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EVALUATION OF THE OXIDATIVE STABILITY OF ASCORBYL OLEATE OVER OLIVE OIL

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ABSTRACT

Natural compounds capable of reducing or neutralizing free radicals are increasingly used as antioxidants in foods and cosmetics. This study aimed to evaluate the stability of enzymatically synthesized ascorbyl oleate in olive oil. The synthesis was conducted using commercial lipase NS 88011 under optimized conditions (molar ratio of 1:9 L-ascorbic acid/oleic acid, at 70 °C for 1 hour). Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method at a wavelength of 515 nm. Ascorbic acid and isolated ascorbyl oleate were applied in an oxidative stability test of olive oil at 80 °C, assessing fatty acid content, acidity, iodine, and refractive indices. The IC_{50} value (concentration required to capture 50% of DPPH radical) for ascorbic acid and ascorbyl oleate were $3.22.10^{-6}$ mg/mL and 0.03671 mg/mL, respectively. Despite its lower antioxidant potential, ascorbyl oleate demonstrated efficacy in enhancing oxidative stability, thereby extending the shelf life of olive oil compared to both the control and ascorbic acid. Hence, the natural antioxidant ascorbyl oleate holds promise for application as an ingredient in the food and pharmaceutical industries.

Keywords: Biocatalysis. Antioxidant. Lipid oxidation

1 INTRODUCTION

Natural antioxidants are increasingly used in industrialized food formulations, emphasizing L-ascorbic acid (Vitamin C), widely used in water-based products, as its polar nature limits its application in products derived from oil and fat. Furthermore, ascorbic acid exposed to oxygen and light is easily oxidized, being degraded first to dehydroascorbic acid and then irreversibly to oxalic acid.[1]

To overcome these limitations, ascorbic acid can be esterified with a fatty acid generating a lipophilic product that combines antioxidant and lipophilic properties of its precursor.[2] This work evaluated the oxidative stability of olive oil treated with ascorbyl oleate, which was synthesized enzymatically.

2 MATERIAL & METHODS

The synthesis of ascorbyl oleate was carried out through biocatalysis under the conditions maximized by Dalla Costa et al.[3] using L-ascorbic acid (99% Sigma-Aldrich) and oleic acid (97% Sigma-Aldrich) as substrates in the molar ratio of 1:9 and lipase NS 88011 (30% w/w substrates) in 50 mL of tert-butanol solvent (P.A. Merck). The reaction's condition was 70 °C, 125 rpm 2% (w/w) molecular sieve (4A Sigma-Aldrich) for 1 h. After, the biocatalyst and molecular sieve were filtered and excess ascorbic acid was removed by liquid-liquid extraction with 20 mL of distilled water (3 times).

Product isolation was performed using column chromatography with flash silica gel 60 and eluent (hexane: ethyl acetate, 1:1 (v/v)). The fractions were monitored by thin layer chromatography (TLC) with a mixture of chloroform: methanol: acetic acid: water (81:9:8:2 v/v), respectively, and as developer a 5% solution of sulfuric acid in ethanol. After, the chromatosheets were heated to 110 °C for 30 min.

Antioxidant activity by the free radical scavenging method DPPH (2,2 diphenyl-1-picryl hydrazyl) at a wavelength of 515 nm. The technique consists of incubating for 30 min 500 µL of 0.1 mM DPPH solution and 500 µL of solution containing increasing concentrations of the sample (0.01; 0.025; 0.05; 0.075; 0.1; 0.25; 0.5; 0.75; 1; 1.25; 5; 7.5; 10 mg/mL) in ethanol (Êxodo Cientifica, 96% purity). For control, 500 µL of DPPH was used with 500 µL of ethanol and the blank was analyzed without DPPH. The percentage of DPPH radical uptake was calculated in terms of percentage of antioxidant activity (AA%) according to Equation 1.

$$AA\% = \frac{100\text{-}[(\text{sample absorbance} - \text{ethanol absorbance}) * 100]}{\text{control absorbance}} \quad (1)$$

With the results of the concentration and the percentage of antioxidant activity, the sample concentration necessary to capture 50% of the DPPH free radical (IC_{50}) was calculated by linear regression analysis.[4]

The accelerated oxidative stability of olive oil was carried out in an oven (Model TE - 393/1, Tecnal®, Brazil) with extra virgin olive oil (Vale Fértil). For the test, a control sample (olive oil without added antioxidants) and two other samples with the addition of ascorbic acid and ascorbyl oleate were evaluated. The olive oil samples (25 g) were placed in beakers and sealed with aluminum foil. The volume of antioxidants added to the olive oil was based on current legislation on natural antioxidants in extra virgin olive oil, which indicates a maximum limit of 0.03 g per 100 g. All tests were randomly distributed in the oven (80 ºC) and, at the end of each established interval (0, 5, 7, and 10 days). A sample from each treatment was collected and subjected to evaluation by physicochemical parameters: index peroxide, free fatty acid content, iodine index (Wijis solution), and refractive index (Refractometer Model 2WAJ, Biobrix®, Brazil) based on the methodology described by the Adolfo Lutz Institute.[5]

3 RESULTS & DISCUSSION

Ascorbic acid had an IC_{50} value of $3.22.10^{-6}$, while ascorbyl oleate had an IC_{50} of 0.03671 mg/mL. The synthetic antioxidants, butylhydroxyanise (BHA) and butylhydroxytoluene (BHT) presented IC_{50} values of 0.00413 and 0.01767 mg/mL by the DPPH method respectively. Although ascorbyl oleate has lower antioxidant potential, it does not have the disadvantages of commercial synthetic antioxidants that have side effects, such as impaired coagulation, mutation, and blood tumors [6], which led to a ban on foods in the United Kingdom, Japan and other European countries.[7]

Table 1 presents the results of peroxide indices, free fatty acid content, iodine, and refraction for pure olive oil, and olive oil with the addition of ascorbic acid and with ascorbyl oleate.

Table 1 Effect of olive oil samples with and without the addition of antioxidant (ascorbyl oleate (AO) and ascorbic acid (AA)) with the indices: peroxide, iodine, refraction, and the content of free fatty acids conditioned in 80 ºC.

	Treatments	Day 0	Day 5	Day 7	Day 10
peroxide indice (meq/g of sample)	Olive oil	$10,178^{dA}$±0,346	$23,895^{cB}$±0,852	$36,457^{bB}$±0,634	$66,216^{aB}$±0,944
	With AA	$11,173^{dA}$±0,302	$25,996^{cA}$±1,037	$38,724^{bA}$±0,600	$69,879^{aA}$±1,151
	With OA	$10,346^{cA}$±0,466	$11,821^{cC}$±0,239	$20,558^{bC}$±0,107	$22,966^{aC}$±0,899
free fatty acid content (% m/m)	Olive oil	$0,776^{cA}$±0,030	$0,891^{bcA}$±0,015	$0,968^{bA}$±0,016	$1,139^{aA}$±0,060
	With AA	$0,770^{cA}$±0,006	$0,880^{bcA}$±0,021	$0,982^{bA}$±0,045	$1,123^{aA}$±0,131
	With OA	$0,759^{bA}$±0,008	$0,869^{abA}$±0,015	$0,944^{aA}$±0,022	$0,950^{aB}$±0,005
Refraction indice	Olive oil	$1,4631^{cA}$±0,0002	$1,4638^{bA}$±0,0002	$1,4643^{abA}$±0,0005	$1,4648^{aA}$±0,0002
	With AA	$1,4631^{cA}$±0,0001	$1,4639^{bA}$±0,0004	$1,4639^{bAB}$±0,0003	$1,4648^{aA}$±0,0001
	With OA	$1,4632^{bA}$±0,0001	$1,4634^{abA}$±0,0002	$1,4635^{abB}$±0,0001	$1,4639^{aB}$±0,0003
Iodine indice (g of I_2/100g of olive oil)	Olive oil	$83,313^{aB}$±1,497	$78,492^{bA}$±0,631	$75,660^{bB}$±0,779	$64,596^{cB}$±2,332
	With AA	$84,750^{aAB}$±2,260	$78,026^{bA}$±1,590	$74,630^{bB}$±1,745	$65,081^{cB}$±2,351
	With OA	$88,991^{aA}$±0,215	$82,505^{bA}$±0,522	$81,107^{bcA}$±1,289	$77,606^{cA}$±1,537

Mean ± standard deviation. Different lowercase letters in the rows and uppercase letters in the columns for each index imply a significant difference ($P<0.05$) by the Tukey Test.

The olive oil sample with the addition of ascorbyl oleate showed better responses in all tests. As expected, ascorbic acid was ineffective in maintaining oxidative stability as it is insoluble in olive oil. The increase in peroxide index indicated the formation of primary products of lipid oxidation. However, the increase in the peroxide index in an olive oil sample with the addition of ascorbyl oleate was 3 times lower (22.966 meq/1000 g of sample) when compared to pure olive oil (66.216 meq/1000 g of sample) after 10 days of incubation in an oven at 80 °C. This demonstrated that the treatment with ascorbic acid greatly exceeded the limit value of the critical oxidation point permitted by Brazilian legislation (20 meq/1000g) [8] suggesting the possible application of ascorbyl oleate with preservative natural, due to better resistance to peroxidation.

The action of light and heating accelerates the hydrolysis and oxidation process of the olive oil. The increase in free fatty acid content from the 5th day onwards, above the maximum acceptable limit (0.8%) by Brazilian legislation, is evidence of this process.[5,8] However, in the sample with ascorbyl oleate, the free fatty acid content was lower than in pure olive oil, as it remained stable and without a significant difference from the 7th to the 10th day of storage under critical conditions.

The refractive index values showed an increasing trend in the analyzed samples, probably due to the breaking of double bonds or polymerization of some compounds caused by heating. [9] The sample containing ascorbyl oleate was the one that showed the smallest variation in the refractive index, statistically differing from the others ($p<0.05$) after 10 days of packaging. Now, the iodine index indicates the preservation of unsaturations in the samples after heating, which was evaluated by the iodine consumption that occurs during a halogenation reaction. As we can see, once again the sample with ascorbyl oleate showed the best response, with a significantly higher iodine index ($p<0.05$) than the others, remaining following what is permitted by legislation (75-94 g of I_2/100 g of olive oil). [8]

The results highlighted the low performance of ascorbic acid, due to its polar nature, being insoluble in products with high lipid content, which makes it unsuitable for application in oils and similar products. In a previous study, Watanabe et al. [10] evaluated the behavior of ascorbic acid and some derivatives and observed that all ascorbates tested exhibited a superior antioxidant effect than ascorbic acid on the oxidative stability of linoleic acid, corroborating our study. This ability of ascorbyl oleate to delay lipid oxidation of olive oil may be related to the ability of ascorbyl esters to interact synergistically with chelators and primary antioxidants, such as tocopherol, present in olive oil, reducing its oxidized form. [11,12]

Previous studies also showed a positive effect of ascorbyl esters on the oxidative stability of various oils. [13-16] Extra virgin olive oil does not initially contain oxidation products due to the antioxidant effect of tocopherol and the low content of polyunsaturated fatty acids, [17] being less susceptible to deterioration due to the lower degree of establishment than other edible oils, such as soy. In many countries, the legislation does not allow preservatives with antioxidant effects on olive oils. However, based on the results obtained in this study, its use can be taken to increase the shelf life of other oils that can be added, such as soybean, canola, sunflower, and corn, among others. [18]

4 CONCLUSION

The IC_{50} values for ascorbic acid and ascorbyl oleate were $3.22.10^{-6}$ and 0.03671 mg/mL, respectively. Although the antioxidant potential of ascorbyl oleate is lower, the olive oil oxidative stability test demonstrated its capability to effectively protect olive oil from lipid oxidation and significantly increase its shelf life. In this context, ascorbyl oleate presents a promising prospect for application in the food and pharmaceutical industries due to its antioxidant properties. Additionally, it is considered a natural preservative since the catalyst and its precursors are of natural origin.

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