_{57}H_{110}O_6$	54:0	6.6	7.0	8.3	5.4	6.7	7.9	7.3	9.3	6.1	10.0

[a]Triacylglycerols abbreviation; [b]Carbon number/number of double bounds of the three fatty acid moieties; [c]Relative percentage. M: Myristic acid; P: Pamitic acid; O: Oleic acid; S: Stearic acid; Po: Palmitoleic acid; V: Vaccenic acid; L: Linoleic acid; Ln: Linolenic acid.

TAG composed of pamitic (P), oleic (O), stearic (S), palmitoleic (Po), vaccenic (V), linoleic (L), linolenic (Ln) acids, dominates the TAG profile of pet food. The major TAG pet food are 52:3, followed by 52:2, 54:4, 54:5, 52:4, and 54:3. These results are according to Porcari *et al.* [24] and Cajka *et al.* [23] for chicken oil lipid profile.

One of the possible TAGs found for *m/z* 874 is PLO, consisting of the FAs 16:0/18:2n-6/18:1n-9. These FAs were also found in greater amounts in the analysis performed by GC-FID (Table 1).

The feed manufactures of samples 1, 2, 4, 7, and 9 reported the presence of EPA and DHA at the guarantee levels on its labels. These two FAs come predominantly from fish oil, such as sardines and salmon.[12] Therefore, to verify and compare the lipid profile of the sardine and salmon oils with the lipid profiles presented by the feed, both oils were extracted by Bligh and Dyer[16] and the direct infusion analysis by ESI(+)-MS was carried out. Figures 4 and 5 exposes the lipid profiles of both fish oils, respectively, in the ratio *m/z* 100-1200.

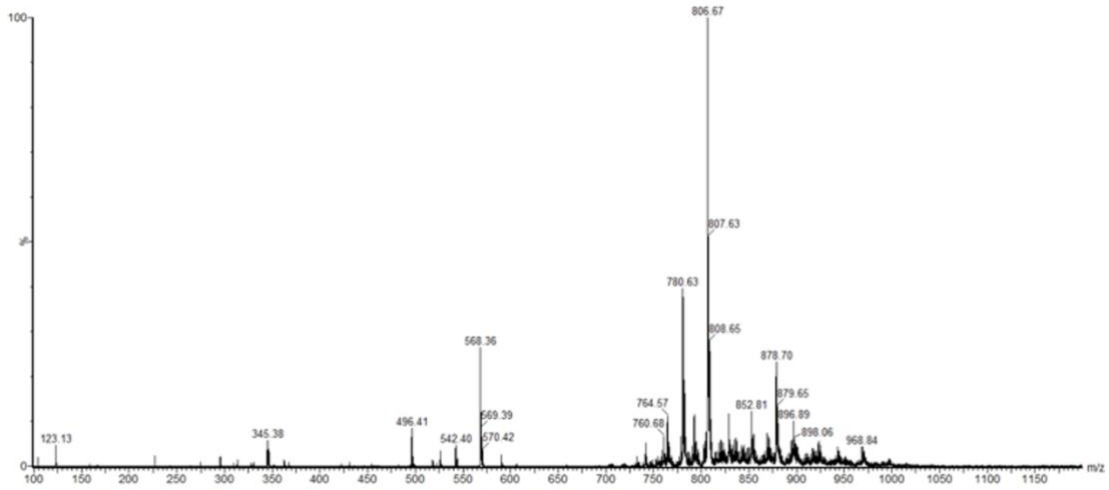


Figure 4: Lipid profile of sardine obtained by ESI (+)-MS,in the region of m/z 100-1200.

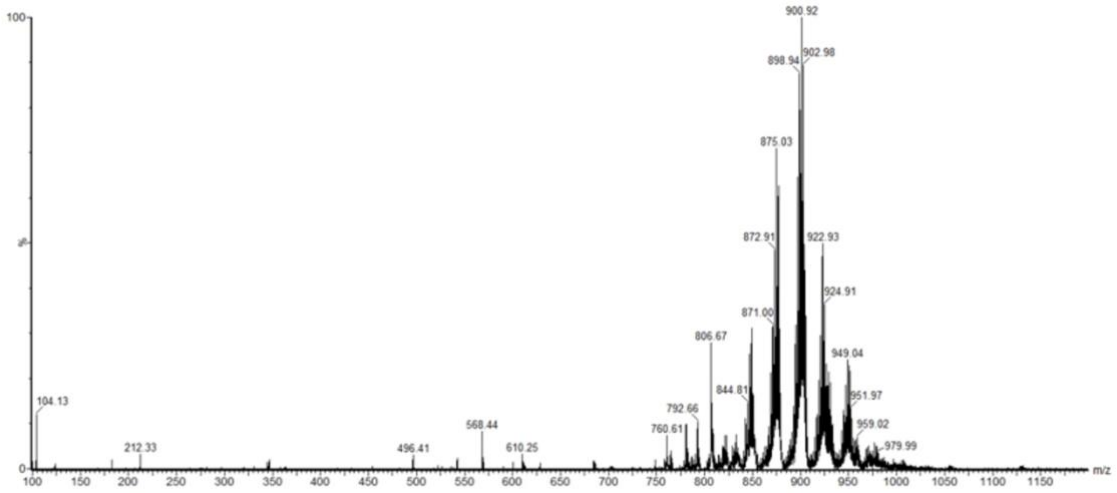


Figure 5: Lipid profile of salmon obtained by ESI (+)- MS, in the region of m/z 100-1200

Hence, it can be observed that none of the feed samples (Figure 1) presented lipid profiles similar to the profiles analyzed for sardine (Figure 4) and salmon (Figure 5). Moreover, the profiles obtained for fish oils were also compared in this work with the lipid profiles of fish oils present in omega-3 capsules analyzed by direct infusion using ESI(+)-MS with ionization $[TAG+NH_4]^+$, and it was observed that the capsules have profiles similar to those obtained for sardine oil.[22]

The results obtained by ESI(+)-MS confirm the data obtained by GC-FID, showing the presence of chicken oil in the feed and the absence of EPA and DHA, since no lipid profiles were found similar to fish oils or TAGs containing these two FAs.

3.3. Principal Component Analysis (PCA)

PCA was performed to clarify the results contribution obtained by ESI(+)-MS. PC1 (65.1%) and PC2 (21.2%) explained 86.3% of the total variance, as proposed in Figure 6.

A separation was observed in two distinct groups, in PC1 negative quadrant one group was formed by samples 1, 2, 3 and 10 due to the closer signal strength *m/z*, and the samples 1 and 2 contributed positively to PC2 and samples 3 and 10 contributed negatively to this

separation.

In PC1 positive quadrant another group was formed by samples 5,6,7 and 8 due to the fact previously exposed. For this group only sample 6 contributed positively to PC2, while samples 5,7 and 8 contributed negatively to PC2.

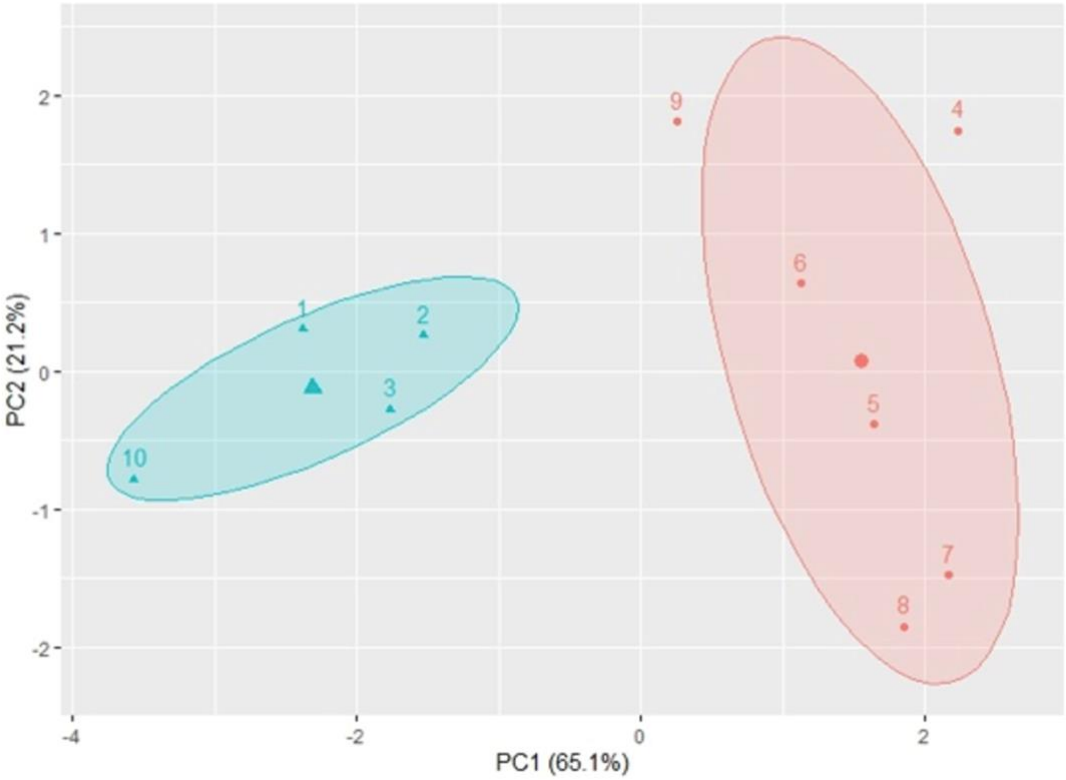


Figure 6: Score plots for m/z of the samples PC1-PC2

4. Conclusions

The fatty acid composition obtained was compared to the dog food packaging labels samples, it was observed that the omega 3 and omega 6 amounts are within the limit determined by each manufacturer, however, the labeling information on the EPA and DHA concentrations are not in accordance with the results obtained by GC-FID nor by ESI-MS. PCA analysis revealed that PC1 and PC2 explained 86.3% of the total variance.

Consequently, the information displayed on the labels are in disagreement with the results obtained for the fatty acid composition analysis by GC-FID and for the lipid profile analysis by ESI(+)-MS.

Acknowledgments

To CNPq, CAPES, Fundação Araucaria for the financial support and grant of the scholarship. To the State and the Universidade Estadual de Maringá for making the necessary technologies available for the development of this research.

References

1. Chandler, M. L.; Pet food safety: Sodium in pet food. *Topics in Companion Animal Medicine*, **2008**, *23*, 148.
2. Thompson, A.; Ingredients: Where pet foods starts. *Topics in companion animal medicine*, New York, **2008**, *23*, 127.
3. Carciofi, A. C.; Teshima, E.; Bazolli, R. S.; Brunetto, M. A.; Vasconcellos, R. S.; Pereira, G. T.; & Oliveira, L. D.; Qualidade e digestibilidade de alimentos comerciais de diferentes segmentos de mercado para cães adultos. *Revista Brasileira de Saúde e Produção Animal*, Salvador, **2009**, *10*, 489.
4. Flores, A. F. C.; Flores, D. C.; Oliveira, G.; Pizzuti, L.; Silva, R. M. S.; Martins, M. A. P.; Bonacorso, H. G.; *J. Braz. Chem. Soc.* **2008**, *19*, 184.
5. Goes, R. H. T. B.; Silva, L. H. X.; Souza, K. A.; *Alimentos e alimentação animal*. Editora UFGD, Dourados, 2013.
6. ABINPET - Associação brasileira de indústria de produtos para animais de estimação. 2016. http://abinpet.org.br/site-cmfv/ (Acessed 03 October 2018)
7. Heinemann, K. M. M.; Waldron, M. K.; Bigley, K. E.; Lees, G. E.; Bauer, J. E.; Long-Chain (n-3) Polyunsaturated Fatty Acids Are More Efficient than -Linolenic Acid in Improving Electroretinogram Responses of Puppies Exposed during Gestation, Lactation, and Weaning. *The Journal of Nutrition*, **2005**, *135*, 1960 .
8. Mueller, R. S.; Fieseler, K. V.; Fettman, M. J.; Zabel, S.; Rosychuk, R. A. W.; Ogilvie, G. K.; Greenwalt, T. L.; Effect of omega-3 fatty acids on canine atopic dermatitis. *Journal of Small Animal Practice*, **2004**, *45*, 293.
9. National Research Council – NRC; *Nutrient requirements of dogs and cats.* The national academies press, Washington, DC, 398, 2006.
10. Olivry, T.; DeBoer, D. J.; Favrot, C.; Jackson, H. A.; Mueller, R. S.; Nuttall, T.; Pre´laud, P.; Treatment of canine atopic dermatitis: 2010 clinical practice guidelines from the International Task Force on Canine Atopic Dermatitis. *Veterinary Dermatology*, **2011**, *21*, 233.
11. Vaughn, D. M.; Reinhart, G. A.; Swain, S. F.; Lauten, D. S.; Garner, C. A.; Bourdeaux, M. K.; Spano, J. S.; Hoffman, C. E.; Conner, B.; Evaluation of Effects of Dietary n-6 to n-3 Fatty Acid Rations on Leukotriene B Synthesis in Dog Skin and Neutrophilis. *Veterinary Dermatology*, **1994**, *4*, 163.
12. Bispo, P.; Batista, I.; Bernardino, R. J.; Bandarra, N. M.; Preparation of Triacylglycerols Rich in Omega-3 Fatty Acids from Sardine Oil Using a Rhizomucor miehei Lipase: Focus in the EPA/DHA Ratio. *Applied Biochemistry and Biotechnology*, **2014**, *172*, 1866.

13. Zeng, Y.; Araujo, P.; Grung, B.; Zhang, L.; Evaluation of different fingerprinting strategies for differentiating marine oils by liquid chromatography ion-trap mass spectrometry and chemometrics. *The Analyst*, **2011**, *136*, 1507.
14. Kralovec, J. A.; Zhang, S.; Zhang, W.; Barrow, C. J.; A review of the progress in enzymatic concentration and microencapsulation of omega-3 rich oil from fish and microbial sources. *Food Chemistry*, **2012**, *131*, 639.
15. Figueiredo I. L.; Claus T.; Santos Junior, O. O.; Almeida V. C.; Magon T. F. S.; Visentainer, J. V.; Fast derivatization of fatty acids in different meat samples for gas chromatography analysis. *Journal of Chromatography A*, **2016**, *1456*, 235.
16. Silveira, R.; Vágula, J. M.; Figueiredo, I. L.; Claus, T.; Galuch, M. B.; Santos Junior, O. O.; Visentainer, J. V.; Rapid methodology via mass spectrometry to quantify addition of soybean oil in extra virgin olive oil: A comparison with traditional methods adopted by food industry to identify fraud. *Food Research International*, **2017**, *102*, 43.
17. Youzbachi, N.; Trabelsi, H.; Elfalleh, W.; Khaldi, A.; Nasri, N.; Tlili, N.; Fatty acids and triacylglycerols composition from Tunisian Acacia species seed oil. *Arabian Journal of Chemistry*, **2015**.
18. R Studio Team, 2015. RStudio: Integrated Development for R. Boston, MA. Retrieved from https://www.rstudio.com/ Acessed 10 August 2018.
19. Ministério da Agricultura - MAPA, 2007. P. e A. DECRETO Nº 6.296, DE 11 DE DEZEMBRO. http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=visualizarAtoPortalMapa&chave=1197672218 (Acessed 15 October 2018)
20. Calviello, G.; Serini, S.; Piccioni, E.; N-3 Polyunsaturated Fatty Acids and the Prevention of Colorectal Cancer: Molecular Mechanisms Involved. *Current Medicinal Chemistry*, **2007**, *14*, 3059.
21. Kvamme, J. L.; Phillips, T. D.; *Pet food Technology*. Watt Publishing Co., Mt. Morris, Illinois, 61054 USA, 2003.
22. Ribeiro, P. M., 2018. Oxidação Lípidica no Processo de Extrusão em Pet Food. Dissertação (Mestrado) Zootecnia - Universidade Estadual de Maringá, Área de Concentração: Produção Animal.
23. Rokey, G.; Plattner, B.; *Process description. In: Pet food production.* Wenger Mfg, Inc., 66534, Sabetha, 1995.
24. Pizzo, J. S.; Galuch, M. B.; Santos, P. D. S.; Santos, O. O.; Visentainer, L.; Eberlin, M. N.; Visentainer, J. V.; Assessment of Adulteration of Cosmetics Based on Vegetable Oils by GC-FID and Lipid Profile Using Direct Infusion Electrospray Ionization Mass Spectrometry (ESI-MS). *Article J. Braz. Chem. Soc*, **2018**, *00*, 1.

25. Galuch, M. B.; Carbonera, F.; Magon, T. F. S.; Silveira, R.; Santos, P. D. S.; Pizzo, J. S.; Santos, O. O.; Visentainer, J. V.; Quality Assessment of Omega-3 Supplements Available in the Brazilian Market. *Journal of the Brazilian Chemical Society*, **2018**, *29*, 631.
26. Cajka, T.; Danhelova, H.; Zachariasova, M.; Riddellova, K.; Hajslova, J.; Application of direct analysis in real time ionization–mass spectrometry (DART–MS) in chicken meat metabolomics aiming at the retrospective control of feed fraud. *Metabolomics*, **2013**, *9*, 545.
27. Bligh E. G.; Dyer W. J.; A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **1959**, *37*, 911.
28. Porcari, A. M.; Fernandes, G. D.; Barrera-Arellano, D.; Eberlin, M. N.; Alberici, R. M.; Food quality and authenticity screening via easy ambient sonic-spray ionization mass

Assessment of grape seed oils lipid quality by GC-FID and ESI-MS: rapid method to detect adulteration

Roberta Silveira[1], Jessica S. Pizzo[2], Luciana P. Manin[1], Adriela A. Rydlewski[1], Marília B. Galuch[2], Patrícia D. S. Santos[2], Oscar O. Santos[1,2], Jesui V. Visentainer[1,2*]

[1]Programa de pós-graduação em Ciência de Alimentos, Universidade Estadual de Maringá (UEM), 87020-900 Maringá-PR, Brasil

[2]Departamento de Química, Universidade Estadual de Maringá (UEM), 87020-900 Maringá-PR, Brasil.

*Corresponding author:
e-mail: jesuiv@gmail.com. Universidade Estadual de Maringá, Avenida Colombo, 5790, 87020-900. Maringá, Paraná, Brasil.
Colocar o tipo de amosttra! COSMETICOS?????

ABSTRACT

Vegetable oils are constantly applied in numerous manufacturing processes, producing expressive amounts of by-products. Grapes (*Vitis vinifera*), one of the most consumed fruits worldwide, produces significant by-products quantities, particularly seeds. These seeds are rich in phytochemicals, bioactive, phenolic compounds and unsaturated FAs, particularly linoleic (18:2) and oleic (18:1) acids, which increases this oil market value, and consequently entice profiteers. Therefore, considering the growing market attributed to grape seed oil, this research focused on monitoring the lipid quality of commercial grape seed oils to detect possible adulteration. Lipid quality was evaluated through direct infusion by electrospray ionization mass spectrometry (ESI-MS) and gas chromatography with flame ionization detection (GC-FID). Refined soybean oil was detected in seven samples, whereas another additive was identified on three other samples. ESI(+)-MS analysis displayed potential as a rapid routine analysis that could be exploited by industries to monitor grape seed oil quality.

Keywords: TAG profile; FA composition; lipid profile; lipid quality; mass spectrometry.

1. Introduction

Vegetable oils are complex mixtures, naturally containing a diversity of compounds. It is mostly composed of triacylglycerols (TAG), followed by diacylglycerols (DAG), fatty acids (FA), phospholipids and minor components (Indelicato et al., 2017). Among the FAs, there are: saturated FAs (SFA, no double bonds), monounsaturated FAs (MUFA, a single double bond) and polyunsaturated FAs (PUFA, ≥2 double bonds), being the last one, generally associated to beneficial health effects (Saini & Keum, 2018).

Considering the PUFAs, some cannot be synthesized by the human body and are considered essential fatty acids, as is the case of linoleic (C18:2) and α-linolenic (C18:3n-3) acids, respectively considered FAs of n-3 and n-6 PUFA families. These FAs possess antiatherogenic and antithrombotic properties and affect the lipoprotein concentration, membrane fluidity, membrane enzyme function and modulation of other compounds (Yang et al., 2018).

Vegetable oil is crucial for the human diet and a major source of edible lipids, accounting for more than 75% of the total lipids consumed in the world. Moreover, it is constantly applied in the manufacturing process as ingredient for several foods (Garavaglia et al., 2016). This manufacturing process constantly produce undesirable by-products.

Grape (*Vitis vinifera*) is one of the most consumed fruit in the world, predominantly as juice (Martin et al., 2020). The juice industry generates an expressive amount of grape pomace, which consists of seeds, stalks and skin that arise as waste during the production of juice, wine, vinegar and molasses. The dry pomace includes approximately 20–26% of seeds (Yalcin et al., 2016).

Grape seeds are rich in phytochemicals, such as catechin and epicatechin; bioactive compounds, such as phytosterol and phenolic compounds; and unsaturated FAs, such as

linoleic (L, 18:2n-6) and oleic (O, 18:1n-9) acids (Crew et al. 2006; Villani et al. 2015; Shinagawa et al. 2017; Ma & Zhang, 2017).

The grape seeds oil content, obtained from cold pressed, can vary from 5.85 to 22.4% (m/m) and it depends on the variety, the environmental factors and the seeds maturation degree (Garavaglia et al. 2016; Oikonomou et al. 2018). Its main characteristic is its elevated content of unsaturated FAs, particularly linoleic (18:2) and oleic (18:1) acids (Yalcin et al., 2016). Plus, it also contains high vitamin E content, which is important for human health (Al Juhaimi et al., 2017).

The unsaturated FAs makes this oil a high-quality and popular for culinary, pharmaceutical, cosmetic and medical industry (Yalcin et al., 2016). Furthermore, grape seed oil is generally used for salad dressings, marinades, deep frying, flavored oils, baking, massage oil, sunburn repair lotion, hair products and hand creams (Al Juhaimi et al, 2017).

Due to its beneficial properties for consumption, as it is rich in PUFAs and MUFAs, grape seed oil assists in the body's energy deposit, with high antioxidant influence, being considered a product with high added value extracted from a by-product (Oikonomou et al. 2018). Nevertheless, it is extensively used on human skin, as it assists in healing, smoothing, calming, antibacterial, antioxidant and normalizing effects (Michalaka & Kiełtyka-Dadasiewicz 2018).

Considering the growing market of products containing grape seed oil (Martin et al., 2020), this research focused on assessing the quality of commercial grape seed oil, once the substitution of vegetable oils with higher prices for oils of lesser value is alarming in Brazil (Silveira et al. 2017; Galuch et al. 2018; Pizzo et al. 2018). Corroborating with the idea, Villani and co-authors (2015) analyzed 21 samples of commercial grape seed extract products and concluded that 42% were adulterated, demonstrating the necessity to develop and use accurate, rapid, and simple analytical methods to assess the authenticity and to determine the

quality control standards for grape seed oils.

Previous research has demonstrated that direct infusion by electrospray ionization mass spectrometry (ESI-MS) and gas chromatography with flame ionization detection (GC-FID) can easily determine adulteration in a vegetable oil due to the TAG profile differentiation and the FA composition characterization, respectively (Silveira et al. 2017; Galuch et al. 2018; Pizzo et al. 2018).

2. Material and methods

2.1.Samples

Ten samples of grape seed oils (GSO) from three different lots were acquired from local market of Maringa – PR, Brazil. All samples were preserved in its original container under refrigeration (6 to 10 °C) and sheltered from light.

Moreover, in order to obtain the pure grape seed oils (PGSO), bunch of grapes were purchased at the local market in Maringá (Paraná, Brasil), the grape seeds were separated from the fruit, placed in a bowl and washed with running water.

Three lots containing three samples each of refined soybean oils (RSO), identified as RSO, was obtained from local market of Maringa – PR, Brazil. All samples were preserved in its original container under refrigeration (6 to 10 °C) and sheltered from light.

Table 1 demonstrates the composition and additional information provided on the label's product by the manufacturer.

Table 1. Composition present on the package labels of the grape seed oils.

Sample	Composition and additional information
U1	Cold pressing/ Pure and natural grape seed oil
U2	Cold pressing/ Pure and natural grape seed oil
U3	Pure and natural grape seed oil
U4	Pure and natural grape seed oil
U5	Pure and natural grape seed oil
U6	Pure and natural grape seed oil
U7	Pure and natural grape seed oil
U8	Pure and natural grape seed oil
U9	Pure and natural grape seed oil
U10	Pure and natural grape seed oil

2.2.Obtaining pure grape seed oil

PGSO was extracted by pressing (Ribeiro et al. 2016). Subsequently, it was ground until a fine flour was obtained, which was placed in a stainless steel cylinder (Metal PEM, PHP 30 tons model), under pressure of 10 tons, for extraction of the PGSO.

2.3.FA composition by GC-FID

FA composition determination of the GSO samples, fatty acid methyl esters (FAMEs) were prior prepared according to Hartman & Lago (1973) and modified by Maia & Rodriguez-Amaya (1993).

A Thermo Scientific Trace Ultra 3300 gas chromatograph (GC), equipped with a flame ionization detector (FID) and a split/splitless injector, was employed. Separations were made on a fused silica capillary column CP-7420 (Select FAME, 100 m x 0.25 mm i.d. x 0.25 µm cyanopropyl film thickness). The H_2, as carrier gas, with flow rate of 1.2 mL min^{-1}, and the N_2, as make-up gas, with flow rate of 30 mL min^{-1} were employed. The flow rates of 30 and 300 mL min^{-1} for H_2 and synthetic air, respectively, were employed to compose the

detector flame. The injector and the detector temperatures were maintained at 230 and 250 ºC, respectively. The initial oven temperature of 165 ºC was maintained for 18 min, raised to 235 ºC at a rate of 4 ºC min^{-1}, being it was continued for 20 min. Samples were injected in split mode, with 1:40 ratio and injection volume was 1.0 µL. FAMEs were identified by comparing the peaks retention time present in the samples with those present in the standard mixture of 37 FAMEs (C4:0-C24:0, Sigma–Aldrich, USA). The results were expressed as a relative percentage of total fatty acids for each peak identified, automatically determined by Chromquest™ 5.0 software.

2.4.TAG lipid profile by direct infusion ESI(+)-MS

Samples were prepared according to Youzbachi et al. (2015) and modified by Silveira et al. (2017). In 50.0 µL of grape seed oils samples were added 950.0 µL of chloroform (HPLC grade, Riedel-de Haën, Germany). After, 5.0 µL aliquot of this solution was added into 1.0 mL of 9:1 (v/v) of methanol/chloroform (HPLC grade, J.T. Baker®, USA) and 20.0 µL of an 0.10 mol L^{-1} ammonium formate (Sigma–Aldrich, USA, solution prepared in methanol) was also added to form the final solution.

TAG profile was obtained by direct infusion of the final solution into a Xevo TQD™ triple quadrupole mass spectrometer (MS) (Waters, USA), equipped with Z spray™ electrospray ionization (ESI) source operating in positive mode (ESI(+)-MS), with continuous flow rate of 50.0 µL min^{-1}, comprising the mass/charge (*m/z*) range of 100–1200. Desolvation temperature was 250 ºC. Capillary and cone voltage were 3.00 kV and 35.0 V, respectively. Mass spectrometry/mass spectrometry (MS/MS) analysis was set at collision energy of 19 V, in order to fragment the main TAGs identified of the GSO, RSO and PGSO. Data were processed using MassLynx™ software.

2.5.Statistical analysis

All analyzes were performed in triplicate and the results were expressed as mean values ± standard deviation (SD). Besides, the results of FA composition were submitted to variance analysis (ANOVA) test followed by Tukey's test with 95% significance level. Data were processed using PAST3 software (Silva & Azevedo, 2016).

3. Results and discussion

3.1.FA composition by GC-FID

FA composition of the GSO samples are demonstrated in Table 2.

Table 2. Fatty acid composition (%) of grape seed oils samples.

Sample	Fatty acid composition (%)[a]													
	14:0	**16:0**	**16:1n-7**	**18:0**	**18:1n-9**	**18:1n-7**	**18:2n-6**	**18:3 n-3**	**18:3n-6**	**20:0**	**20:1n-11**	**22:0**	**22:1n-9**	**24:0**
U1	0.09 ± 0.00^{BC}	10.91 ± 0.03^{ABCD}	0.09 ± 0.00^{CD}	4.00 ± 0.04^{A}	25.35 ± 0.11^{E}	1.45 ± 0.02^{ABC}	50.65 ± 0.13^{F}	5.66 ± 0.03^{BCD}	0.29 ± 0.00^{G}	0.28 ± 0.01^{DEF}	0.37 ± 0.01^{AB}	0.26 ± 0.00^{BCD}	0.44 ± 0.01^{BC}	0.15 ± 0.00^{B}
U2	0.11 ± 0.01^{BC}	9.26 ± 0.00^{E}	0.16 ± 0.00^{B}	3.26 ± 0.06^{D}	37.42 ± 0.08^{A}	1.17 ± 0.05^{D}	41.08 ± 0.05^{H}	5.98 ± 0.09^{B}	0.24 ± 0.00^{H}	0.22 ± 0.01^{F}	0.35 ± 0.01^{B}	0.25 ± 0.01^{CD}	0.38 ± 0.01^{C}	0.13 ± 0.00^{BC}
U3	0.10 ± 0.01^{B}	10.71 ± 0.60^{BCD}	0.09 ± 0.01^{CD}	3.96 ± 0.05^{AB}	21.43 ± 0.39^{H}	1.37 ± 0.09^{C}	53.95 ± 0.36^{C}	6.63 ± 0.51^{A}	0.36 ± 0.01^{F}	0.30 ± 0.06^{DE}	0.33 ± 0.08^{BC}	0.23 ± 0.04^{DE}	0.41 ± 0.09^{BC}	0.13 ± 0.04^{BC}
U4	0.09 ± 0.00^{C}	10.85 ± 0.04^{ABCD}	0.09 ± 0.00^{CD}	3.92 ± 0.10^{AB}	28.04 ± 0.03^{C}	1.54 ± 0.06^{A}	48.14 ± 0.08^{G}	5.31 ± 0.06^{DE}	0.30 ± 0.01^{G}	0.26 ± 0.01^{EF}	0.44 ± 0.03^{A}	0.31 ± 0.00^{B}	0.55 ± 0.00^{A}	0.16 ± 0.01^{B}
U5	0.09 ± 0.00^{BC}	11.21 ± 0.03^{AB}	0.10 ± 0.00^{CD}	3.20 ± 0.03^{DE}	23.26 ± 0.02^{F}	1.50 ± 0.03^{AB}	53.46 ± 0.04^{D}	5.13 ± 0.03^{E}	0.44 ± 0.00^{E}	0.42 ± 0.00^{C}	0.33 ± 0.01^{BC}	0.29 ± 0.02^{BC}	0.42 ± 0.01^{BC}	0.14 ± 0.01^{BC}
U6	0.09 ± 0.00^{C}	10.97 ± 0.09^{ABC}	0.09 ± 0.00^{CD}	3.80 ± 0.07^{B}	23.62 ± 0.18^{F}	1.51 ± 0.03^{AB}	52.35 ± 0.10E	5.60 ± 0.11^{BCDE}	0.36 ± 0.01^{F}	0.33 ± 0.01^{D}	0.37 ± 0.02^{AB}	0.27 ± 0.02^{BCD}	0.48 ± 0.02^{AB}	0.17 ± 0.01^{AB}
U7	0.09 ± 0.00^{C}	11.07 ± 0.04^{ABC}	0.09 ± 0.00^{D}	3.92 ± 0.06^{AB}	22.60 ± 0.03^{G}	1.35 ± 0.01^{C}	52.67 ± 0.07^{E}	5.90 ± 0.05^{BC}	0.55 ± 0.00^{C}	0.55 ± 0.00^{B}	0.35 ± 0.00^{B}	0.29 ± 0.02^{BC}	0.43 ± 0.01^{BC}	0.15 ± 0.01^{B}
U8	0.09 ±	10.56 ±	0.08 ±	3.95 ±	25.79 ±	1.44 ±	50.40 ±	5.46 ±	0.51 ±	0.49 ±	0.36 ±	0.25 ±	0.46 ±	0.16 ±

	0.01^{C}	0.02^{CD}	0.01^{D}	0.09^{AB}	0.02^{D}	0.03^{ABC}	0.03^{F}	0.15^{CDE}	0.01^{D}	0.01^{B}	0.04^{AB}	0.02^{CD}	0.04^{ABC}	0.03^{B}
U9	0.07 ± 0.00^{D}	5.81 ± 0.01^{F}	0.09 ± 0.00^{CD}	3.43 ± 0.02^{C}	33.99 ± 0.10^{B}	0.73 ± 0.04^{F}	54.35 ± 0.06^{B}	0.26 ± 0.00^{F}	0.23 ± 0.01^{H}	ND^{H}	0.22 ± 0.00^{D}	0.60 ± 0.01^{A}	ND^{E}	0.23 ± 0.04^{A}
U10	0.14 ± 0.01^{B}	10.40 ± 0.03^{D}	0.11 ± 0.01^{C}	3.08 ± 0.01^{EF}	22.75 ± 0.00^{G}	1.39 ± 0.02^{BC}	53.42 ± 0.10^{D}	6.62 ± 0.08^{A}	0.59 ± 0.00^{B}	0.72 ± 0.03^{A}	0.24 ± 0.00^{CD}	0.18 ± 0.01^{E}	0.28 ± 0.03^{D}	0.08 ± 0.01^{CD}
PGSO	0.15 ± 0.00^{A}	11.38 ± 0.01^{A}	0.38 ± 0.00^{A}	3.03 ± 0.02^{F}	21.24 ± 0.02^{H}	0.99 ± 0.01^{E}	61.57 ± 0.03^{A}	0.13 ± 0.01^{F}	0.69 ± 0.01^{A}	0.14 ± 0.00^{G}	0.03 ± 0.00^{E}	ND^{F}	0.06 ± 0.00^{E}	0.03 ± 0.00^{D}
GSO Codex	ND-0.3	5.5-11.0	ND-1.2	3.0-6.5	12.0-28.0		58.0-78.0	ND-1.0		ND-1.0	ND-0.3	ND-0.5	ND-0.3	ND-0.4
RSO Codex	ND-0.2	8.0-13.5	ND-0.2	2.0-5.4	17-30		48.0-59.0	4.5-11		0.1-0.6	ND-0.5	ND-0.7	ND-0.3	ND-0.5

[a]The results are expressed as mean ± standard deviation of three replicates. The different uppercase letters in the same column are significantly different (p<0.05) by Tukey's test. GSO Codex: Grape seed oil fatty acid composition ranged obtained by *Codex Alimentarius*. RSO Codex: Refined soybean oil fatty acid composition ranged by *Codex Alimentarius* (CX-STAN 210-1999). PGSO: Pure grape seed oil. ND: not detected.

Fourteen FAs were identified in the samples. Linoleic acid (L, 41.08 – 61.57%), an omega-6 FA, predominated in the FA composition, followed by oleic (O, 21.42 – 37.42%), palmitic (P, 16:0; 5.81 – 11.38%) and stearic (S, 18:0; 3.03 – 4.00%) acids. Santos et al. (2011) analyzed the FA composition of seed extracts of four grape varieties: Isabel, Niagara, Benitaka and Brazil, and a total of eleven FAs were detected. Among it, those with higher proportions were also L, O and P FA.

Due to the high content of omega-6, resveratrol, vitamin E and phenolic, GSO is established as an important antioxidant and antimicrobial activity, and anti-aging effects (Lin et al., 2017; Michalaka & Kiełtyka-Dadasiewicz, 2018).

According to the Codex Standard for Named Vegetable Oils (Codex Alimentarius 1999), the content of L acid in GSO varies between 58.0 and 78.0%; the content of O acid varies between 12.0 and 28.0%; the content of P acid varies between 5.5 and 11.0%; and the content of S acid varies between 3.0 and 6.5%. The maximum content allowed by the Codex Standard for linolenic acid (Ln, 18:3n-3) is 1%. In this study, only the PGSO sample presented all FAs according to *Codex Alimentarius*.

The highest L acid content was found in PGSO (61.57%) followed by U9 (54.35%) > U3 (53.95%) > U5 (53.46%) > U10 (53.42%) > U7 (52.67%) > U6 (52.35%) > U1 (50.65%) > U8 (50.40%) > U4 (48.14%) > U2 (41.08%). Results obtained in this study indicated that the L acid content was outside of the range established by the *Codex Alimentarius* for samples U1, U2, U3, U4, U5, U6, U7, U8, U9 and U10.

Samples U2, U4 and U9 exhibited O acid content above the allowed by *Codex Alimentarius*.

Ln acid content in all samples varied between 0.13 and 6.33%. The highest Ln acid content was found in the U3 (6.33%) sample followed by U10 (6.62%) > U2 (5.98%) > U7 (5.90%) > U1 (5.66%) > U6 (5.60%) > U8 (5.46%) > U4 (5.31%) > U5 (5.13%) > U9

(0.26%) > PGSO (0.13%). Therefore, samples U1, U2, U3, U4, U5, U6, U7, U8, and U10 revealed Ln acid content above the allowed by *Codex Alimentarius*.

Usually, Ln acid contents in GSOs are lower than in RSOs. Furthermore, low Ln acid levels are desired in GSOs for better oxidative stability, which is important for health protection and economic reasons (Göktürk Baydar et al., 2007). In this study, it can be observed that the Ln acid composition of samples U1, U2, U3, U4, U5, U6, U7, U8, and U10 were similar to the Ln acid composition of RSOs samples, indicating possible adulteration. Besides, the FA composition results obtained by PGSO sample are close to data reported by other authors. For the other samples (U1, U2, U3, U4, U5, U6, U7, U8, U9 and U10), the results are distinct to data reported by other authors (Beveridge et al., 2005; Crew et al., 2006; Lachman et al., 2015; Shinagawa et al., 2017).

Beveridge and coauthors evaluated the extraction of the seed oil from eight grapes varieties (Barbera, Malbec, Gamay, Cabernet Sauvignon, Pinot Noir, Merlot, Cabernet Franc, and Syrah), which were crushed for British Columbia wine production by supercritical carbon dioxide (SCE) and petroleum ether (PE), and the FA composition results revealed that L acid content varied from 67.56 – 73.23%, O acid content ranged from 12.71 – 18.47%, and P acid content ranged from 6.28 – 8.26% for SCE. While for PE, the L acid content varied from 66.76 – 73.61%; O acid content varied from 12.63 – 18.95%, and P acid content ranged from 6.35 – 8.61% (Beveridge et al., 2005).

Crew and coauthors described the composition of 30 grape seed oils obtained from France, Italy and Spain during 2002-2003. The highest FA was L acid, ranging from 69.3 − 74.6%, 63.1 − 69.0%, and 61.3 − 70.2% for France, Italy and Spain, respectively; followed by the O acid, ranging from 14.0 − 17.6%, 17.2 − 20.9%, and 16.2 − 20.0% for France, Italy and Spain, respectively; then P acid content, ranging from 6.6 − 8.4% (France), 7.9 − 9.6% (Italy), and 7.1 − 11.6% (Spain); and Ln acid content varied from 0.4 − 1.8%, 0.4 −

0.8% and 0.3 − 0.6% for France, Italy and Spain grape seed oil, respectively (Crew et al., 2006).

Shinagawa and coauthors evaluate the FA composition of cold pressed grape seed oil in the Brazilian markets between July 2012 and August 2013. All analyzed samples exhibited higher L acid concentrations (72.19 - 75.02%), followed by O acid (14.80 - 17.34%), P acid (9.72 - 10.22%) and Ln acid (0.21 - 0.49%). The values in all studies mentioned above were within the limits allowed by the Codex Standard for Named Vegetable Oils (Shinagawa et al., 2017).

In the study presented by Lachman and coauthors, FAs from 41 grape seeds samples from the gene collection of Viticulture Research Station Karlstejn, Czech Republic, were determined. Results showed that L acid was the most abundant FA in all the analyzed oils (68.10 to 78.18%). The O acid content ranged between 8.67 and 16.78%; and the P acid varied from 4.93 to 8.02%. The values found in this work were above the limits allowed by the Codex Standard for Named Vegetable Oils (Lachman et al., 2015).

3.2.TAG lipid profile by direct infusion by ESI(+)-MS

The TAG profile of the GSOs samples were acquired by direct infusion ESI(+)-MS. This method is rapid, simple, short and easy, as well as it has been used to the rapid characterization of the vegetable oils and fats, with little sample preparation (Catharino et al., 2005; Galuch et al., 2018). Moreover, vegetable oils have a characteristic TAG profile, and adulterations result in significant modifications on these profiles (Silveira et al., 2017; Pizzo et al., 2018).

Figure 1 displays the TAG profile of GSO comprising the region between 100 to 1200 *m/z*.

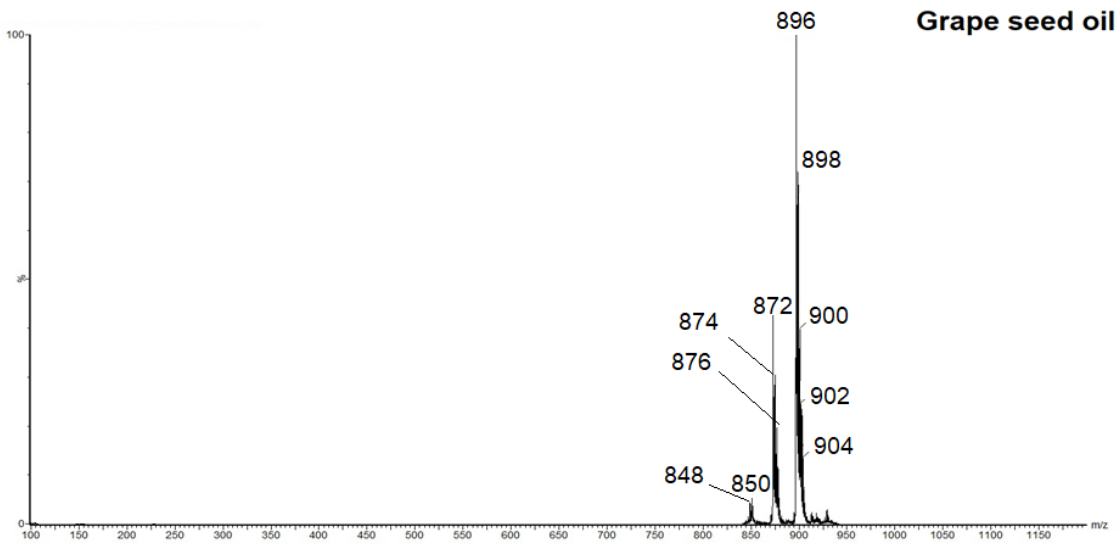


Figure 1. TAG lipid profile by direct infusion ESI(+)-MS of the pure grape seed oil sample.

It is possible to observe in Figure 1 that the PGSO presented higher intensities of the TAG LLL (896 *m/z*), followed by LLO (898 *m/z*) and LLP (872 *m/z*). These results are agreeing with those found in the literature. Jakab et al. reported that the LLL content was the highest (38.41 - 41.19%), followed by LLO (19.76 – 22.20%) and PLL (14.35 – 16.93%). Other TAGs, as LLLn (894 *m/z*), LnLP (870 *m/z*), OOL (900 *m/z*), PLO (874 *m/z*), PLP (848 *m/z*), OOO (902 *m/z*), POO (876 *m/z*), POP (850 *m/z*) and SOO (904 *m/z*), varied from 0.44 to 7.23% (Jakab et al. 2002). Ovcharova et al. reported that the LLL content in grape seed oil was the highest (40.4 - 57%), followed by LLO (16.4 – 23.3%) and LLP (8.4 – 15.4%). Other TAGs, as LOO, LOP, LLS, OOO, OOP and LOS, varied from 1.7 to 6.7%, while LPP was detect at the trace level (Ovcharova et al. 2016).

Figures S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 (supplementary information) displays the TAG profile of GSOs samples U1, U2, U3, U4, U5, U6, U7, U8, U9 and U10, respectively. The samples U1, U3, U5, U6, U7, U8 and U10 presented higher intensities of TAG LLL followed by LLO and OOL, while for sample U9, it was found that the major component was LLO followed by OOL and LLL. The U4 sample presented the TAG

sequence: LLO, LLL, and OOL, while the U2 sample presented as the major component the LLL followed by LLO and OOO.

According to the lipid profiles (Figures S1-S10), it is evident that the samples U1, U3, U5, U6, U7, U8 and U10 are similar to each other, although distinct from the PGSO sample. Furthermore, samples U2, U4 and U9 are distinct from all other samples and from each other. It could be observed in the region 898 - 904 *m/z*, 896 – 904 *m/z* and 800 - 900*m/z*, respectively. PGSO was distinct from all other samples (848 - 950 *m/z*). All GSOs samples presented higher intensities in the region 910 - 1000 *m/z*, being different from the PGSO sample, which present lower intensities in this region. These differences are in accordance with the results obtained in the FA analyzes by GC-FID.

Figure 2 displays the TAG profile of all GSOs samples for a better visualization. Figure 3 displays the TAG profile of RSOs.

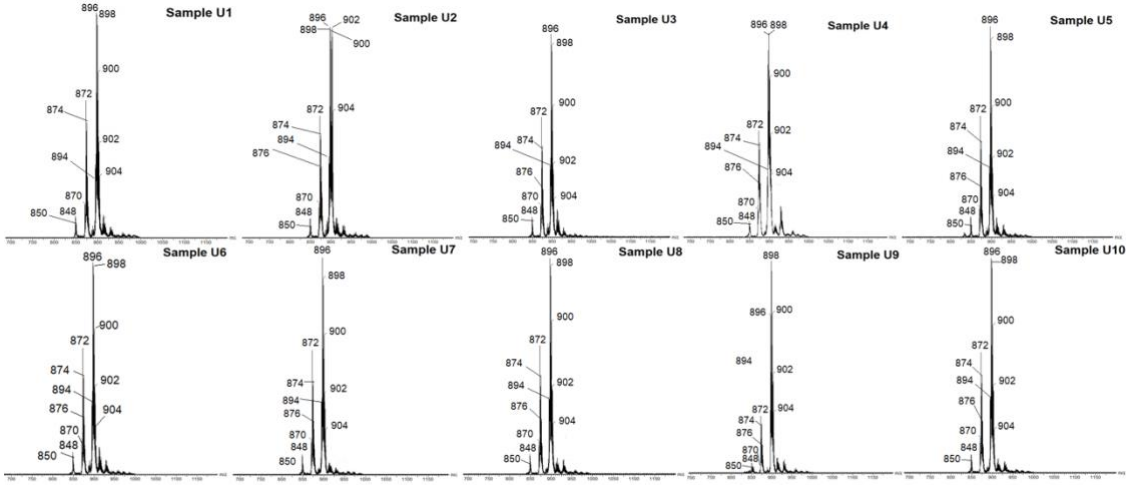


Figure 2 TAG lipid profile by direct infusion ESI(+)-MS of all grape seed oils samples.

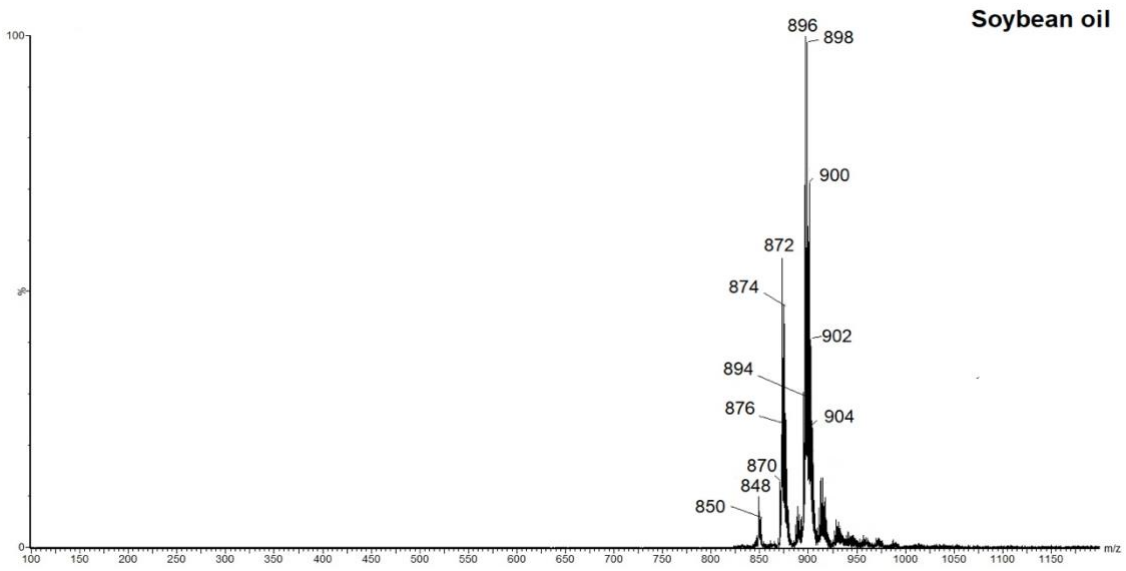


Figure 3 TAG lipid profile by direct infusion ESI(+)-MS of the refined soybean oil sample.

Therefore, it is observed that the TAG profile of samples U1, U3, U5, U6, U7, U8 and U10 are similar to RSO and, consequently, adulterated with RSO, while U2, U4 and U9 are adulterated with another vegetable oil, which modify its TAG profile.

3.3.TAGs lipid profile by direct infusion ESI(+)-MS

Table 3 demonstrates the main TAGs identified from the direct infusion ESI(+)-MS analysis of GSO samples, RSO and PGSO. The results were expressed as relative percentage of the main TAG.

Table 3. The main TAGs identified from the direct infusion ESI(+)-MS analysis of the grape seed oils samples, soybean oil and pure grape seed oil in relative percentage (%).

TAGs	DAG 1,2	DAG2,3	DAG1,3	$[TAG+NH_4]^+$	RSO	PGSO	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10
LLL	-	599	-	896	19.82	28.06	18.29	15.36	19.92	16.95	19.90	19.01	20.21	19.22	18.91	19.59
LLO	599	601	-	898	17.85	20.19	18.11	15.19	18.72	19.26	19.28	18.58	18.41	18.49	25.49	19.35
LLP	599	575	-	872	11.78	12.11	10.98	8.93	11.40	9.81	11.98	11.31	11.59	11.44	6.80	10.53
OOL	603	601	-	900	12.52	11.17	13.57	13.73	12.73	15.02	12.63	12.87	11.61	13.56	19.10	13.37
PLO	575	601	577	874	8.55	8.55	9.43	7.44	8.90	8.42	8.72	8.78	8.92	8.25	5.90	8.88
OOO	-	603	-	902	8.16	6.86	8.19	14.74	7.08	9.25	7.63	7.76	7.75	7.72	11.34	7.87
LLLn	599	597	-	894	6.54	0.61	5.87	5.89	7.06	5.67	6.79	6.52	6.57	6.72	0.56	6.32
POO	577	603	-	876	4.74	5.34	4.95	4.98	4.34	5.02	4.68	4.62	4.67	4.67	3.33	4.72
SOO	605	603	-	904	4.53	3.77	4.99	9.14	3.66	5.50	3.90	4.35	4.14	4.41	7.24	3.88
PPO	551	577	-	850	1.06	1.57	1.06	0.83	1.11	0.98	1.06	1.09	1.00	1.03	0.34	0.97
PPL	551	575	-	848	1.76	1.22	1.85	1.50	2.00	1.70	0.67	2.02	1.92	1.76	0.63	1.65
PLLn	575	597	573	870								3.10		2.74		2.85
LnLP	597	575	573	870				2.26	3.08		2.75		3.22			
LLnP	597	573	575	870	2.72											
LPLn	575	573	597	870		0.54	2.71								0.34	
LnPL	573	575	597	870						2.41						

It is observed that samples U1, U3, U5, U6, U7, U8 and U10 present relative percentage similar to RSO, which is in accordance with the TAG profile and the FA composition analysis. Samples U2, U4 and U9 are distinct from all other samples, which confirms the GC-FID analysis results.

4. Conclusion

The FA composition by GC-FID and the TAG profile by direct infusion ESI(+)-MS provide a valuable assessment of the grape seed oils lipid composition. In this study, ten samples of GSO, RSO and PGSO were analyzed and an adulteration was verified by the addition of RSO in seven GSO samples, and by the addition of another vegetable oil in three grape seed oils. The results obtained in this study demonstrate the quality control importance of GSO, which are susceptible to adulteration and, therefore, have reduced benefits to human consumption, since it is a high cost product. In addition, ESI(+)-MS analysis revealed that it is a very attractive candidate in rapid and routine analyzes in industries to verify the quality of grape seed oil in industries.

Acknowledgments

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Araucária for financial support.

Declarations of interest: none

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico [307056/2015-5]; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior [88887.339593/2019-00] and Fundação Araucária [039/19].

5. References

Al Juhaimi, F., Geçgel, Ü., Gülcü, M., Hamurcu, M., & Özcan, M.M. (2017). Bioactive properties, fatty acid composition and mineral contents of grape seed and oils. South African Journal of Enology and Viticulture, 38(1), 103-108.

Beveridge, T. H. J., Girard, B., Kopp, T., & Drover, J. C. G. (2005). Yield and Composition of Grape Seed Oils Extracted by Supercritical Carbon Dioxide and Petroleum Ether: Varietal Effects. Journal of Agricultural and Food Chemistry, 53(5), 1799–1804. https://doi.org/10.1021/jf040295q

Catharino, R. R., Haddad, R., Cabrini, L. G., Cunha, I. B. S., Sawaya, A. C. H. F., & Eberlin, M. N. (2005). Characterization of Vegetable Oils by Electrospray Ionization Mass Spectrometry Fingerprinting: Classification, Quality, Adulteration, and Aging. Analytical Chemistry, 77(22), 7429–7433. https://doi.org/10.1021/ac0512507

Codex Alimentarius. (1999). Standard for Named Vegetable Oils Codex Stan 210-1999. Codex Alimentarius, 1–13.

Crew, C., Hough, P., Godward, J., Brereton, P., Lees, M., Guiet, S., & Wilfried, W. (2006). Quantitation of the Main Constituents of Some Authentic Grape-Seed Oils of Different Origin. Journal of Agricultural and Food Chemistry, 54, 6261–6265. https://doi.org/10.1021/JF060338Y

Galuch, M. B., Carbonera, F., Magon, T. F. S., Silveira, R., Santos, P. D. S., Pizzo, J. S., et al (2018). Quality assessment of omega-3 supplements available in the Brazilian market. Journal of the Brazilian Chemical Society, 29(3), 631. https://doi.org/10.21577/0103-5053.20170177

Garavaglia, J., Markoski, M. M., Oliveira, A., & Marcadenti, A. (2016). Grape Seed Oil Compounds: Biological and Chemical Actions for Health. Nutrition and Metabolic Insights, 9, 59–64. https://doi.org/10.4137/NMI.S32910

Göktürk Baydar, N., Özkan, G., Sema Çetin, E., & Çetin, E. S. (2007). Characterization of grape seed and pomace oil extracts. Grasas y Aceites, 58(1), 29–33. https://doi.org/10.3989/gya.2007.v58.i1.5

Hartman, L., & Lago, R. C. (1973). Rapid preparation of fatty acids methyl from lipids. Laboratory Practice, 22(October), 474–476.

Indelicato, S., Bongiorno, D., Pitonzo, R., Di Stefano, V., Calabrese, V., Indelicato, S.,

& Avellone, G. (2017). Triacylglycerols in edible oils: Determination, characterization, quantitation, chemometric approach and evaluation of adulterations. Journal of Chromatography A, 1515, 1–16.doi:10.1016/j.chroma.2017.08.002

Jakab, A., Héberger, K., & Forgács, E. (2002). Comparative analysis of different plant oils by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A, 976(1–2), 255–263. https://doi.org/10.1016/S0021-9673(02)01233-5

Lachman, J., Hejtm, A., Aborský, J. T., Kotíkov, Z., Pivec, V., St Ralkov, R., et al (2015). Evaluation of oil content and fatty acid composition in the seed of grapevine varieties. LWT - Food Science and Technology, 63, 620–625. https://doi.org/10.1016/j.lwt.2015.03.044

Lin, T.-K., Zhong, L., Santiago, J., Lin, T.-K., Zhong, L., & Santiago, J. L. (2017). Anti-Inflammatory and Skin Barrier Repair Effects of Topical Application of Some Plant Oils. International Journal of Molecular Sciences, 19(1), 70. https://doi.org/10.3390/ijms19010070

Ma, Z., & Zhang, H. (2017). Phytochemical Constituents, Health Benefits, and Industrial Applications of Grape Seeds: A Mini-Review. Antioxidants, 6(3), 71. doi:10.3390/antiox6030071

Maia, E. L., & Rodriguez-Amaya, D. B. (1993). Avaliação de um método simples e econômico para metilação de ácidos graxos com lipídios de diversas espécies de peixes. Revista Instituto Adolfo Lutz, 53, 27–35.

Martin, M. E., Grao-Cruces, E., Millan-Linares, M. C., & La Paz S. M. (2020) Grape (*Vitis vinifera L.*) Seed Oil: A Functional Food from the Winemaking Industry. Foods, 9, 1360. doi:10.3390/foods9101360

Michalaka, M., & Kiełtyka-Dadasiewicz, N. (2018). Oils from fruit seeds and their dietetic and cosmetic significance. Herba Polonica, 64, 63–70.

Oikonomou, I., Daferea, D., Kanakis, C., Pappas, C., Tarantilis, P. A., Avramouli, A., et al (2018). Quality Evaluation of Grape Seed Oils of the Ionian Islands Based on GC-MS and Other Spectroscopic Techniques. International Scholarly and Scientific Research & Innovation, 12, 288–292.

Ovcharova, T., Zlatanov, M., & Dimitrova, R. (2016). Chemical composition of seeds

of four Bulgarian grape varieties Composição química das grainhas de quatro castas búlgaras. Ciência Téc. Vitiv, 31(1), 31–40. https://doi.org/10.1051/ctv/20163101031

Pizzo, J., Galuch, M., Santos, P., Santos, O., Visentainer, L., Eberlin, M., et al (2018). Assessment of Adulteration of Cosmetics Based on Vegetable Oils by GC-FID and Lipid Profile Using Direct Infusion Electrospray Ionization Mass Spectrometry (ESI-MS). Journal of the Brazilian Chemical Society, 29(12), 2457–2465. https://doi.org/10.21577/0103-5053.20180123

Ribeiro, S. A. O., Nicacio, A. E., Zanqui, A. B., Biondo, P. B. F., Abreu-Filho, B. A. de, Visentainer, J. V., et al (2016). Application of Enzymes in Sunflower Oil Extraction: Antioxidant Capacity and Lipophilic Bioactive Composition. Journal of the Brazilian Chemical Society, 27(5), 834–840.

Saini, R. K., & Keum, Y. S. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance — A review. Life Sciences, 203, 255–267. doi:10.1016/j.lfs.2018.04.049

Santos, L. P., Morais, D. R., Souza, N. E., Cottica, S. M., Boroski, M., & Visentainer, J. V. (2011). Phenolic compounds and fatty acids in different parts of Vitis labrusca and V. vinifera grapes. Food Research International, 44(5), 1414–1418. https://doi.org/10.1016/J.FOODRES.2011.02.022

Shinagawa, F. B., Santana, F. C. de, Araujo, E., Purgatto, E., Mancini-Filho, J., Shinagawa, F. B., et al (2017). Chemical composition of cold pressed Brazilian grape seed oil. Food Science and Technology, 38(1), 164–171. https://doi.org/10.1590/1678-457x.08317

Silva, F. de A. S. E., & Azevedo, C. A. V. (2016). The Assistat Software Version 7.7 and its use in the analysis of experimental data. African Journal of Agricultural Research, 11, 3733–3740. https://doi.org/10.5897/AJAR2016.11522

Silveira, R., Vágula, J. M., Figueiredo, I. L., Claus, T., Galuch, M. B., Santos Junior, O. O., & Visentainer, J. V. (2017). Rapid methodology via mass spectrometry to quantify addition of soybean 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

Villani, T. S., Reichert, W., Ferruzzi, M. G., Pasinetti, G. M., Simon, J. E., & Wu, Q.

(2015). Chemical investigation of commercial grape seed derived products to assess quality and detect adulteration. Food Chemistry, 170, 271–280. https://doi.org/10.1016/j.foodchem.2014.08.084

Yalcin, H., Kavuncuoglu, H., Ekici, L., & Sagdic, O. (2016). Determination of Fatty Acid Composition, Volatile Components, Physico-Chemical and Bioactive Properties of Grape (*Vitis vinifera*) Seed and Seed Oil. Journal of Food Processing and Preservation, 41(2). doi:10.1111/jfpp.12854

Yang, R., Zhang, L., Li, P., Yu, L., Mao, J., Wang, X., & Zhang, Q. (2018). A review of chemical composition and nutritional properties of minor vegetable oils in China. Trends in Food Science & Technology, 74, 26–32. doi:10.1016/j.tifs.2018.01.013

Youzbachi, N., Trabelsi, H., Elfalleh, W., Khaldi, A., Nasri, N., & Tlili, N. (2015). Fatty acids and triacylglycerols composition from Tunisian Acacia species seed oil. Arabian Journal of Chemistry. https://doi.org/10.1016/J.ARABJC.2015.08.020

Supplementary Information

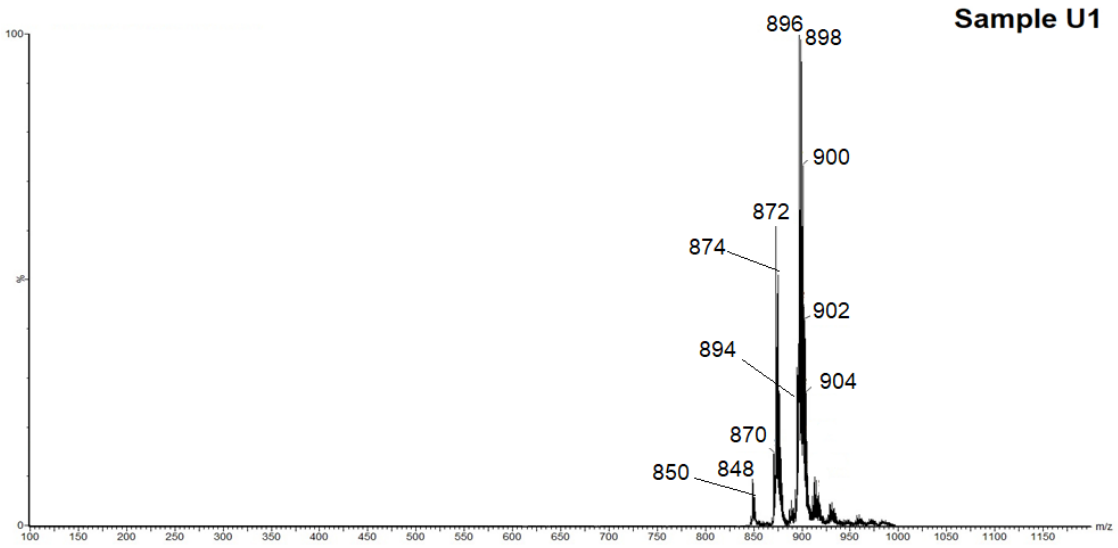


Figure S1 TAG profile by direct infusion ESI(+)-MS of the U1 grape seed oil sample.

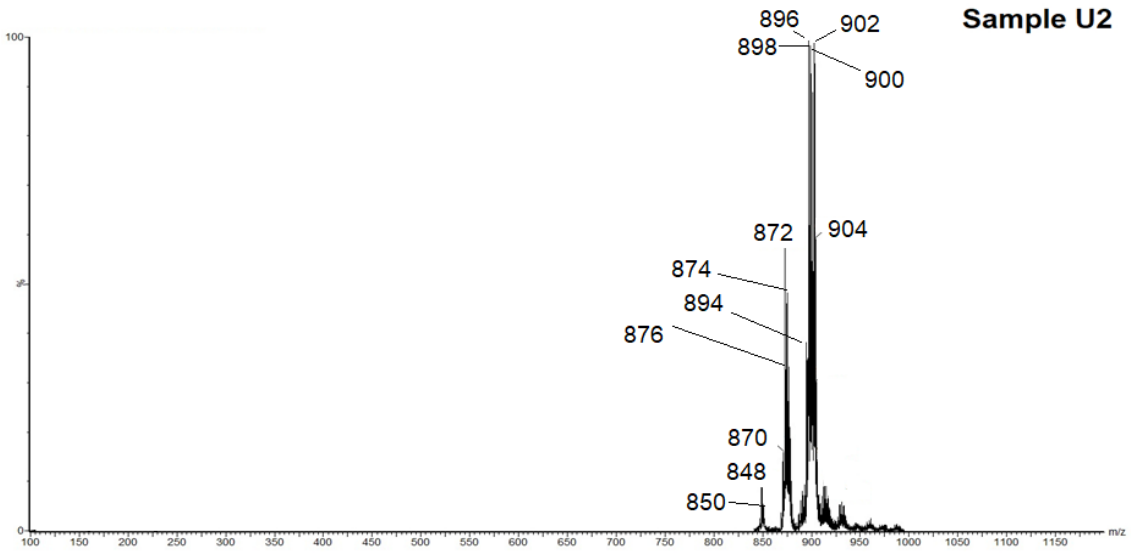


Figure 2 TAG profile by direct infusion ESI(+)-MS of the U2 grape seed oil sample.

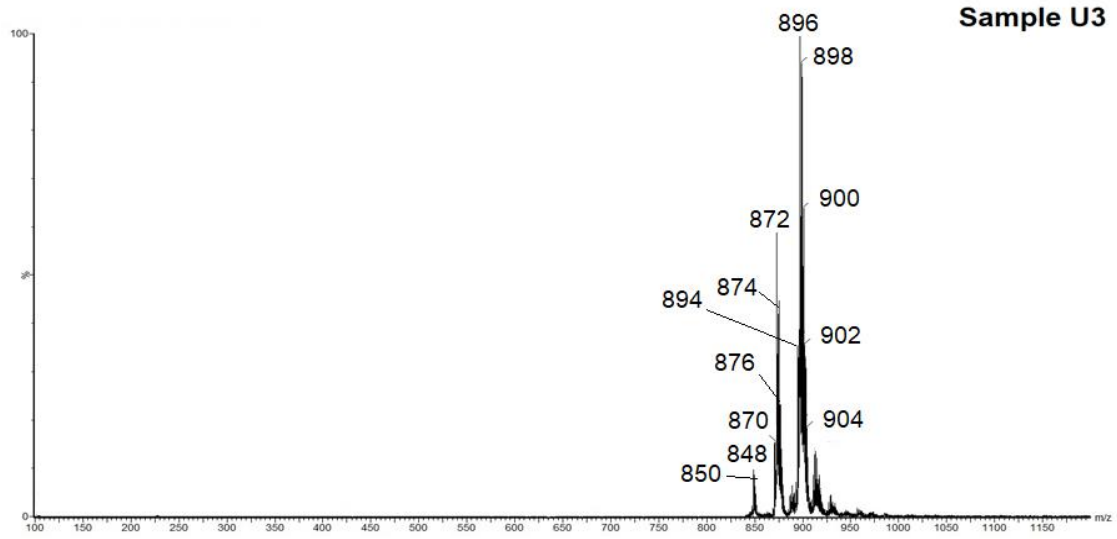


Figure S3 TAG profile by direct infusion ESI(+)-MS of the U3 grape seed oil sample.

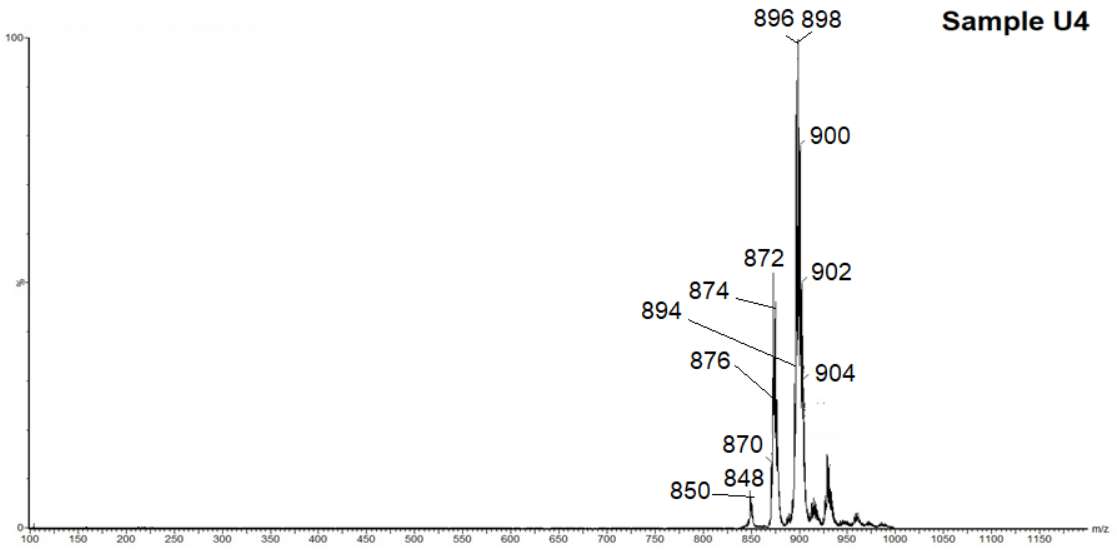


Figure S4 TAG profile by direct infusion ESI(+)-MS of the U4 grape seed oil sample.

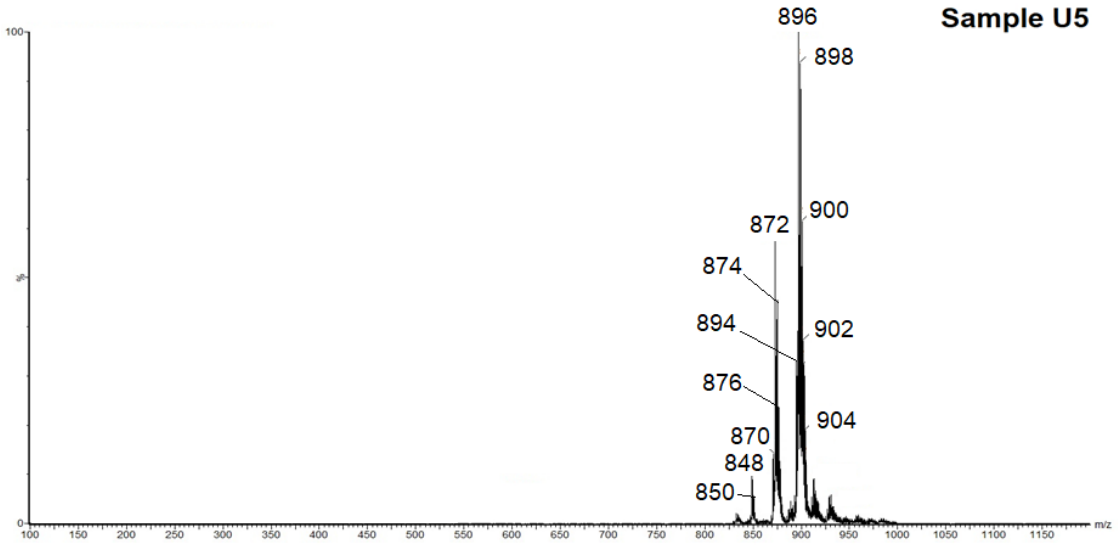


Figure S5 TAG profile by direct infusion ESI(+)-MS of the U5 grape seed oil sample.

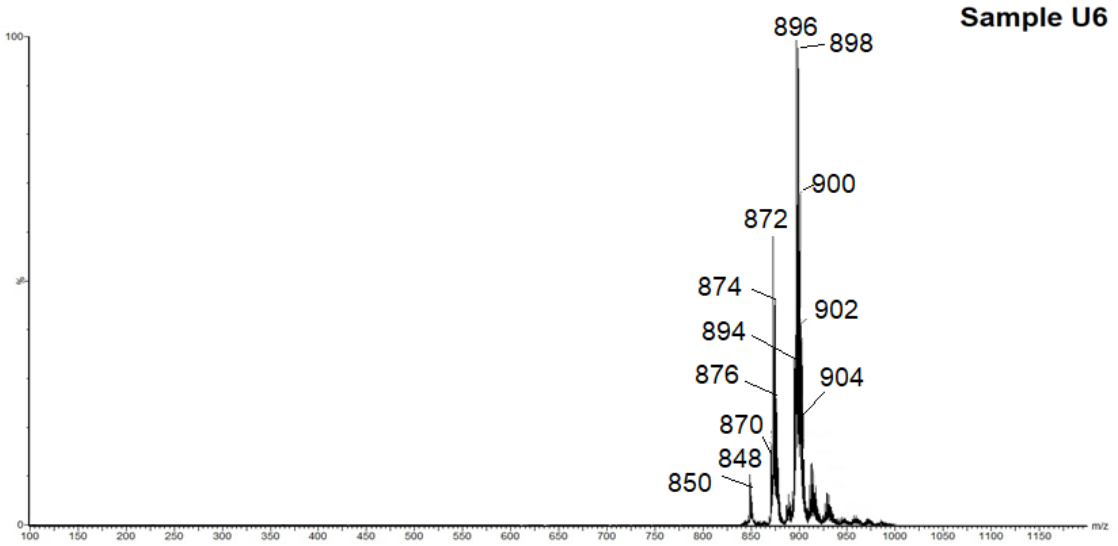


Figure S6 TAG profile by direct infusion ESI(+)-MS of the U6 grape seed oil sample.

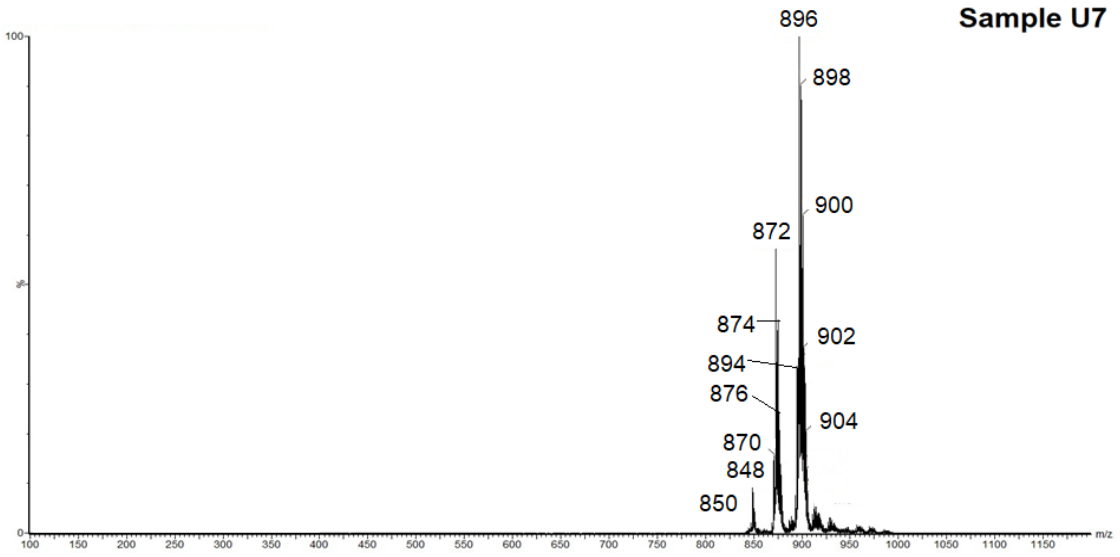


Figure S7 TAG profile by direct infusion ESI(+)-MS of the U7 grape seed oil sample.

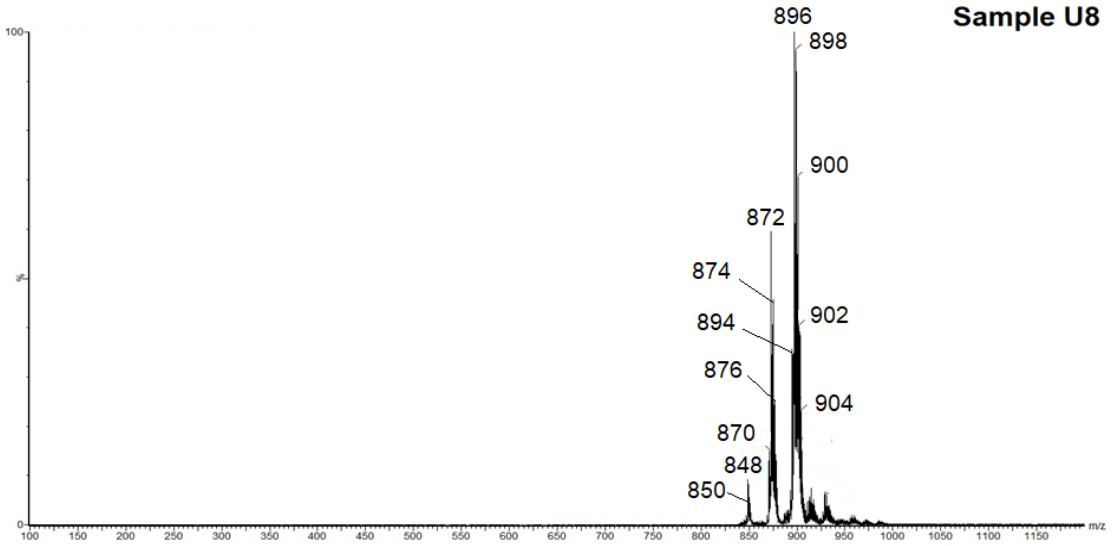


Figure S8 TAG profile by direct infusion ESI(+)-MS of the U8 grape seed oil sample.

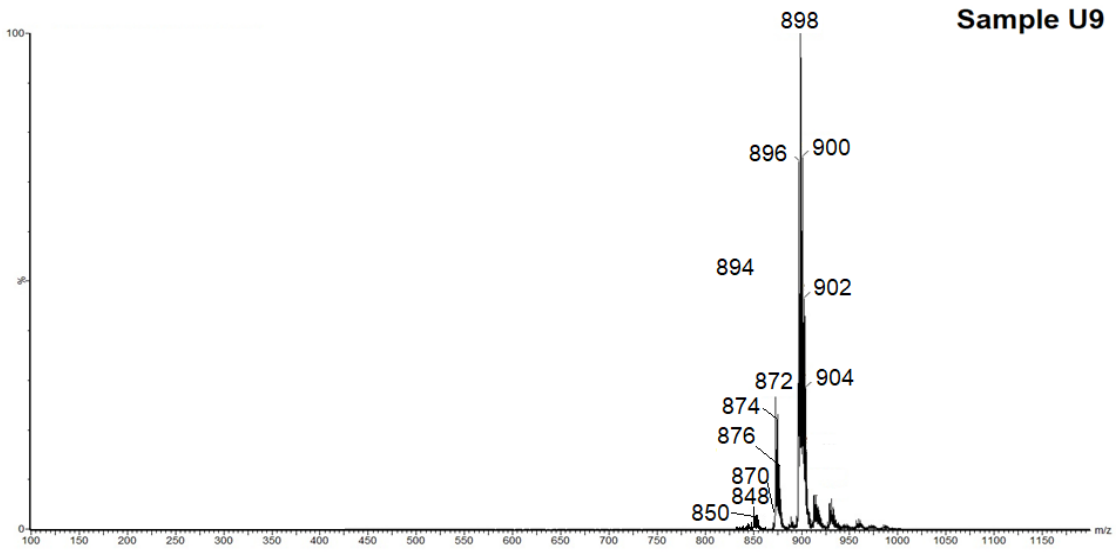


Figure S9 TAG profile by direct infusion ESI(+)-MS of the U9 grape seed oil sample.

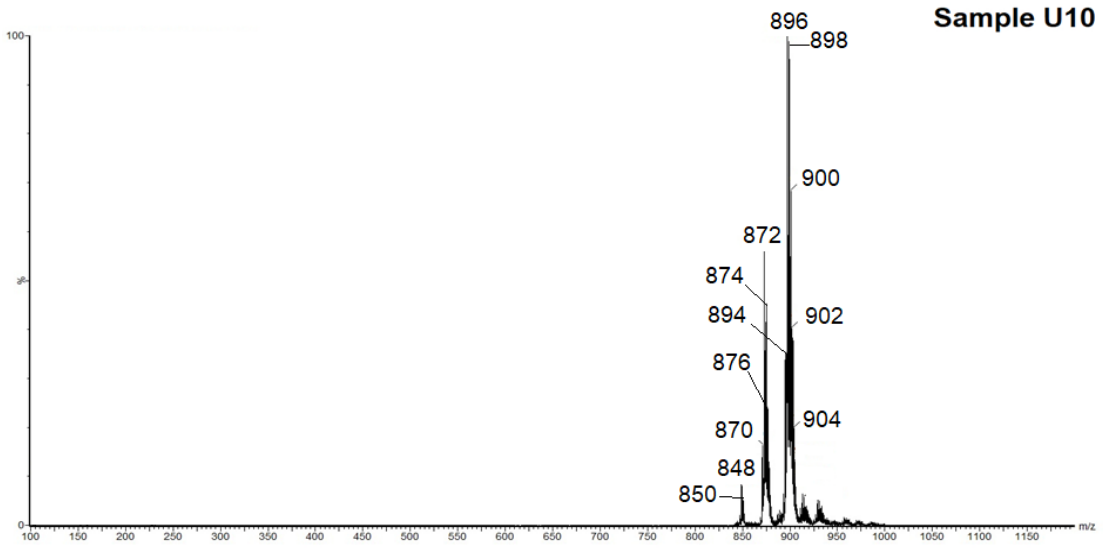


Figure S10 TAG profile by direct infusion ESI(+)-MS of the U10 grape seed oil sample.