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Short Communication

Evaluation of antioxidant activity of seaweed through accelerated oxidation methods

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Abstract

This study investigated the effect of Nori and Hijiki extracts on the stability of soybean oil and minced tilapia. The extracts were applied at 25, 50 and 100 µg GAE/g. In Oxipres, the induction period (IP) for tilapia minced without antioxidant was 4.6 h. With the addition of Nori extract at increasing concentrations, the IP was 6.6 h, 6.8 h and 5.7 h. For Hijiki, the IP was, respectively, 5.8 h, 5.4 h and 5.3 h. In the Rancimat, the IP was 7.05 h for oil without antioxidant and 7.31 h for the oil with BHT. For Nori, the IP reached 8.28, 8.63 and 8.72 h; and for Hijiki, 7.58, 7.46 and 3.39 h, respectively. The results show that there is potential for the use of seaweed extracts to increase the oxidative stability of oil, as found in tilapia minced and soybean oil.

Introduction

Lipid oxidation is a inevitable phenomenon, with direct implications on the commercial of foods. In recent years, a constant concern to provide consumers with high quality products, has led to the adoption of measures that can limit the oxidative degradation during the processing and storage of products. The use of substances with antioxidant capacity has been one of the most commonly adopted (Silva *et al*., 1999).

Recent research has been conducted to replace the chemical additives used in oils and meat products for natural products, as described in references (Medina *et al.*, 2003; Luther *et al.*, 2007; Nielsen *et al.*, 2007; Sanchez-Alonso *et al*., 2007). These studies aimed to control the quality of fatty acids by determining their oxidation degree and evaluating the antioxidant activity of new compounds.

The induction period, also called oxidative stability index, is a comparative parameter widely used for the quality control of raw materials and processes and for the evaluation of different types of frying oils, changes in fatty acid composition, efficiency of antioxidants use, among others (Antoniassi, 2001).

The Oxipres method is considered an accelerated method where the oxidation process is monitored by oxygen consumption, recorded through changes in pressure in a hermetically sealed container containing the gas. The oxidation product is accelerated due to the high pressure (5 MPa) and high temperature (typically 100°C) to which the product is subjected. The oxidation of the sample is graphically recorded providing the induction period (moment of sharp pressure drop that characterizes the moment oxidation is accelerated) allowing, also, to estimate the product shelf life (Reblová, 2006).

The Rancimat method is also an accelerated oxidation method based on the rapid and automatic determination of the time elapsed to reach the maximum oxidation rate of the oil. However, this time, also called induction time or stability rate of the oil is determined by measuring the conductivity increase of deionized water, due to the final compounds of oxidation that bubble in the water (Farhoosh, 2007).

Seaweed is a source of compounds with biological activity that allows its use as functional ingredients. The use of metabolites with biological activity from seaweed increased significantly over the last three decades. A series of phenolic compounds such as catechins, flavonols and flavonol glycosides have been identified in methanol extracts of red and brown seaweed, and have shown significant antioxidant activity (Yoshie-Stark *et al.*, 2003). However, there are no reports in the literature regarding the investigation of seaweed antioxidant activity through accelerated oxidation methods. So, this is the first study aiming to investigate the antioxidant effect of extracts of Nori and Hijiki seaweeds, commonly used in Japanese food, through two accelerated oxidation

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methods: Oxipres and Rancimat.

Materials and Methods

Materials

Red Nori (*Porphyra tenera*) and brown Hijiki (*Hijikia fusiformis*) were obtained already dried from enterprises that supply oriental foods. The tilapias (*Oreochromis niloticus*) were obtained from a supermarket chain in the State of São Paulo, Brazil. The refined, bleached, and deodorized (RBD) soybean oil was provided by Cargill, batch: 19229 (shelf life: 05/16/2011), without addition of antioxidants.

Preparation of seaweed extracts

The dried seaweeds were milled, weighed 10 g of each and 100 mL of ethanol 60% (v/v) was added as solvent extractor. The flasks were sealed and kept in the dark at room temperature for 48 hours for the extraction of phenolic compounds. The extracts were filtered through qualitative filter paper, concentrated in a rotary evaporator, frozen, lyophilized and stored at -8ºC (Cabral, 2012).

Phenolic compounds content

The ethanolic extracts of Nori and Hijiki, previously lyophilized, were resuspended in ethanol (0.5 ml) was mixed with 2.5 mL of Folin-Ciocalteau (diluted in distilled water 1:10) and 2.0 mL of 4% Na_2CO_3 (m/v) in distilled water. After two hours of incubation in the dark at room temperature, the absorbance was measured at 740 nm in a Fenton 600 Plus spectrophotometer. The results were calculated using a curve constructed with concentrations ranging from 5 to 100 µg/mL of the gallic acid equivalents (mg GAE/g of seaweed) (Singleton *et al.*, 1999).

Processing the tilapia Minced

Approximately 3 kg of tilapia (*Oreochromis niloticus*) were weighed, washed with water, with 0.5 mg/L of residual chlorine, eviscerated and beheaded. After further wash, the fish were processed in mechanical pulping, HIGH TECH brand, model HT 100-C, to obtain the mechanically deboned meat - MDM (Cabral, 2012). The MDM obtained was washed with potable water at 10ºC at a ratio of 3 L water for 1 Kg of MDM. The homogenization was performed by hand for 3 minutes, and then, the material remained at rest for 3 minutes. The MDM was placed in a nylon bag and centrifuged to drain water excess and to obtain the tilapia minced.

Oxidative stability of the tilapia Minced - Oxipres

Samples of 50 g of tilapia minced were mixed with extracts lyophilized of Nori and Hijiki seaweeds corresponding to 25, 50 and 100 µg GAE/g of minced. A control without antioxidant and a control containing the synthetic antioxidant BHT at a concentration of 100 µg/g of minced were also subjected to this analysis. Afterwards, the samples were placed into the Oxipres (Mikrolab Asrhus) at a temperature of 100ºC and oxygen pressure of 0.5 MPa (Danisco, 2007). The readings of the oxygen pressure drop caused by its reaction with free radicals produced by the sample oxidation comprised the curve, which was plotted versus time, allowed to calculate the induction period.

Oxidative stability in oil - Rancimat

Samples of 5 g of soybean oil were added with extracts lyophilized of Nori and Hijiki seaweeds corresponding to 25, 50 and 100 µg GAE/g based on the phenolic compounds content. Afterwards, the material was subjected to temperature of 110 ± 1ºC under dry airflow at a rate of 9 L/h, in accordance to the method cd12b-92 (American Oil Chemist's Society, 2003) in a Rancimat 743, Metrohm AG, CH-9100 Herisau-Switzerland. The readings of increased conductivity, due to the oxidation accumulation, which comprised the curve, plotted as a function of reaction time, allowed to calculate the induction period (IP). A control prepared with soybean oil samples containing no antioxidant and samples with synthetic antioxidant BHT at a concentration of 100 µg/g were also subjected to this analysis.

Fatty acids content of tilapia minced and soybean oil

Preparation of methyl esters of fatty acids: The samples of soybean oil and minced without antioxidant had their fatty acids profile analyzed only for characterization. After lipid extraction by Soxhlet, it followed with the preparation of methyl esters of fatty acids. Approximately 100 mg of sample were weighed in a centrifuge tube and was added 2 mL of n-hexane and then 0.2 mL of 2 M methanolic KOH. The tube was sealed and stirred for 30 sec. In this was added 3 mL of saturated sodium chloride. The mixture was allowed to stand until separation of the phases. The upper phase was used for analysis by gas chromatography.

Chromatographic conditions: The Gas chromatograph, with a flame ionization detector, with the following operating conditions: 2560 Supelco column with dimensions 10 m x 0.25 mm x 0.2 mm; programming the column temperature for 45ºC for 4 min, the first ramp 13ºC / min to 175ºC (27 min), the second ramp of 4ºC / min to 215ºC (35 min), injector

temperature 220ºC, detector temperature 220ºC and sample split ratio 1:50. It was injected 1 uL of sample into the chromatograph. The peaks were identified by comparison with the retention times of standards of the methyl esters (C4 to C24) with the separate components of the sample, using software-YL Clarity (Instituto Adolfo Lutz, 2008). The method was validated, and the limits of detection (LOD) and limits of quantification (LOQ) were related to the oil content of samples, of the 0.3% and 0.5%, respectively.

Statistical analysis

The statistical analysis was carried out in the Software SAS 9.2 (2002; 2008) through the variance analysis (ANOVA) and the Tukey test ($p < 0.05$).

Results and Discussion

In this study, Nori and Hijiki seaweeds presented phenolic compounds, respectively, 7.95 and 6.01 mg GAE/g of seaweed. Cabral (2012) found, in Nori, chlorogenic, caffeic and vanillic acids. In Hijiki seaweed were detected chlorogenic, caffeic and cinnamic acids by liquid chromatography. The protective effect in oil systems can be attributed, according to Rice-Evans *et al*. (1996), to the properties of the phenolic compounds whose ability to donate hydrogen and inhibit oxidation rises as the number of hydroxyl groups in the molecule increases. This property also confirmed by Réblová (2012).

For extracts of red seaweed Nori and brown seaweed Wakame, phenolic compounds content were determined, 0.0019 mg GAE/g and 0.001 mg GAE/g, respectively, according to Onofrejová *et al.* (2010). Among these phenolic compounds, the authors found a high concentration of p-hydroxybenzoic acid, acid 2,3 - diidroxibenzoic, 3,4-diidroxibenzaldeid, besides the salicylic, cinnamic acid and caffeic acids. Raymundo *et al*. (2004) found values for total phenolic for the green seaweed Enteromorpha intestinalis of 6.01 mg GAE/g, equivalent to results in this experiment for the brown seaweed Hijiki. This variation in the phenolic content quantification can be attributed to different species of seaweeds. Furthermore, these compounds are secondary metabolites and are strongly influenced by the soil and climatic conditions where they are inserted.

The samples of tilapia minced added with seaweed extracts showed a significant induction period compared to the sample without antioxidant. Regarding the effect of extract concentration, we observed that the Nori seaweed at 100 µg GAE/g of minced, showed antioxidant effect lower than 25 and 50 µg GAE/g (Table 1). This same feature was observed for the Hijiki seaweed. Even so, the concentrations tested in this experiment showed an antioxidant effect, since the protection factor is greater than one (Table 1).

Table 1. Induction period (IP) and protection factor (PF) of minced samples analyzed in Oxipres

Treatments	Induction Period (h)	Protection factor
without antioxidants	4.6 ± 0.21^{d}	-
BHT 100 µg GAE/g	10.8 ± 0.23^{a}	2.35
Nori 25 µg GAE/g	6.6 ± 0.07^{b}	1.43
Nori 50 µg GAE/g	6.8 ± 0.15^{b}	1.48
Nori 100 µg GAE/g	5.7 ± 0.13^{c}	1.24
Hijiki 25 µg GAE/g	5.8 ± 0.14^{c}	1.26
Hijiki 50 µg GAE/g	5.4 ± 0.07^{c}	1.17
Hijiki 100 µg GAE/g	5.3 ± 0.07^{c}	1.15

Means (n = 3) and standard deviation are indicated.
Different letters indicated statistically differ at 5% probability (Tukey test).

Table 2. Induction period and protection factor of the samples analyzed in Rancimat

Treatments	Induction Period (h)	Protection factor
without antioxidant	7.05 ± 0.06^{d}	-
BHT 100 µg GAE/g	7.31 ± 0.02^{cd}	1.03
Nori 25 µg GAE/g	8.28 ± 0.04^{b}	1.17
Nori 50 µg GAE/g	8.63 ± 0.06^{ab}	1.22
Nori 100 µg GAE/g	8.72 ± 0.15^{a}	1.23
Hijiki 25 µg GAE/g	7.58 ± 0.23^{c}	1.07
Hijiki 50 µg GAE/g	7.46 ± 0.07^{cd}	1.06
Hijiki 100 µg GAE/g	3.39 ± 0.09^{e}	0.48

Means (n = 3) and standard deviation are indicated.
Different letters indicated statistically differ at 5% probability (Tukey test).

There are no studies in the literature that investigated the induction period of seaweed extracts used in products through the Oxipres method. According to Trojaková *et al*. (2001), the ethanol extract of rosemary, when applied to sunflower oil showed a protective factor in the Oxipres method of 0.3 (in this case, the BHT protection factor was 0.56). The effect of temperature on the antioxidant activity of phenolic acids (gallic, gentisic, protocatechuic, syringic, vanillic, ferulic, caffeic, and sinapic) was studied by Réblová (2012) in pork lard, using an Oxipres apparatus. The results showed that gallic, gentisic, protocatechuic, and caffeic acids showed a significant antioxidant activity at 150ºC and vanillic acid was active at 90ºC.

For the soybean oil analyzes through the Rancimat method, the time required for the occurrence of the maximum oil oxidation rate, without the addition of antioxidants sources (control) was 7.05 h (Table 2). The time required for the free radicals formation, highly reactive molecules, which trigger the oxidation process (initiation phase), was higher in soybean oil added with seaweed extracts, delaying the onset of the propagation phase and consequently the phase of oxidation termination. The Nori seaweed showed concentration-dependent antioxidant effect, i.e., the higher the extract concentration applied to the oil, the more developed the induction period, and for 100 µg GAE/g of oil, the induction period was 8.72 h. The Hijiki seaweed showed the opposite effect, and the pro-oxidant effect was observed at the highest concentration tested, with the induction period of 3.39 h. Thus, seaweed extracts, with the exception

Table 3. Fatty acids content of tilapia minced and soybean oil (mg/100 g)

Fatty acids	Minced	Soybean oil
C4	0,28	0,44
C14:0	0,21	0,08
C16:0	1,49	11,09
C16:1	0,26	0,10
C17	0,00	0,08
C17:1	0,00	0,06
C18:0	0,51	4,01
C18:1n9c	1,46	28,93
C18:2n6c	0,47	47,23
C18:3n3	0,02	3,93
C18:3n6	0,00	0,72
C20:0	0,00	0,45
C20:1	0,07	1,16
C21:0	0,00	0,11
C22:6n3	0,00	0,14
C24:0	0,00	0,59
C24:1	0,00	0,04
Trans Fatt	0,00	0,00

Means (n = 3) and standard deviation are indicated. Different letters indicated statistically differ at 5% probability (Tukey test).

of Hijiki 100 µg GAE/g of oil, protected the oil from oxidation. All treatments with seaweed extracts with antioxidant effect showed a protection factor greater than that of BHT (Table 2). It is known that Hijiki has a high concentration of polysaccharides, including polyalcohols such as xylitol and ribitol in its chemical profile, and fatty acids (Cabral, 2012). With the increase the concentration of this seaweed, these compounds also increased its concentration. Since these are easily oxidized by the temperature, even Maillard reaction may occur in the case of sugars, this may have influenced the antioxidant activity of the same on soybean oil.

As occurred for Oxipres, no studies that evaluate the seaweed antioxidant activity in oils through the Rancimat method were found in the literature. The accelerated tests allow to quickly estimate the oxidative stability of a fatty acid and a selection of antioxidants doses, isolated or associated. Given that the natural phenomena of oxidative processes are slow and often occurring over several months, the stability tests in real time become sometimes incompatible with the quality control for the industry (Frankel, 1993).

When evaluating the antioxidant capacity of grape extracts of Isabel and Niagara varieties through the Rancimat method, Shirahigue *et al.* (2010) found protective factors of 1.79 and 1.59, respectively. Therefore, the results of this study were similar to those found by the authors, also pointing to the antioxidant activity of the seaweed analyzed.

It was observed that the total saturated fatty acids for the minced was 2.49 mg/100 g and for the soybean oil, the total was 16.85 mg/100 g, and as the major fatty acids was the palmitic acid (C16:0) in both samples. For monounsaturated fats, oil and the minced showed levels of 1.79 and 30.29 mg/100 g, respectively, and the oleic acid (C18:1n9c) was the majority. With respect to polyunsaturated fats, the values for the minced and the oil were respectively 0.49 and 52.02 mg/100 g, linoleic acid being predominant (Table 3). For soybean oil, the results confirm the data obtained by Aued-Pimentel *et al.* (2009).

The levels of fatty acids found in this study explain the differences in results presented in the accelerated oxidation tests. Soybean oil has significantly higher levels of fatty acids with a high degree of unsaturation, and this has as a consequence a high reactivity and oxidative instability, caused by the presence of double bonds. Therefore, it becomes necessary to apply more potent antioxidants in soybean oil compared to minced tilapia. This explains the fact that the protective factors of seaweed found in the Rancimat test (in soybean oil) to be relatively small that the protection factors obtained in Oxipres (in minced tilapia).

Conclusions

In conclusion, it was observed differences in response between the two methods used for the tested products. In the Rancimat method, soybean oil with seaweed extracts remained stable longer than the control that used in the synthetic antioxidant BHT. In the Oxipres, the BHT was considered more efficient. However, based on these methods, extracts of Nori and Hijiki seaweeds can be considered as promising antioxidants in foods, as the protective factor was higher than one, in particular minced and soybean oil, since these products are considered to have nutritionally important fatty acids.

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