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Antioxidative effect of *Arthrospira platensis* biomass on the lipid oxidation

Efeito antioxidante da biomassa de Arthrospira platensis sobre a oxidação lipídica

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ABSTRACT

Introduction: Cyanobacteria are promising natural sources of antioxidants. Environmental conditions that influence synthesis of substances in cultures of cyanobacteria have been studied. **Objective:** This investigation aimed to evaluate the antioxidative effect of *Arthrospira platensis* biomass on the process of lipid oxidation in bulk oil and oil/water emulsion. **Method:** *Arthrospira platensis* growth potential for antioxidant production under different cultivation conditions using an experimental design. The antioxidative effect of the methanolic extracts and the biomass on preventing lipid oxidation was measured by peroxide value (PV). **Results:** The results showed that the growing conditions to obtain the biomass promoted change in the protective ability of different biomass extracts. Extracts obtained from the cultivated biomass grown under 150 μmol photons/m^2s^{-1}, 1.875 $g.L^{-1}$ $NaNO_3$; 13.5 $g.L^{-1}$ $NaHCO_3$ (assay14) and 50 μmol photons/m^2s^{-1}, 2.5 $g.L^{-1}$ $NaNO_3$; 18.0 $g.L^{-1}$ $NaHCO_3$ (standard) showed the most antioxidative effect on preventing lipid oxidation, therefore used in the formulation of mayonnaise. The mayonnaise made with soybean oil and 0.5% biomass was preserved against lipid photodegradation during seven days of storage, but the peroxide value related to control varied from 2.9 to 3.1 $mEqO_2.Kg^{-1}$, with no significant difference in the preservation afforded by tert-butilhydroquinone, in the same period of storage. In mayonnaise made with sunflower oil, biomass, independent of concentration, was unable to protect the product against photooxidation. **Conclusions:** The results demonstrate the protective ability on preventing lipid oxidation of *Arthrospira platensis* biomass and its potential for use in food lipid-based of soybean oil.

KEYWORDS: Natural Antioxidant; *Spirulina*; Vegetable Oil; Mayonnaise

RESUMO

Introdução: Cianobactérias são promissoras fontes naturais de antioxidantes. Condições ambientais que influenciam síntese de substâncias em cultivos de cianobactérias tem sido estudadas. **Objetivo:** O objetivo deste estudo foi avaliar o efeito antioxidante da biomassa de *Arthrospira platensis* no processo de oxidação lipídica do óleo e da emulsão óleo/água. **Método:** A produção de antioxidantes por *Arthrospira platensis* em função das condições de cultivo foi avaliada por meio de planejamento experimental. O efeito antioxidante dos extratos metanólicos e das biomassas foi avaliado através do índice de peróxidos. **Resultados:** Os resultados mostram que as condições de crescimento para se obter a biomassa promoveram mudança na capacidade protetora dos diferentes extratos da biomassa. Extratos obtidos a partir da biomassa cultivada sob 150 μmol fótons/m^2s^{-1}, 1,875 $g.L^{-1}$ $NaNO_3$; 13,5 $g.L^{-1}$ $NaHCO_3$ (ensaio 14) e 50 μmol fótons/m^2s^{-1}, 2,5 $g.L^{-1}$ $NaNO_3$; 18,0 $g.L^{-1}$ $NaHCO_3$ (padrão) mostraram maior efeito antioxidante contra a oxidação lipídica, portanto, utilizados para formulação da maionese. A maionese feita com óleo de soja e 0,5% de biomassa foi preservada da fotodegradação lipídica durante sete dias de armazenamento, mas o índice de peroxido em relação ao controle variou de 2,9 para 3,1 $mEqO_2.Kg^{-1}$, não havendo diferença significativa da preservação proporcionada pela *terc*-butil hidroquinona, no mesmo período de armazenamento. Na maionese feita com

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óleo de girassol, a biomassa, independentemente da concentração, não foi capaz de proteger o produto contra a foto-oxidação. **Conclusões:** Os resultados demonstram a capacidade protetora contra a oxidação lipídica da biomassa de *Arthrospira platensis* e seu potencial para uso em alimentos ricos em lipídeos à base de óleo de soja.

PALAVRAS-CHAVE: Antioxidante Natural; *Spirulina*; Óleo Vegetal; Maionese

INTRODUCTION

High fat food has lipids in a major part of its composition, being very susceptible to lipid oxidation, which could contribute for changes of flavor and color, being an important economic concern in the food industry, because this affects some quality characteristics of food products[1,2]. Beyond that, this changes nutritional quality due degradation of fat-soluble vitamins and essential fatty acids[3]. The lipid degradation could occur in different forms, being the most common auto-oxidation, in which fat reacts with atmospheric oxygen in three stages: initiation, propagation and termination[3,4]. To prevent the lipid oxidation, antioxidants are added in manufactured food; antioxidants of synthetic origin (as butil hydroxyanisole (BHA), butil hydroxytoluene (BHT) and tert-butilhydroquinone (TBHQ)) are the most used; however, they have restrict use in some countries, due to possible dangerous effects for human health[3,5]. Studies have reported some of these effects, such as DNA damages caused by the use of synthetic antioxidants[6,7]. In addition, Braeuning et al.[8] showed that the higher the TBHQ concentration, the greater its toxic effect, and that this leads to tumor formation in rodents; however, the exact mechanisms of this process have not been fully elucidated yet. This potential toxicological aspect of the synthetic antioxidants encourages a great interest to obtain and use antioxidants of natural sources that are supposed to be safer since they are present in plants[9,10] and/or microorganisms[11].

In this sense, cyanobacteria have been identified as one of the most promising groups of organisms for the discovery and identification of natural products with antioxidative effect[12,13]. Araújo et al.[14] showed that cyanobacteria was able to produce substances with antioxidant activity that was capable of protecting soybean oil on preventing lipid oxidation when their extract was added to the oil and compared with BHT. Another group of researchers showed that biomass of *Spirulina* when added to pasta promotes high phenolic content and antioxidant activity compared to control pasta - without *Spirulina*[15]. Moreover, Ismaiel et al.[16] demonstrated that *Arthrospira platensis* has a good production of chlorophyll-a, carotenoids and phycocianin, which are powerful antioxidants.

The genus *Arthrospira* was officially included in the Bergey's Manual of Systematic Bacteriology in 1989[17] and the species *Arthrospira maxima* and *Arthrospira platensis*, which are cultivated at industrial level in several regions of the world, are often referred to as *Spirulina maxima* and *Spirulina platensis*, and the biomass of these cyanobacteria is marketed under the name 'spirulina'[18]. The Food and Drug Administration (FDA) authorizes the use of Spirulina like food, without health risk, as well as GRAS (Generally Recognized as Safe) certificated this genus of cyanobacteria[19,20]. Thus, the biomass of *Arthrospira platensis* could be used as food additive without health risk. Therefore, this work aimed to evaluate the antioxidative effect of *Arthorspira platensis* biomass on the process of lipid oxidation in bulk oil and oil/water emulsion (mayonnaise).

METHOD

Chemicals

Methanol and dichloromethane were purchased from ProQuimicos (Brazil); BHT and TBHQ were purchased from Vogler Ingredients (Brazil); Activated carbon, Potassium sorbate, potassium benzoate, guar gum, potassium iodide, starch and EDTA were purchased from Vetec Química Fina LTDA (Brazil); Vegetable oils used were refined oils without antioxidants, produced by Cargill ® Company (Brazil), pasteurized whole egg was produced by Fleischmann® Company (Brazil).

Arthrospira platensis biomass

For the development of this work, we used freeze-dried biomass of cyanobacteria *Arthrospira platensis* grown in our previous study (unpublished data) about the influence of culture conditions on the biomass production, at different conditions of sodium bicarbonate ($NaHCO_3$), sodium nitrate ($NaNO_3$) and light intense, which totalize 17 cultivation conditions and plus the standard cultivation condition (S) (Table).

Table. Experimental design of growing conditions of *Arthrospira platensis*.

Assay	$NaHCO_3$ (g.L^{-1})	$NaNO_3$ (g.L^{-1})	Irradiation (μmol de photons.m^{-2}s^{-1})
S	18.00	2.500	50.00
1	10.82	1.500	70.20
2	16.18	1.500	70.20
3	10.82	2.250	70.20
4	16.18	2.250	70.20
5	10.82	1.500	129.80
6	16.18	1.500	129.80
7	10.82	2.250	129.80
8	16.18	2.250	129.80
9	9.00	1.875	100.00
10	18.00	1.875	100.00
11	13.50	1.250	100.00
12	13.50	2.500	100.00
13	13.50	1.875	50.00
14	13.50	1.875	150.00
15	13.50	1.875	100.00
16	13.50	1.875	100.00
17	13.50	1.875	100.00

Arthrospira platensis extract

Eighteen methanolic extracts were prepared by sonication and the solvent was removed from the extract with a rotating evaporator at 35°C. Subsequently, the extracts were purified by open column chromatography using activated carbon as stationary phase. The mobile phase consisted of a gradient of dichloromethane and methanol, in proportions of 9:1 to 1:9 (v:v), which were eluted by 20 ml of each solution. The solvents were subsequently removed with a rotating evaporator.

Evaluation of the antioxidative effect of extracts on preventing soybean oil oxidation

The antioxidative effect of the extracts on preventing lipid oxidation as compared to the synthetic antioxidant BHT and TBHQ was evaluated using soybean oil added standard antioxidant (100 ppm) or purified extract (1,000 ppm)[14]. Oil without addition of antioxidant or extracts was used as a control. Oil samples were subjected to oxidation induced thermally in Schaal oven test for seven days[6] and peroxide value (PV) was measured [21].

Evaluation of the antioxidative effect of biomass on preventing mayonnaise oxidation

Based on the results obtained in the first step, an experiment was conducted to evaluate the antioxidative effect of the biomass to oxidation front of a high fat food. Handmade mayonnaise was developed and used like model.

Product formulation

For formulating the mayonnaise, refined vegetable oil without antioxidants (70%) of two types, soybean and sunflower oil, was used. The pasteurized egg was used as a source of lecithin and water. Vinegar (acetic acid) was added as acidulate in the emulsion formulation while maintaining pH low. Provided food additives were added in the mayonnaise, under Brazilian law[22].

The additives were added based on 100 g of product, except TBHQ that was added based on 100 g of oil. The *Arthrospira platensis* biomass was also added based on 100 g of product, in concentrations of 0.5% and 0.75%. For each type of vegetable oil, four samples of mayonnaise were formulated, namely: control (without added antioxidant or biomass), synthetic antioxidant (TBHQ), *Arthrospira platensis* biomass 0.5% and *Arthrospira platensis* biomass 0.75%.

Evaluation of oxidative stability of mayonnaise emulsion

For this assay, 45 g of each sample was stored in plastic tubes Falcon type 50 ml in storage conditions that favored the lipid degradation in accelerated form, under light incidence 25 μmol photon.$m^{-2}.s^{-1}$ and temperature of 30°C ± 1°C, for 14 days[23]. The protect capability of biomass was evaluated in times 0, 7 and 14 days of storage by PV determination by the method of Alemán et al[24] with small modifications, so for this analysis, the samples of each time were lyophilized to separate the lipid fraction and thereafter the PV was determined.

Statistical Analysis

The evaluation of the effect of variables (concentration of sodium bicarbonate, sodium nitrate and irradiance) on the protective ability of the extracts derived from the biomass was performed with the aid of Statistica version 8.0. The effect of the variables was considered statistically significant when $p < 0.1$. The peroxide value results obtained in the evaluation tests of the antioxidative effect of the biomass extracts were statistically analyzed to assess significant differences among samples. Analysis of variance (ANOVA one way) was used. In case of significant difference, the means were compared by Tukey test. Differences were considered statistically significant at $p < 0.05$. Data were analyzed using the GraphPad Prism version 5.0.

RESULTS AND DISCUSSION

Evaluation of the antioxidative effect of *Arthrospira platensis* extracts on preventing lipid oxidation

The test to measure the protective ability of the extracts on preventing lipid oxidation was conducted with soybean oil added of the synthetic antioxidant TBHQ or BHT or 18 purified extracts of biomass produced in different cultivation conditions. The oil samples were subjected to oxidation thermally induced and during this process the formation of peroxides occurred, which is one of the intermediates of the mechanism of reaction of lipid deterioration. Thus, after the incubation period, the PV of the samples was measured, to evaluate the ability of the extracts in protecting the oil on preventing oxidation. Figure 1 shows these results. The control is the soybean oil without antioxidant. The non-peroxide formation by the addition of antioxidants TBHQ or BHT or extracts, indicates the ability of these additives to increase the oxidation stability of the oil. The results are expressed in "peroxide value percent of control".

It was possible to observe in Figure 1 that the PV of soybean oil was lower by adding 100 ppm synthetic antioxidant TBHQ and BHT and 1,000 ppm *Arthrospira platensis* extracts E7; E10; E11; E12; E14; E15; E16; E17 and ES, than the control and others treatments. This means that these extracts increased the oxidative stability, being capable to minimize the lipid oxidation that results in peroxide formation. The others extracts were not capable to increase the stability since the PV of oil added of these extracts was significantly equal to the control. Through statistical analysis by analysis of variance (ANOVA) followed by Tukey test, we could verify that the antioxidant TBHQ and BHT gave significantly different protection than the protection promoted by the extracts, except E16 that did not differ statistically by BHT. The antioxidant TBHQ showed the most protective capacity. The oil with added 1,000 ppm of extracts E14 and ES obtained results that did not differ statistically and had the highest antioxidant effect among the tested extracts, followed by E10 and E15 and by BHT and E16 that also did not differ statistically from each other. In the same way, the oil added to extracts E7, E11, E12 and E17 did not differ statistically, but had the lowest antioxidant effect. Therefore, the treatments E14 and ES, in the same period to evaluate the

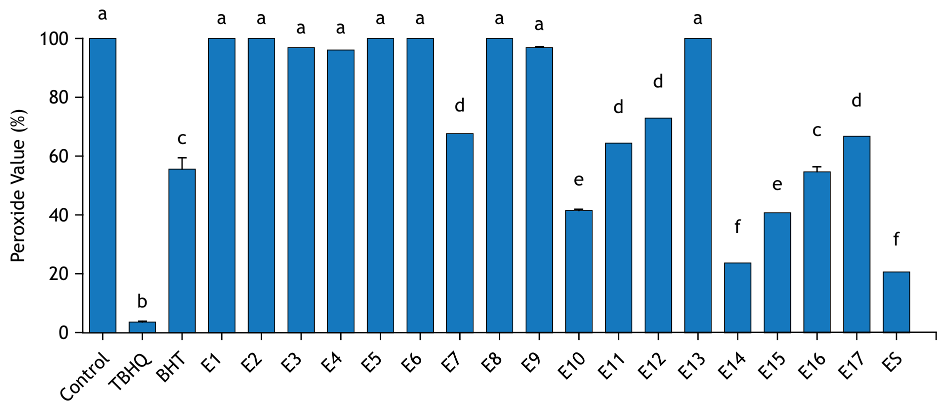

Figure 1. Peroxide value of soybean oil without antioxidant (control) and added of 100 ppm synthetic antioxidant (TBHQ or BHT) or 1,000 ppm *Arthrospira platensis* extract (E1: extract one; E2: extract two; E3: extract three; E4: extract four; E5: extract five; E6: extract six; E7: extract seven; E8: extract eight; E9: extract nine; E10: extract ten; E11: extract eleven; E12: extract twelve; E13: extract thirteen; E14: extract fourteen; E15: extract fifteen; E16: extract sixteen; E17: extract seventeen; ES: standard extract). Equal letters with no significant difference ($p < 0.05$).

oxidation of soybean oil, showed PV below the other extracts, conferring 76.41% and 79.48% of soybean oil protection on preventing the formation of hydroperoxides, respectively.

The effect of the three variables of cultivation of *Arthrospira platensis* on the protective ability of the extracts was measured. From these data, analysis of variance was made and the statistically significant factors could be observed, in addition to observing which were the more relevant to antioxidative effect, expressed by the protective capacity measured by the PV. Thus, Figure 2 shows that the variables light and concentration of bicarbonate ($NaHCO_3$) showed a significant effect ($p = 0.085517$ and $p = 0.096482$, respectively), whereas the variable concentration of nitrate ($NaNO_3$) showed marginally significant effect ($p = 0.100306$). Thereby, the linear term of the light is the most important variable for antioxidative effect, followed by the quadratic terms of $NaHCO_3$ and $NaNO_3$.

Through the modification of culture conditions differences in antioxidative effect of biomass were observed. The conditions that produced biomass extract with more antioxidative effect on preventing oxidation, for the most part, were those with higher light levels (≥ 100 μmol photon.m^{-2}.s^{-1}), regardless of the concentration of $NaNO_3$ and major $NaHCO_3$ concentrations (≥ 13.50 g.L^{-1}). The biomass growth condition 14 (150 μmol photon.m^{-2}.s^{-1}, 1.875 g.L^{-1} $NaNO_3$ and 13.5 g.L^{-1} NaHCO3) showed the best antioxidative effect and the biomass from S condition was significantly equal. The standard condition was produced with low irradiance (50 μmol photon.m^{-2}.s^{-1}), but with maximum concentration of $NaHCO_3$ (18 g.L^{-1}) and $NaNO_3$ (2.5 g.L^{-1}). In this case, the high protection capacity of biomass could be related to the effect of bicarbonate and nitrate concentrations, in accordance with Pareto chart (Figure 2). The larger substrate offer could reflect in the accumulation of substances that influenced the protective capability and that showed a different biosynthetic route from that initiated by oxidative stress induced for high photosynthetic rate, in high light level.

Some studies were conducted to evaluate the best conditions of cyanobacteria cultivation related with its antioxidant potential. Shalaby et al.[25] reported that *Arthrospira platensis* under

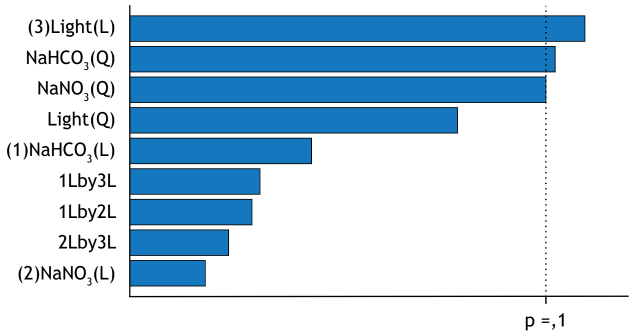

Figure 2. Pareto chart for the effect of the variables light, sodium bicarbonate ($NaHCO_3$) and sodium nitrate ($NaNO_3$) on the peroxide value.

salt stress conditions, the algal polar extracts displayed the highest antioxidative effect including phycobilins pigments, sulfated polysaccharides and phenolics compounds.

Researchers found that the antioxidative effect of the extracts obtained from cyanobacteria biomass is influenced by the strain of *Arthrospira* used for cultivation and the composition of the growth medium. In their study, they evaluated antioxidative effect of ethanolic extracts and reported that higher values of antioxidative effect were achieved when the culture medium had reduced concentrations of $NaNO_3$ and/or $NaHCO_3$[26]. In the present study, the biomass of extract E14 was cultivated with reduced concentrations of $NaNO_3$ and $NaHCO_3$.

Other study demonstrated the existence of significant interactions with the content of phenolic compounds of methanolic extracts of *Arthrospira platensis* and antioxidant potential. The authors demonstrated that higher $NaNO_3$ concentrations (1.875 and 2.500 g.L^{-1}) promoted higher levels of phenolic compounds in *Arthrospira platensis* grown at 35°C. These $NaNO_3$ concentrations are equal to culture conditions of biomass obtained for E14 and ES, respectively, which showed a better antioxidative effect on preventing oxidation for E14, than for other *Arthrospira platensis* extracts[27].

Araújo et al.[14] studied the occurrence of antioxidant compounds in *Anabaena* PCC 7119 cyanobacteria which was cultivated under different light intensities (100, 200 and 300 μmol photon.m^{-2}.s^{-1}). These researchers found good antioxidative effect in methanolic extracts from the cultures with incident light 100 and 200 μmol photon.m^{-2}.s^{-1}, irradiance values similar to those studied in this work, which were tested in soybean oil.

Evaluation of the antioxidative effect of *Arthrospira platensis* biomass applied in emulsion food product

As there was no significant difference between the 14 and S tests that showed the best protective capacity on preventing oxidation in soybean oil, the standard assay (S) was selected for the development of the evaluation test to protective capacity of biomass on preventing the oxidation of mayonnaise.

Figure 3 shows the results of PV on 0, 7 and 14 days for the mayonnaise samples made with soybean and sunflower oil. It can be seen that the initial PV (time 0) of mayonnaise produced with soybean oil is higher than the mayonnaise produced with sunflower oil; this is probably due to the fatty acid composition of these oils. In the study made by Castelo Branco and Torres[28], the results showed that more than 50% of the fatty acid in soybean and sunflower oil are polyunsaturated, but the sunflower oil has a higher content of monounsaturated. Monounsaturated fatty acids are less susceptible to oxidation than polyunsaturated[29], justifying the highest concentration of peroxides in the soybean oil used to prepare the mayonnaise.

In the Brazilian law there is not a limit of PV to mayonnaise, but 10 mEqO_2.Kg^{-1} is the maximum level for PV to vegetable refined oil[30]. Based on this law, all samples were classified as suitable for consumption by PV.

Based on statistical analysis, it was observed that in mayonnaise made with soybean oil the control sample (without synthetic antioxidant or biomass of *Arthrospira platensis*) at the end of the storage period (time 14) did not differ significantly from initial PV (time 0). This result is probably related to the protective capability of the components present in soybean oil, such as tocopherols. Castelo Branco[31] investigated the influence of the initial composition of vegetable oils on the oxidative stability and total antioxidant capacity; it was observed that the soybean oil has a high content of γ-tocopherol, being also the oil with a higher content of total tocopherol, which is naturally present in vegetable oils and is a natural effective antioxidant as also described by Chaiyasit et al[2].

Even with the mayonnaise prepared with soybean oil, it was observed that TBHQ was able to preserve the photo-degradation of mayonnaise by 14 days of storage, while the biomass of *Arthrospira platensis* at concentration of 0.5% was able to preserve only for the first 7 days. The statistical analysis shows that the PV measured on the seventh day, to mayonnaise added of 0.5% biomass, had no significant difference of PV measured for mayonnaise added of TBHQ. This demonstrates that the protection offered by the biomass was equal to that conferred by TBHQ, during the first 7 days. This result also demonstrates the ability of this biomass for minimizing lipid oxidation during 7 days of storage, under the test conditions. It is worth emphasizing that in this experiment mayonnaise was kept in conditions that accelerated the degradation rate (25 μmol photon.m^{-2}.s^{-1}, 30° C), which does not correspond to those in which the product is usually kept for marketing, differing mainly on the light intensity. Thus, it can be inferred that the addition of biomass in this concentration could be able to protect the product for a longer period of time, enhancing the potential use of biomass, in that concentration, as a substitute for synthetic antioxidant.

In the mayonnaise added of 0.75% biomass, it was observed that the PV increased significantly until the seventh day of storage, followed by a decrease of the same to the fourteenth day of storage, showing results significantly equal to the initial value. This fact occurs because the PV gives a measure about the degree

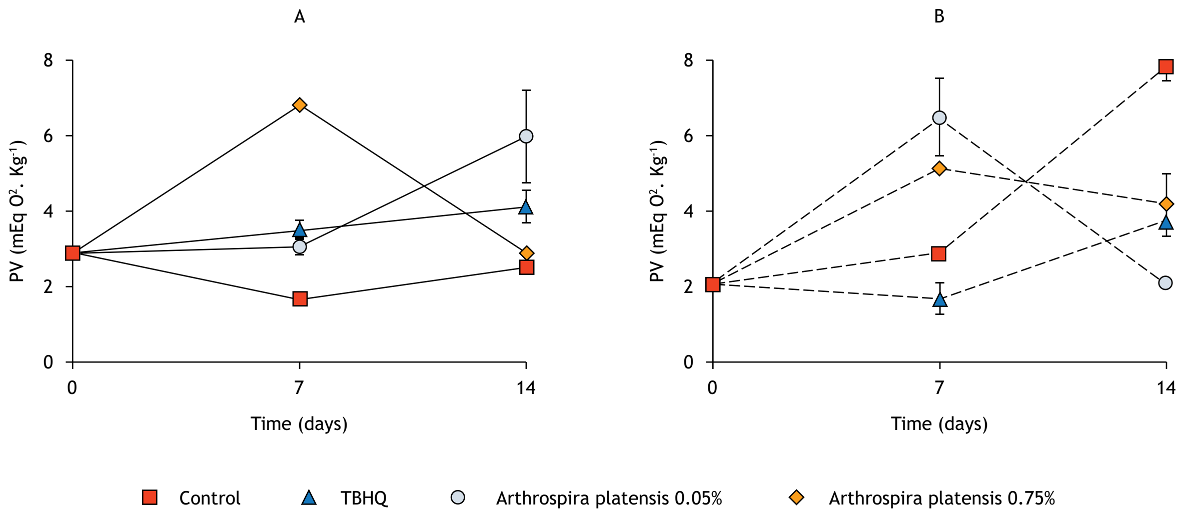

Figure 3. Peroxide Value of mayonnaise prepared with (A) soybean oil and (B) sunflower oil.

of oxidation produced by peroxide and hydroperoxide that react with potassium iodate[32], but the peroxides are produced during an initial stage of oxidation and in the following stages (propagation and termination) they are complexed to form secondary products that are more stable[2], resulting in a PV reduction. Abreu et al.[32] explain that the PV tends to increase during the initial stages of oxidation because it is when the hydroperoxide rate formation is higher than the decomposition rate, but when a maximum value is achieved, the PV decreases due to lower accessibility of substrate and to instability of peroxide molecules.

The increase of biomass concentration, 0.5% to 0.75%, did not represent a greater capacity to protect the product of the photo-oxidation, which would signify that this increment, beyond promoting higher concentration of antioxidant substances at mayonnaise, also represents the increase of concentration of substances that could be degraded in the same conditions, such as chlorophyll and carotenoids. This degradation produces instable products, among which reactive oxygen species, that oxidize other molecules, like lipid[33], which can explain why the PV increases in mayonnaise with 0.75% biomass.

The results of the mayonnaise prepared with sunflower oil show that in the control sample there was lipid degradation throughout the storage period, being more intense after the seventh day. This degradation could be related with the greater vulnerability of sunflower oil, as demonstrated by Castelo Branco[31] that verified the initial quality of vegetable oil and found that sunflower oil shows higher PV than that showed by the soybean oil (1.14 mEq $O_2.Kg^{-1}$ and 0.38 mEq$O_2.Kg^{-1}$, respectively). In other results in the same study, it was verified that sunflower oil showed greater susceptibility to oxidation and lower oxidative stability.

TBHQ ensures protection to mayonnaise prepared with sunflower oil until seven days of storage, showing degradation after this time, but this degradation was significantly lower compared to control, which showed on the fourteenth day the highest degradation among all tested samples of sunflower oil. For samples with 0.5% and 0.75% biomass, it was observed that the PV in the seventh day has a significant increase, followed by reduction at the fourteenth day of storage. The biomass, independent of the added concentration, was not able to protect from the photo-degradation the mayonnaise produced with sunflower oil.

Some studies were performed to investigate the oxidative stability of emulsions, such as the study of Pavlović et al.[34] that evaluates the effect of ascorbic acid and EDTA in emulsified dressings salad made with different types of sunflower oil (with different tocopherol profiles) and stored for three months at 25°C protected from light. The results showed that in the sample control (without antioxidant) the PV increased rapidly during the first month of storage; then the increase was slower, probably due to formation of secondary oxidation products. Another study about oxidative stability of emulsion was performed by Altunkaya et al[35], and evaluated mayonnaise added with grape seed extract, which is rich in phenolic compounds. They investigated the oxidative alterations in mayonnaise by various methods, including PV, during storage in conditions that protect from light at 20° C for 8 weeks. The mayonnaise added with grape seed extract showed PV significantly lower compared with the control mayonnaise, after 8 weeks of storage. However, the progress in oxidation follows a pattern that is normally found in the auto-oxidation, such as initial increase in lipid peroxidation products followed by a subsequent increase in secondary products of lipid oxidation.

Castelo Branco and Torres[36] described that the tocopherol profile of refined vegetable oils is one of the determinants of total antioxidant capacity, being the concentration of γ- and δ-tocopherol responsible for this feature. When evaluating different samples of refined vegetable oils, the following values were found in soybean oil : 93.3 to 129.4 and 24.0 to 49.7 mg.100g^{-1} of γ- and δ-tocopherol, respectively, while for sunflower oil the values were 4.50 to 5.97 and 0.23 to 0.57 mg.100g^{-1} of γ- and δ-tocopherol, respectively. The findings of Castelo Branco and Torres[36] study are complementary to that found in this work, pointing to a possible synergistic effect of tocopherols present in soybean oil with substances that promote antioxidative effect of biomass, promoting the protection of mayonnaise produced with soybean oil. These results also contribute to elucidate the behavior observed in mayonnaise produced with sunflower oil. As sunflower oil inherently has the lowest total antioxidant capacity and is therefore more prone to oxidation, the substances conveyed through the biomass, irrespective of the added concentration, were not able to promote oxidation prevention of mayonnaise in this study's conditions.

CONCLUSIONS

In view of the results obtained in the present work, it is possible to conclude that the biomass of *Arthrospira platensis* presents compounds displaying antioxidant effect and that such compounds do not decrease their protecting capacity against the soybean oil and mayonnaise oxidation when they were submitted to experimental conditions. The main objective of our research is discovering new non-toxic natural compounds as an alternative to the food additives nowadays available, against which there have been restrictions regarding their toxic effects. In this context, *Arthrospira platensis* is a promising new source of antioxidant compounds. These results demonstrate the potential of this cyanobacteria as a source of new antioxidant compounds to be applied on food processing.

REFERENCES

1. Li CY, Kim HW, Li H, Lee DC, Rhee HI. Antioxidative effect of purple corn extracts during storage of mayonnaise. Food Chem. 2014;152:592-6. https://doi.org/10.1016/j.foodchem.2013.11.152
2. Chaiyasit W, Elias RJ, McClements DJ, Decker EA. Role of physical structures in bulk oils on lipid oxidation. Crit Rev Food Sci Nutr. 2007;47(3):299-317. https://doi.org/10.1080/10408390600754248

3. Ramalho VC, Jorge N. Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos. Quim Nova. 2006;29(4):755-60. https://doi.org/10.1590/S0100-40422006000400023
4. Freire PC, Mancini-Filho J, Ferreira TA. Principais alterações físico-químicas em óleos e gorduras submetidos ao processo de fritura por imersão: regulamentação e efeitos na saúde. Rev Nutr. 2013;26(3):353-68. https://doi.org/10.1590/S1415-52732013000300010
5. Shahidi F, Zhong Y. Lipid oxidation and improving the oxidative stability. Chem Soc Rev. 2010;39(11):4067-79. https://doi.org/10.1039/b922183m
6. Kashanian S, Ezzati Nazhad Dolatabadi J. DNA binding studies of 2-tert-butylhydroquinone (TBHQ) food additive. Food Chem. 2009;116(3):743-7. https://doi.org/10.1016/j.foodchem.2009.03.027
7. Nagai F, Okubo T, Ushiyama K, Satoh K, Kano I. Formation of 8-hydroxydeoxyguanosine in calf thymus DNA treated with tert-butylhydroquinone, a major metabolite of butylated hydroxyanisole. Toxicol Lett. 1996;89(2):163-7. https://doi.org/10.1016/S0378-4274(96)03800-3
8. Braeuning A, Vetter S, Orsetti S, Schwarz M. Paradoxical cytotoxicity of tert-butylhydroquinone in vitro: what kills the untreated cells? Arch Toxicol. 2012;86(9):1481-7. https://doi.org/10.1007/s00204-012-0841-3
9. Almeida-Doria RF, Regitano-D'Arce MA. Antioxidant activity of Rosemary and oregano ethanol extracts in soybean oil under thermal oxidation. Food Sci Technol (Campinas). 2000;20(2):2. https://doi.org/10.1590/S0101-20612000000200013
10. Yang Y, Song X, Sui X, Qi B, Wang Z, Li Y et al. Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability. Ind Crops Prod. 2016;80:141-7. https://doi.org/10.1016/j.indcrop.2015.11.044
11. Bertolin TE, Margarites AC, Giacomelli B, Fruetti A, Horst C, Texeira DM. Ficocianina, tocoferol e ácido ascórbico na prevenção de oxidação lipídica em charque. Braz J Food Technol. 2011;14(4):301-7. https://doi.org/10.4260/BJFT2011140400036
12. Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC. Marine cyanobacteria - a prolific source of natural products. Tetrahedron. 2001;57(46):9347-77. https://doi.org/10.1016/S0040-4020(01)00931-0
13. Shanab SM, Mostafa SS, Shalaby EA, Mahmoud GI. Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. Asian Pac J Trop Biomed. 2012;2(8):608-15. https://doi.org/10.1016/S2221-1691(12)60106-3
14. Araújo KGL, Domingues JR, Sabaa Srur AU, Silva AJ. Production of antioxidants by *Anabaena PCC 7119* and evaluation of their protecting activity against oxidation of soybean oil. Food Biotechnol. 2006;20(1):65-77. https://doi.org/10.1080/08905430500524200
15. De Maco ER, Steffolani ME, Martínez CS, León AE. Effect of spirulina biomass on the technological and nutritional quality of bread wheat pasta. Lebensm Wiss Technol. 2014;58(1):102-8. https://doi.org/10.1016/j.lwt.2014.02.054
16. Ismaiel MM, El-Ayouty YM, Piercey-Normore MD. Antioxidant characterization in selected cyanobacteria. Ann Microbiol. 2014;64(3):1223-30. https://doi.org/10.1007/s13213-013-0763-1
17. Castenholz RW. Subsection III, order oscillatoriales. In: Staley JT, Bryant MP, Pfennig N, Holt JG. Bergey's Manual of Systematic Bacteriology. Baltimore: William & Wilkins; 1989. p. 1771-80.
18. Araújo KGL, Facchinetti AD, Santos CP. Influência da ingestão de biomassa de *Spirulina* (*Arthrospira sp.*) sobre o peso corporal e consumo de ração em ratos. Food Sci Technol (Campinas). 2003;23:1.
19. U.S. Department of Health and Human Services. Food and Drug Administration. Agency response letter GRAS Notice No. GRN 000127. Washington: Food and Drug Administration; 2003[cited 2014 Jun 18]. Available from: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm153944.htm
20. Moraes IO, Arruda RO, Maresca NR, Antunes AO, Moraes RO. *Spirulina platensis*: process optimization to obtain biomass. Food Sci Technol (Campinas). 2013;33(1):179-83. https://doi.org/10.1590/S0101-20612013000500026
21. Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. São Paulo: Instituto Adolfo Lutz; 2008[cited 2014 Jun 18]. Available from: http://www.crq4.org.br/sms/files/file/analisedealimentosial_2008.pdf
22. Agencia Nacional de Vigilância Sanitária – Anvisa. Resolução RDC Nº 4, de 15 de janeiro de 2007. Aprovar o regulamento técnico sobre atribuição de aditivos e seus limites máximos para a categoria de alimentos 13: molhos e condimentos. Diário Oficial União 17 jan 2007.
23. American Oil Chemist Society - AOCS. Official methods and recommended pratices of the AOCS. (5th ed). Champaing: American Oil Chemist Society; 2003. Accelerated light exposure of edible vegetable oils, Cg 6-01.
24. Alemán M, Bou R, Guardiola F, Durand E, Villeneuve P, Jacobsen C et al. Antioxidative effect of lipophilized caffeic acid in fish oil enriched mayonnaise and milk. Food Chem. 2015;167:236-44. https://doi.org/10.1016/j.foodchem.2014.06.083
25. Shalaby EA, Shanab SM, Singh V. Salt stress enhancement of antioxidant and antiviral efficiency of *Spirulina platensis*. J Med Plants Res. 2010;4(24):2622-32. https://doi.org/10.5897/JMPR09.300
26. Tarko T, Duda-Chodak A, Kobus M. Influence of growth medium composition on synthesis of bioactive compounds and antioxidant properties of selected strains of Arthrospira cyanobacteria. Czech J Food Sci. 2012;30:258-67.
27. Colla LM, Furlong E, Costa JA. Antioxidant properties of *Spirulina* (*Arthospira*) *platensis* cultivated under different temperatures and nitrogen regimes. Braz Arch Biol Technol. 2007;50(1):161-7. https://doi.org/10.1590/S1516-89132007000100020

28. Castelo Branco VN, Torres AG. Capacidade antioxidante total de óleos vegetais comestíveis: determinantes químicos e sua relação com a qualidade do óleo. Rev Nutr. 2011;24(1):173-87. https://doi.org/10.1590/S1415-52732011000100017
29. Berton-Carabin CC, Ropers MH, Genot C. Lipid oxidation in oil-in-water emulsions: involvement of the interfacial layer. Compr Rev Food Sci Food Saf. 2014;13(5):945-77. https://doi.org/10.1111/1541-4337.12097
30. Agencia Nacional de Vigilância Sanitária – Anvisa. Resolução RDC Nº 270, de 22 de setembro de 2005. Regulamento técnico para óleos vegetais, gorduras vegetais e creme vegetal. Diário Oficial União, 23 set 2005.
31. Castelo Branco VN. Transformações químicas na oxidação acelerada de óleos vegetais e sua relação com a capacidade antioxidante total, uma abordagem multivariada [tese]. Rio de Janeiro, Universidade Federal do Rio de Janeiro; 2013.
32. Abreu DA, Losada PP, Maroto J, Cruz JM. Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (Salmo salar L.). Food Res Int. 2010;43(5):1277-82. https://doi.org/10.1016/j.foodres.2010.03.019
33. Streit NM, Canterle LP, Canto MW, Hecktheuer LH. As Clorofilas. Cienc Rural. 2005;35(3):748-55. https://doi.org/10.1590/S0103-84782005000300043
34. Pavlović MD, Pucarević M, Mićović V, Zivić M, Zlatanović S, Gorjanović S et al. Influence of sunflower oil qualities and antioxidants on oxidative stability on whey-based salad dressings. Acta Chim Slov. 2012;59(1):42-9.
35. Altunkaya A, Hedegaard RV, Harholt J, Brimer L, Gökmen V, Skibsted LH. Oxidative stability and chemical safety of mayonnaise enriched with grape seed extract. Food Funct. 2013;4(11):1647-53. https://doi.org/10.1039/c3fo60204d
36. Castelo Branco VN, Torres AG. Generalized linear model describes determinants of total antioxidant capacity of refined vegetable oils. Eur J Lipid Sci Technol. 2012;114(3):332-42. https://doi.org/10.1002/ejlt.201100181

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.

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