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Effect Of Oregano Essential Oil On Oxidative Stability Of Low-Acid Mayonnaise

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Abstract : Mayonnaise is an oil-in-water emulsion considered one of the most popular condiments in the world. Because of the high fat content, it is susceptible to deterioration due to autoxidation, thus requiring use of antioxidants to delay or prevent this process. The use of synthetic antioxidants has been largely replaced by the use of essential oils because they have good antioxidant properties that can protect food against rancidity. This study aimed to evaluate the effect of using oregano essential oil as a natural antioxidant in mayonnaise preparations with low acidity. The essential oil's antioxidant activity was evaluated using the DPPH assay. Samples were also evaluated for acidity, pH and water activity. The results showed that oregano essential oil is rich in phenolic compounds, with good antioxidant activity and acts to protect mayonnaise against oxidation reactions. We conclude that oregano essential oil can be used as a natural antioxidant.

Keywords - *lipid oxidation; natural antioxidant; peroxides; phenolic compounds*

I. INTRODUCTION

In Brazil, as in most countries, there is a tendency to use processed sauces in both homes and restaurants or other eating establishments. Mayonnaise is one of the oldest commercially produced condiments and also one of the most widely used worldwide [1]. According to Brazilian regulations, mayonnaise is a creamy product in the form of a stable oil-in-water emulsion prepared from vegetable oil, water and egg, plus other other ingredients within certain limits, and it must be sufficiently acidic to prevent hazardous deterioration under normal conditions [2]. During the manufacture of mayonnaise, vinegar and lactic acid are used to acidify the product and protect it from possible microbial contamination. Vinegar is used to keep the pH low (between 3.3 and 3.8), while lactic acid serves as a complementary source of acidity to reduce the vinegar taste [3]. In this respect, regular mayonnaise is sometimes rejected by consumers and replaced by low-acid mayonnaise (LAM), often homemade, which does not pass through the pasteurization process and has pH of around 4.5.

Because of its composition, low-acid mayonnaise is susceptible to deterioration, since the oil (which represents approximately 79%) makes the product vulnerable to lipid oxidation, in which free radicals are generated in the food matrix [4]. This oxidation is a major cause chemical deterioration of foods, particularly mayonnaise, which reduces the shelf life due to changes in color, aroma, taste and even viscosity, making it unacceptable to consumers and/or hazardous to eat. Preventing this requires the use of antioxidants to delay or prevent this process [5-6]. The use of synthetic antioxidants in foods has been questioned because of safety concerns. Therefore, natural products rich in phenolic compounds have gained attention because they exhibit a range of activities such as antioxidant, antimicrobial, antimutagenic and anti-inflammatory activities, as alternatives to prevent the oxidative deterioration of foods and reduce the use of synthetic antioxidants [7].

Tert-Butylhydroquinone, better known as TBHQ, is a potent antioxidant that can be added to processed foods. However, some toxicological studies have shown carcinogenic effects of this compound in animal experiments. For this reason, its use is not permitted in Canada and the European Union, since there is a possibility of harmful effects on human health. In Brazil, the use of synthetic antioxidants is controlled by the Ministry of Health, which determines maximum allowable concentrations for butyl-hydroxy-toluene, BHT (100 mg/kg) and butyl-hydroxy-anisole, BHA (200 mg/kg) [6].Due to the increase of public aversion to synthetic additives and preference for natural compounds, the so-called "green consumerism", many researchers have turned their attention to natural antioxidants, which can be obtained from essential oils of herbs and spices, medicinal plants, bark, fruit, mushrooms and cereals [8, 9, 10]. Oregano is a spice of great commercial

importance that has long been used as a flavor ingredient for salads, soups and meats. However, other biological properties of the extracts and essential oil of oregano (OEO) are of great interest to industry due to their radical scavenging properties [11, 12]. Essential oils (EO) are complex mixtures with low molecular weight, obtained from plant substances by steam distillation, water distillation or solvent extraction. The flavonoids, terpenoids and phenolic components of EO have significant antioxidant effects [13]. Essential oils have shown superior antioxidant activity in foods in relation to BHT. Oregano essential oil has been studied as an antioxidant in different types of food products, to increase their oxidative stability [14]. The phenolic compounds present in OEO, such as thymol and carvacrol, are able to donate electrons to reactive free radicals, making them more stable and non-reactive, giving the oil antioxidant activity [11].

Based on growing global concern over the use of synthetic antioxidants, this study aimed to evaluate the effect of using oregano essential oil as a natural antioxidant in low-acid mayonnaise.

II. MATERIAL AND METHODS

2.1 Raw material

Mayonnaise was made from soybean oil, egg yolk, vinegar and salt. Three different types of mayonnaise were produced and used in this experiment: control mayonnaise (CM, without the addition of antioxidant); mayonnaise with BHT (BHTM, adding 0.1% BHT during preparation); and mayonnaise with oregano essential oil (OEOM, adding 0.4% OEO during preparation). Eight samples were prepared of each kind of mayonnaise, stored in Falcon tubes (45 mL) and kept under refrigeration at ± 10 ° C for eight weeks. Each week on the day that preceded the analysis, 1 tube of each type of mayonnaise was removed from refrigeration and subjected to freezing.

2.2 Methods

2.2.1 Chemical analysis of OEO

The composition of OEOs was determined by gas chromatography coupled to mass spectrometry with electron impact ionization (GC-MS-IE). To identify the constituents of OEO, dilution of sample with hexane of was injected automatically (ALS injector HP-1100; Hewlett Packard, Palo Alto, CA, USA) in split mode (100:1). Separation and analysis were performed with HP 5890 gas chromatograph II coupled to mass spectrometer (HP 5973 MSD) and EI at 70 eV, monitored by the ChemStation software. Helium was used as carrier gas with constant flow of 1 cm3 /min. An HP-5 fused silica capillary column (30 m x 0.32 mm x 0.25 mm) was used, at temperatures of 250°C for the injector and 250°C for the interface between the chromatograph and the detector. The temperature cycle was initial column temperature of 40°C for 2 min, increased at the rate of 5°C/min up to 90°C and then at 10°C/ min to 250°C, which was maintained for 5 minutes, for a total run of 35 minutes.

2.2.2 Oil extraction method

According to the procedure described by Lagunes-Galvez and co-authors [15], the samples were frozen at ± 20 ° C for 24 hours and unfrozen for 2 h at ± 4 °C to break the emulsion. Then the mixtures were centrifuged at 2,000 rpm for 15 minutes. The lipid phase of the emulsion was separated from the residue and stored in a closed glass jar for later analysis.

2.2.3 Antioxidant activity of mayonnaise: DPPH radical elimination assay

The mayonnaise's antioxidant capacity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and stable as methanol extracts were prepared and samples of DPPH and Trolox solutions [16]. To determine free radical scavenging capacity, the IC_{50} value was defined as the amount of sample in mg/ml that produced a 50% decrease of the initial concentration of DPPH. A high IC_{50} value indicates low capacity to eliminate free radicals, while a low IC_{50} value indicates high capacity to scavenge free radicals.

2.2.4 Determination of the peroxide value

The peroxide content, expressed as milliequivalents of active oxygen per kg of oil (meq/kg), was determined by a standard procedure for mixtures of oil and acetic acid: chloroform was allowed to react with a potassium iodide solution in the dark, and then the free iodine was titrated with a sodium thiosulfate solution [17]. The amount of peroxide present in the sample was calculated using the equation:

Peroxide value (mEq/kg sample) = S x M x f x 1000/w

S = quantity in ml of sodium thiosulphate

M = molarity of the sodium thiosulphate solution

f = normal correction factor

w = sample weight in grams

2.2.5 Conjugated dienes and trienes

The content of conjugated dienes and trienes was measured by spectrophotometry (Shimadzu mini 1240 UV-Vis spectrophotometer) at 234 nm and 268 nm using as hexane as blank (AOCS Method Ti 1a-64). Oil samples were diluted 1:10 with hexane for subsequent reading [18]. Conjugated dienes and trienes were assessed once per week for eight weeks during storage. The concentration was expressed as percentage of dienes and trienes conjugated with dienoic acids. The results were expressed according to the formula [19]:

Conjugated dienes and trienes = B x V/w
B = absorbance reading at 234 and 268 nm
V = the volume (ml) of hexane employed
w= the mass (g) of the sample

2.2.6 pH value, acidity and water activity

The pH was determined by a Metrohm 785 DMP Titrino pH meter with direct reading at 25 °C (AOAC, 2005). The water activity (A_w) was determined using a Novasina Aw Center 503-C meter (Novasina AG, Zurich, Switzerland) at 25 °C. The acidity was determined with a Metrohm DMP 785 Titrino meter with NaOH reagent. All experiments were performed in triplicate, expressing the results as averages of the three readings.

2.3 Statistical analysis

The data were submitted to analysis of variance (ANOVA) with 95% significance level ($p < 0.05$) and the Tukey test with 95% significance level ($p < 0.05$) using the statistical software XLSTAT version 7.5 (2007).

III. RESULTS AND DISCUSSION

Using GC-MS-IE, nine volatile components were identified. Figure 1 shows the chromatogram of OEO. Carvacrol was the major component of OEO sample (70.3%), other compounds were identified (Figure 2). Authors identified ten volatile components in two samples of OEO: carvacrol, α-pinene, β-pinene, β-caryophyllene, D-limonene, linalool, caryophyllene oxide, and the monoterpene hydrocarbons γ-terpinene and p-cymene [20]. One of the most important factors in the study of essential oils applicability is the chemical composition, which can vary within the same plant due to factors related to biology (genetics, nutrition and development phase) besides those edafoclimatic (local climate and soil) [21].

Antioxidant activity is a complex process involving a number of different mechanisms, such as sequestration of free radicals, hydrogen donation and chelating of metal ions.

Phenolic compounds present in the oil act as antioxidants by donation of a hydrogen atom (which acts as a receiver of free radicals) by interrupting the chain reactions of oxidation or of chelating metals [22]. Oregano essential oil is rich in phenolic compounds. These compounds play a vital role in the neutralization and inhibition of free radicals [23]. Have been detected in OEO 87 monoterpenes, including carvacrol (5-Isopropyl-2-methylphenol), the oil's main component (representing 69.0% to 92.6% of the total oil composition), which is a monoterpenic phenol that acts to reduce lipid peroxidation, and thus has antioxidant activity in different lipid systems [20, 23].

Other author, studying *Origanum vulgare* L., found total phenolic content ranging between 32.05 and 72.82 mg/g [24]. These data reinforce the idea that carvacrol has antioxidant potential [25]. The methanol extract of the samples exhibited antioxidant activity in the DPPH test. Table 1 shows the IC50 results of the control, BHT and OEO samples, respectively.

BHTM showed antioxidant activity that was 4.8 and 1.7 times higher than the CM and OEOM samples, respectively, while CM had 2.9 times higher antioxidant activity than OEOM. Mitropoulou and co-authors found an IC50 value for carvacrol of 2.679± 0.127mg/mL [26]. This result suggests that the expected antioxidant activity of oregano essential oil is mainly attributed to carvacrol, its main constituent. Other authors have found different IC50 values than those found in our mayonnaise samples containing OEO. Hussain and co-authors found for *Origanum vulgare* L. an IC50 value of 65.5 mg/mL and for *Origanum majorana* L. a value of 89.2 mg/mL [27]. This difference may be due to the use of different solvents for extraction, geographical location of the plants, fertilization and climatic conditions, as well as to the fact that we studied OEO added to mayonnaise, whereas the last authors [27] analyzed OEO extracts alone. The activity of essential oils as antioxidants depends not only on their structure, but also the concentration, light, temperature, substrate type and physical state of the system.

Lipid oxidation occurs when atmospheric oxygen or oxygen dissolved in the oil reacts with unsaturated fatty acids. Food constituents, mainly lipids, always react with surrounding oxygen and cause rancidity in foods [28]. It has been classified as the main form of deterioration that affects the sensory and nutritional quality of foods [22]. Hydroperoxides are the primary oxidation products and are measured by means of peroxide index

values (PI). This index is very useful in predicting the onset of rancidity. Most methods of measuring lipid hydroperoxides are based on the ability of peroxide to oxidize indicator compounds [29].

In this study, we observed that the peroxide value of the samples increased with the storage time over the eight weeks, and during the entire period of observation, mayonnaise plus OEO had the lowest peroxide index (Table 2). According to Depree and Savage peroxide values tend to increase 15 days after storage [30].

Other products of the oxidation of lipids, such as conjugated dienes and trienes, can be measured by ultraviolet absorption at 232-270 nm (Figures 3 and 4). These measurements provide a good indication of changes occurring during the oxidation process, so their analysis is considered complementary to peroxide value, since because they are unstable, peroxides are quickly formed and broken into smaller compounds, while the conjugated dienes that simultaneously form remain in food [31].

The results of determinations of conjugated dienes do not report the degree of deterioration of oil because the effect of different oxidation of unsaturated fatty acids varies in quality and magnitude. However, variations in concentrations of conjugated dienes over time provide sufficient data for monitoring the oxidation of the same sample [32].

After the second week, the results showed significant difference between treatments. The oregano essential oil showed a protective effect against the formation of dienes compared to the control sample.

The behavior of the samples observed in the analysis of conjugated dienes was the same for the peroxide value: the value of conjugated dienes increased with storage time and the sample containing OEO had the lowest value of conjugated dienes. Conjugated diene hydroperoxides are produced during the first phase of lipid oxidation by the reaction between peroxyl radicals and oxygen ($^3O_2$). The autoxidation produces conjugated products [33], which absorb UV light at 232-234 nm, and their second oxidation products, such as ketones, which absorb at 268-272 nm [32].

The UV absorbance at 268 nm is a measure of conjugated trienes and secondary oxidation products such as dienals and cetodienos conjugates. The oxidation of polyunsaturated fatty acids can be analyzed by increasing the absorptivity in the range of ultraviolet spectrum [32].

The pH (Table 3) did not change statistically ($p > 0.05$) during storage, remaining between 4.5 and 3.5, in comparison with the recommended range of 3.0 to 3.5 [34]. Other author considered as a safe pH range between 4.0 and 4.10 or less than 4.5 [3]. These values cannot be considered very accurate, since they are affected by other factors acting simultaneously, like water activity. The low pH of all samples is due to the acetic acid present in the vinegar used to acidify the mayonnaise and assure microbiological control. The pH value has a significant effect on the viscosity and elasticity of mayonnaise, where pH above 3.9 makes mayonnaise viscoelastic [30].

The water activity (Table 4) values were not statistically different. The availability of water in a food can influence the metabolism and proliferation of microorganisms. The addition of NaCl to a food tends to reduce its water activity, as noted at the beginning of the storage of mayonnaise samples [35].

In the refining process, the acidity of vegetable oils is reduced, so its measurement can be used as a quality control measure. Non-enzymatic oxidation increases the acidity. Greater acidity (Table 5) can be observed in the control mayonnaise, which indicates the beginning of decomposition of lipids and the formation of free fatty acids. Thus, the value of acidity of the sample determines its condition [36].

IV. FIGURES AND TABLES

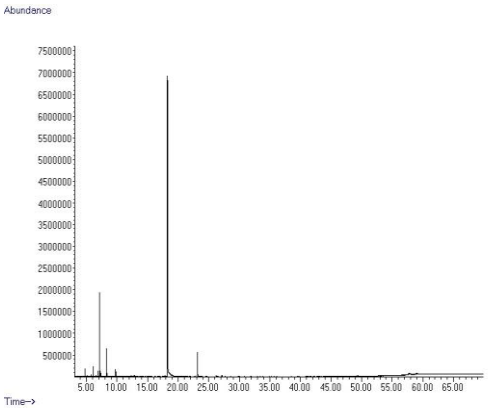

Figure 1- Chromatogram of OEO

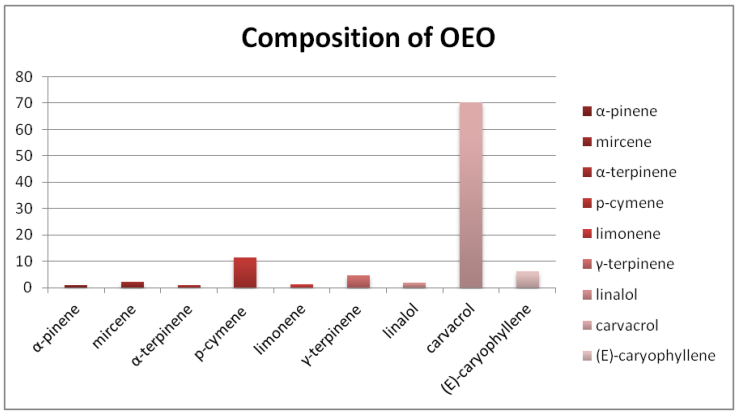

Figure 2- Composition of OEO

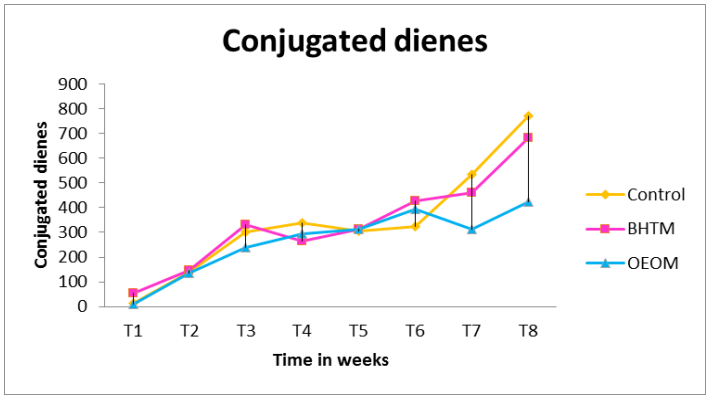

Figure 3- Evolution of formation of conjugated dienes during storage mayonnaise

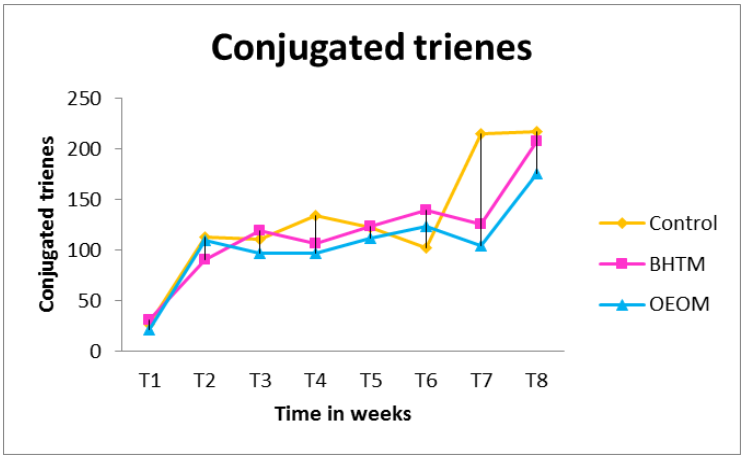

Figure 4- Evolution of formation of conjugated trienes during storage mayonnaise

TABLE 1. Antioxidant activity of the mayonnaise samples

Sample	**IC_{50}**	**µmol Trolox (equivalent/mg)**
CM	326 mg/mL	0.26
BHTM	67 mg/mL	1.25
OEOM	111 mg/mL	0.76

Average of two analytical results. CM = control mayonnaise; BHTM = mayonnaise with BHT; OEOM = mayonnaise with OEO

TABLE 2. Peroxide index (meq/kg) of mayonnaise samples

Time (weeks)	Mayonnaise Control	Mayonnaise BHT	Mayonnaise OEO
T_1	$1.35 \pm 0.07^{A,h}$	$1.44 \pm 0.32^{A,g}$	$1.02 \pm 0.03^{B,i}$
T_2	$1.41 \pm 0.17^{A,g}$	$1.38 \pm 0.30^{A,h}$	$1.38 \pm 0.13^{B,h}$
T_3	$1.55 \pm 0.63^{B,g}$	$1.34 \pm 0.21^{A,h}$	$1.24 \pm 0.07^{B,h}$
T_4	$1.77 \pm 0.62^{B,f}$	$1.55 \pm 0.08^{B,g}$	$1.48 \pm 0.05^{B,g}$
T_5	$3.44 \pm 0.68^{C,b}$	$2.30 \pm 0.31^{C,e}$	$1.77 \pm 0.15^{C,f}$
T_6	$3.07 \pm 0.11^{C,c}$	$3.22 \pm 1.07^{C,b}$	$2.12 \pm 0.03^{D,e}$
T_7	$3.67 \pm 1.46^{C,f}$	$3.18 \pm 0.94^{C,d}$	$2.23 \pm 0.60^{D,e}$
T_8	$3.70 \pm 0.99^{C,a}$	$3.25 \pm 0.99^{C,d}$	$2.68 \pm 0.51^{E,c}$

Values followed the same capital letter in the rows showed no statistical difference ($p < 0.05$). Values followed the same lower-case letter in the column showed no statistical difference ($p < 0.05$).

TABLE 3. Variation of the pH of the mayonnaise samples Control, BHT and OEO

Time (weeks)	Mayonnaise Control	Mayonnaise BHT	Mayonnaise OEO
T_1	$4.26\pm0.01^{A,m}$	$4.40\pm0.01^{A,n}$	$4.20\pm0.03^{A,m}$
T_2	$4.29\pm0.02^{A,j}$	$4.19\pm0.01^{B,l}$	$4.30\pm0.02^{A,j}$
T_3	$4.09\pm0.19^{B,h}$	$4.18\pm0.06^{B,i}$	$4.12\pm 0.03^{B,h}$
T_4	$4.18\pm0.12^{B,g}$	$4.19\pm0.02^{B,g}$	$4.16\pm0.05^{B,g}$
T_5	$4.53\pm0.04^{B,e}$	$3.90\pm0.02^{C,f}$	$3.90\pm0.01^{C,f}$
T_6	$4.22\pm 0.01^{A,c}$	$3.95\pm0.02^{C,d}$	$3.96\pm0.02^{C,d}$
T_7	$3.91\pm0.01^{C,b}$	$3.90\pm0.02^{C,b}$	$3.90\pm0.01^{C,b}$
T_8	$3.70\pm0.03^{C,a}$	$3.63\pm0.03^{D,a}$	$3.59\pm0.02^{D,b}$

Values followed the same capital letter in the rows showed no statistical difference ($p < 0.05$). Values followed the same lower-case letter in the column showed no statistical difference ($p < 0.05$).

TABLE 4. Variation of the water activity of the mayonnaise samples Control, BHT and OEO

Time (weeks)	Mayonnaise Control	Mayonnaise BHT	Mayonnaise OEO
T_1	$0.975\pm0.003^{A,a}$	$0.979\pm0.003^{A,a}$	$0.977\pm0.004^{A,d}$
T_2	$0.974\pm0.002^{A,e}$	$0.979\pm0.004^{A,d}$	$0.977\pm0.011^{A,a}$
T_3	$0.977\pm0.003^{A,d}$	$0.979\pm0.004^{A,d}$	$0.978\pm0.001^{A,b}$
T_4	$0.975\pm0.002^{A,d}$	$0.975\pm0.001^{A,d}$	$0.976\pm0.001^{A,g}$
T_5	$0.975\pm0.003^{A,d}$	$0.980\pm0.002^{B,f}$	$0.980\pm0.002^{A,f}$
T_6	$0.975\pm0.001^{A,d}$	$0.977\pm0.002^{A,d}$	$0.978\pm0.002^{A,d}$
T_7	$0.978\pm0.002^{A,b}$	$0.980\pm0.014^{C,c}$	$0.978\pm0.002^{A,b}$
T_8	$0.977\pm0.002^{A,d}$	$0.979\pm0.002^{A,a}$	$0.979\pm0.003^{A,a}$

Values followed the same capital letter in the rows showed no statistical difference ($p < 0.05$). Values followed the same lower-case letter in the column showed no statistical difference ($p < 0.05$).

TABLE 5. Variation of the acidity of the mayonnaise samples Control, BHT and OEO

Time (weeks)	Mayonnaise Control	Mayonnaise BHT	Mayonnaise OEO
T_1	$2.42\pm0.08^{A,g}$	$2.51\pm0.08^{E,a}$	$2.33\pm0.05^{C,e}$
T_2	$2.24\pm0.06^{A,g}$	$2.50\pm0.09^{E,a}$	$2.33\pm0.03^{C,e}$
T_3	$2.36\pm0.07^{B,f}$	$2.52\pm0.03^{E,a}$	$2.36\pm0.02^{C,e}$
T_4	$2.37\pm0.07^{B,f}$	$2.47\pm0.02^{E,a}$	$2.10\pm0.01^{C,e}$
T_5	$2.30\pm0.07^{D,d}$	$2.27\pm0.04^{D,d}$	$2.19\pm0.01^{F,b}$
T_6	$2.37\pm0.07^{B,f}$	$2.52\pm0.04^{E,a}$	$2.00\pm0.01^{F,b}$
T_7	$2.40\pm0.02^{A,g}$	$2.09\pm0.01^{F,b}$	$2.13\pm0.08^{F,b}$
T_8	$2.54\pm0.02^{A,g}$	$2.46\pm0.09^{G,c}$	$2.13\pm0.06^{G,c}$

Values followed the same capital letter in the rows showed no statistical difference ($p < 0.05$). Values followed the same lower-case letter in the column showed no statistical difference ($p < 0.05$).

V. CONCLUSION

In the low-acid mayonnaise, the most likely form of deterioration is lipid oxidation, so the oregano essential oil used as a natural antioxidant can delay the oxidation of oxidizable substrates such as foods like mayonnaise that are rich in lipids. The antioxidant activity of OEO is primarily correlated to the phenolic compounds, so the higher the concentration of phenolic compounds, the higher the antioxidant activity will be.

VI. ACKNOWLEDGEMENTS

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