OXIDATIVE STABILITY AND FATTY ACID PROFILE OF REFINED ECHIUM (*ECHIUM PLANTAGINEUM*) OIL

Estabilidade oxidativa e perfil lipídico do óleo de Echium (*Echium plantagineum*) refinado

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The refined *Echium* oil could be a new option of vegetable oil to be added in manufacture products, that's contents not only alpha-linolenic (ALA) ω3 fatty acid but also the stearidonic (SDA) ω3 fatty acid. However, this highly unsaturated fatty acids profile could lead to a limited shelf life due to lipid oxidation. In this context, this research aimed to analyze the oxidative stability (peroxide index and Rancimat method) and fatty acid profile of Refined *Echium* oil. The value of peroxide index found (0.242meq/kg) was lower than that established by Brazilian legislation (less than 10meq/kg), and the accelerated oxidation through Rancimat (8.79 hours) showed a high value. The oil presented a high value of total PUFAS (65%), containing approximately 40% of ω3 (28% of ALA and 12% of SDA). The results indicated that this Refined *Echium* Oil has a good stability oxidative and a great lipid profile, so a good alternative oil.

KEYWORDS: STEARIDONIC ACID; OMEGA-3; PUFAS.

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INTRODUCTION

Echium plantagineum belongs to the *Boraginaceae* family, native to Western and Southern Europe, North Africa, and Southwest Asia. It is cultivated because of the fatty acids present in its seeds, containing approximately 33% alpha-linolenic ω3 acid (ALA), 14.5% oleic acid, 15% ω6 linoleic acid, 13-15% omega-stearidonic ω3 (SDA) acid, and 12% gamma-linolenic ω6 acid (GLA) (BERTI et al., (2007)(Boraginaceae. JOHANSSON et al., (1997) suggested that *Echium* seed oil may contain 8-15% SDA. Therefore, it is an excellent vegetable oil source of ω3 because contains the important ω3 fatty acids: ALA (C18:3 alpha-linolenic) and SDA (C18:4 stearidonic).

Although, information about *Echium* oil refined did not found in the literature. Once this refined *Echium* Oil (>10% SDA) was authorized by the European Commission as a new food (2008) (BARROS, 2008).

The ALA, found in some vegetables and vegetable oils, is converted to EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) that promote beneficial effects on human health. This metabolic process is mediated by enzymes called elongases and desaturases, which participate in the transformation of ω3 and ω6 PUFAS (polyunsaturated fatty acids), resulting in a metabolic competition between the two omega fatty acids. Linoleic fatty acid (LA), found in some vegetable oils, is a source of ω6. An excess of this fatty acid competes with the metabolic process of ALA and the transformation into derivatives as EPA and DHA. This means that there should be an adequate ω-6:ω-3 ratio in the diet (SIMOPOULOS, 2004)and have excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA.

The Food and Agriculture Organization of the United States and the World Health Organization (FAO/WHO, 2003) recommends a ω6:ω3 ratio of 5:1, whereas the proportion of 10:1 (HARRIS et al., 2008) (TEICHERT & AKOH, 2011) or even 7:1 (SANTOS et al., 2013) may cause adverse effects so could be considered harmful to health.

Review research about ω6:ω3 ratio intake in Occidental countries showed ratios between 10:1, 20:1, and 50:1 (SIMOPOULOS, 2004). In Brazil, according to the guidelines of the Cardiology Brazilian Society (SANTOS et al., 2013), the ω6:ω3 ratio should be lower than that of the present one that is between 15:1 to promote general health.

For this reason, other natural sources of ω3 have been extensively studied to increase their consumption in the population, including studies of processed foods: linseed oil in mortadella (BERASATEGI et al., 2011) (CÂMARA & POLLONIO, 2015), sausage with olive, linseed and fish oil (SALCEDO-SANDOVAL et al., 2013), sausage with encapsulated fish oil (LORENZO et al., 2016), canola, linseed, soybean and olive oils in mortadella (YUNES et al., 2013), soybean oil enriched with SDA in margarine (PANDE et al., 2012), *Echium* oil in yogurt (COMUNIAN et al., 2017).

Besides that, some researchers have been shown that biosynthesis converting ALA to EPA is deficient, around 0.01-8%, and even lower from ALA to DHA due to the Δ6 desaturase enzyme, which is limited in humans and also due to the affinity of that enzyme for both fatty acids ω6 and ω3, although the enzyme has a higher specificity for ω3 than ω6, for this reason, a smaller quantity of ω3 are necessary (HARRIS et al., 2008) (TEICHERT & AKOH, 2011).

The SDA is a better precursor in the PUFAS biosynthesis of EPA and DHA in the human body than the source of ALA that demonstrates less effectiveness in this conversion is due to the non-competition of SDA by the enzyme Δ6 desaturase (JAMES et al., 2003). HARRIS et al. (2008) confirmed the increase of EPA in the body in individuals with diets of soybean oil enriched with 10.59% of ALA and 16.56% of SDA, compared to individuals that consumed only soybean oil (7.60% of ALA). LEMKE et al. (2010) also obtained similar results when comparing a diet of soybean oil enriched with SDA with a diet of regular soybean oil and also with oil containing EPA (HARRIS et al., 2008) (JAMES et al., 2003) (KRUL et al., 2012).

WALKER et al. (2013) reviewed the effects of SDA supplementation on human health,

stating that three studies positively assessed the increase of EPA in subjects that intake a genetically modified soybean oil enriched with SDA. However, to the best of our knowledge, there are no published studies in the scientific literature relating aspects of the addition of *Echium* oil, as a source of SDA, in a food matrix to evaluate the oxidation stability.

The SDA is a fatty acid with four unsaturations (18:4, ω-3), which makes it more susceptible to lipid oxidation that could affect the characteristics of the processed food (GRAY et al., 2010).

In this context, the main objective of this research was to evaluate the oxidative stability and lipid profile of *Echium* Oil to check if this could be considered as a new oil with health benefits and could be a good alternative to increase the consumption of ω3 in the population.

MATERIAL AND METHODS

MATERIAL

Echium Oil (15200 Newmega™ *Echium* Oil Ref) was imported from *De Wit Specialty Oils* headquartered in the Netherlands, batch number: 0808, shelf-life: march/2018.

DETERMINATION OF THE FATTY ACID PROFILE BY GAS CHROMATOGRAPHY

Briefly, 4 g samples were mixed in proportion to 1:1:2:0.8 with methanol, chloroform, and water respectively. The solution was homogenized with 4 mL of chloroform, following the addition of 4 mL of 0.88% NaCl solution. The mixture was centrifuged at 13.000 x g for 2 min, and the lower layer was collected and evaporated under nitrogen. Oil extracted from the samples (10 mg) was esterified as described by SHIRAI et al. (2005). Oil samples (10 mg) were added in tubes containing 1 mg of IS (Tricosanoic Acid Methyl Ester (C23:0), 50 µL 0.5% BHT, and 1 mL 0.5 M methanolic NaOH. The solution was vortexed for 15 sec and heated in a water bath at 100oC/5 min. After cooling, samples were mixed with 2 mL 14% BF3 in methanol, vortexed, and heated in a water bath at 100ºC for 5 min more. After cooling, 1 mL isooctane was added. The tubes were vigorously shaken for 30 sec, 5 mL of a saturated solution of NaCl were added, and the tubes were gently homogenized. After centrifugation at 13,000 x g/5 min, the organic phase was transferred to a new vessel and dried under a nitrogen stream. The recovered lipids were reconstituted in 0.5 mL isooctane

Echium oil fatty acid quantification was determined by GC chromatography, equipped with a G3243A MS detector (Agilent 7890 A GC System, Agilent Technologies Inc., Santa Clara, USA). Fatty acids were identified by NIST 11 by comparing the retention time with four purified standards mixed with fatty acid methyl ester (Sigma Chemical Co.: 4-7801; 47085-U; 49453-U and 47885-U). Results were expressed as g fatty acid/100g oil.

DETERMINATION OF THE PEROXIDE VALUE (PV)

The peroxide value was determined to quantify the formation of the primary compounds of the lipid oxidation process, the peroxides. This evaluated all the substances, in milliequivalents of peroxide per kg of the sample (meq/kg), that oxidize the potassium iodide. The determination was performed using official methodology: AOCS Ca 5 a-40 (1996).

ACCELERATED OXIDATION ANALYSIS BY THE RANCIMAT METHOD

The Official Method from International Organization for Standardization (ISO 6886) (1996) was used to determine the induction period (IP) in hours (the time when the product remained stable to oxidation). A higher IP value suggests greater stability of the oil or fat evaluated. The determination

of oxidative stability by Rancimat (743, Metrohm, Switzerland) is based on increased electrical conductivity developed by HADORN & ZURCHER (1974). Rancimat is an accelerated oxidation method. The acceleration was caused by heating (temperature = 90ºC in the present study) the fat or oil (2.5 g ± 0.01) and injecting atmospheric air (20 L/h ± 1). The IP result was determined by the conductivity reading generated by the volatile substances (ketones, esters, ether) formed during the oxidation.

RESULTS AND DISCUSSION

The peroxide value obtained for the *Echium* oil was 0.242 ± 0.044 (meq/kg). The peroxide value obtained for the *Echium* oil presented fewer results than the technical regulatory (BRASIL, 2005) for vegetable oils and fats from Brazil, and the maximum peroxide value for refined oils is 10 meq/kg. This shows that the *Echium* oil evaluated was in good conditions to use, presenting peroxide value below this established oxidation parameter. According to SILVA *et al*. (1999), this oxidation parameter evaluation determination should occur in the oxidative process first stages because the peroxide value could vary during the product storage.

Usually, the commercial oils show peroxide values between 0.1 and 0.70, such as soybean oil, 0.27 meq/kg, presented by LIMA & GONÇALVES (1994), 0.62 meq/kg of sunflower oil (ANGELO & JORGE, 2008) and 0.10 meq/kg of palm oil (GRIMALDI, 2005). Once that, antioxidants are added to the commercial oils to keep the oxidation stability during the storage period. Although, SILVA et al. (1999) affirmed that peroxides values do not constitute a guarantee of good oxidative stability of oils.

The Rancimat index result (accelerated oxidation method) for *Echium* oil was 8.79 ± 0.08 hours of induction period (IP). Similarly, ANTONIASSI (2001) found an induction period value of 6.2, 12.4, and 14.0 h for sunflower, corn, and canola oils, respectively. The value found for the *Echium* oil of 8.79 h can be considered good since its composition contains stearidonic acid. This presents four unsaturation, which could increase its autoxidation potential exponentially. According to SILVA et al. (1999), oils autoxidation depends on the unsaturation in the fatty acid For this reason, vegetable oils exhibited greater susceptibility to deterioration than animal fats.

This result of the induction period of *Echium* oil could be explained by antioxidants contained in this refined oil, present naturally or added by a producer (that did not inform). GRAY et al. (2010) showed the quantity of 6% and 16.5% of Alpha-Tocopherol (natural antioxidant) in seed and crude oil of *Echium*. The prevention of lipid oxidation can be done by adding antioxidants to maintain the quality and prolong the oils and foods' shelf life (RAMALHO & JORGE, 2006).

The fatty acid profile of *Echium* oil can be observed in Table 1. The lipid profile of *Echium* Oil presented 65 g/100g of polyunsaturated fatty acids, 40 g/100g of these from ω3 and 24 g/100g from ω6, a larger quantity of ω3. According to NETTLETON (1994), vegetable oils with the highest polyunsaturated fatty acids (excluding monounsaturated fatty acids) proportion are safflower, sunflower, and soybean oils values of 78, 69, and 61 g/100g, respectively. Among these three vegetable oils, soybean oil has the highest amount of ω-3 (7%), coming from alpha-linolenic acid, almost six times lower than *Echium* oil.

Similar to *Echium* oil, linseed oil presented 66.8 g/100g polyunsaturated fatty acids in its composition being 46 g/100g from ω3 (NOVELLO & POLLONIO, 2012). However, all the linseed oil ω3 comes from alpha-linolenic acid, which competes metabolically with ω6 (MARTIN et al., 2006). GRAY et al. (2010) evaluated the seed's fatty acid profile, and crude oil bodies of *Echium* presented similar values of ALA and SDA of this present study: 33.77 and 33.65 g ALA/100g and 13.31 and 13.66 g SDA/100g to seed and crude oil, respectively.

Thus, it can be stated that the *Echium* oil of this present study could be a better source of ω3 because it contains 40% of this relevant fatty acid, and it does not come exclusively from C18:3 (ALA). A good amount (12%) comes from stearidonic acid (C18:4 SDA), which has no metabolic

competition by the Δ6-desaturase enzyme used.

This Refined Oil presented an excellent ω6:ω3 ratio of 1:1.6, comparing with other oils such as 7.7:1 of soybean oil and 2.6:1 of canola oil (NETTLETON, 1994), both with a higher quantity of ω6 than ω3 than *Echium* oil. On the other hand, according to NOVELLO & POLLONIO (2012), linseed oil could present a 1:3 ratio. Oils containing good ω3 proportion compared with ω6 regards to nutritional indexes: atherogenic index (AI) and thrombogenic index (TI), that are related to the risk of cardiovascular diseases, serum triacylglycerols, blood pressure, and glucose modulation in metabolism (GARAFFO et al., 2011). High values of these indices indicate an increase in these health risks.

In this context, and evaluating the *Echium* oil lipid profile results, it can be affirmed that this oil is a suitable nutritional source.

CONCLUSION

The Refined *Echium* Oil presented good oxidation stability analysis parameters according to the Brazilian regulatory of vegetable oils. Besides that, this oil showed an excellent lipid profile, with a high value of ω3, is an option to improve the lipid profile of products foods and better alternative enriching with a new and better source of ω3, compared with traditional vegetable oils.

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DECLARATION OF CONFLICTING INTERESTS

We have no conflict of interest to declare.

AUTHOR'S CONTRIBUTION

Manoela Alves Pires ex-student from PhD of Food Science and Engineering of São Paulo University (FZEA/USP). Responsible to had analyzed the *Echium* Oil and wrote the article.

Marco Antonio Trindade a Professor from Department of Food Engineering of São Paulo University (FZEA/USP). Advisor of research and wrote the article.

RESUMO

O óleo de *Echium* refinado pode ser uma nova opção de óleo vegetal a ser adicionado em produtos industrializados, contendo na sua composição não somente o ácido graxo alfa-linolênico (ALA) ω3, como também o ácido graxo estearidônico (SDA) ω3. No entanto, esse perfil de ácidos graxos, altamente insaturados, pode levar a um prazo de validade limitado devido à oxidação lipídica. Nesse contexto, esta pesquisa teve como objetivo analisar a estabilidade oxidativa (índice de peróxido e método de Rancimat) e o perfil de ácidos graxos do óleo de *Echium* refinado. O valor do índice de peróxido encontrado (0,242 meq/kg) foi inferior ao estabelecido pela legislação brasileira (inferior a 10 meq/kg) e a oxidação acelerada pelo Rancimat (8,79 horas) apresentou alto valor. O óleo apresentou alto valor total de ácidos graxos poli-insaturados (65 g/100g) contendo, aproximadamente, 40 g/100g de ω3 (28 g/100g de ALA e 12 g/100g de SDA). Os resultados indicaram que este óleo de *Echium* refinado possui uma boa estabilidade oxidativa e um ótimo perfil lipídico, sendo uma boa alternativa de óleo vegetal.

Palavras-chave: Ácido estearidônico; Omega-3; PUFAs.

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TABLE 1 - LIPID PROFILE OF THE REFINED ECHIUM OIL (G/100G)

Fatty Acid	Mean	± Error
Palmitic acid C16:0	11.612	0.350
Stearic acid C18:0	7.567	0.449
Arachidic acid C20:0	0.095	0.013
Σ Saturated	**19.274**	
Elaidic acid C18:1 ω9*trans*	14.147	0.092
Oleic acid C18:1 ω9*cis*	0.498	0.033
Gadoleic acid C20:1 ω9	0.864	0.037
Erucic acid C22:1 ω9	0.150	0.002
Σ Monounsaturated	**15.659**	
Linoleic acid C18:2 ω6 (LNA)	14.212	0.163
Gamma-linolenic acid C18:3 ω6 (GLA)	10.110	0.122
Σ ω6	**24.323**	
Alpha-linolenic acid C18:3 ω3 (ALA)	28.646	0.399
Stearidonic acid C18:4 ω3 (SDA)	12.098	0.118
Σ ω3	**40.744**	
ω6/ω3	**1:1.6 (0.625)**	
Σ polyunsaturated	**65.067**	