Influence of *Lentinus edodes* and *Agaricus blazei* extracts on the prevention of oxidation and retention of tocopherols in soybean oil in an accelerated storage test

Ana Carolina da Silva · Neuza Jorge

Revised: 28 November 2011 / Accepted: 16 January 2012 / Published online: 26 January 2012

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Abstract This study aimed to evaluate the influence of the methanol extracts of mushrooms *Lentinus edodes* and *Agaricus blazei* on the retention of tocopherols in soybean oil, when subjected to an accelerated storage test. The following treatments were subjected to an accelerated storage test in an oven at 60 °C for 15 days: Control (soybean oil without antioxidants), TBHQ (soybean oil+100 mg/kg of TBHQ), BHT (soybean oil+100 mg/kg of BHT), *L. edodes* (soybean oil+3,500 mg/kg of *L. edodes* extract) and *A. blazei* (soybean oil+3,500 mg/kg of *A. blazei* extract). The samples were analyzed for tocopherols naturally present in soybean oil and mass gain. The results showed, the time required to reach a 0.5% increase in mass was 13 days for TBHQ and 15 days for *A. blazei*. The content of tocopherols for TBHQ was 457.50 mg/kg and the *A. blazei*, 477.20 mg/kg.

Keywords Mushrooms · Antioxidants · Mass gain · Tocopherols

Introduction

The lipid oxidation is the main cause of quality loss of fatty foods during processing and storage. This degradation can cause changes of taste, odor and color, destroying fat soluble vitamins and polyunsaturated fatty acids. The food industry tries to minimize such damage with the use of synthetic antioxidants, namely BHA, BHT and TBHQ. However, the use of additives is strictly controlled because of the human

health damage that it can cause (González-Montelongo et al. 2010).

In Canada and the European Community the use of TBHQ is not allowed (Reische et al. 2002). The *Codex Alimentarium* limited to 200 mg/kg the use of BHT in vegetable oils (FAO 2010). The concern regarding food safety among consumers leads the food industry to seek safer alternatives that can replace synthetic antioxidants. Many researchers have been devoted to the search for natural compounds from by-products such as grape marc (Lafka et al. 2007; Pinelo et al. 2005), orange peel (Xu et al. 2008), pomegranate (Li et al. 2006; Devatkal et al. 2011) and star fruit (Shui and Leong 2006).

Mushrooms have also been the target of research because they have significant amounts of phenolic compounds and some varieties, like *L. edodes* and *A. blazei*, provide the strengthening of the immune system when consumed frequently (Choi et al. 2006; Huang and Mau 2006). The in vivo action of mushrooms was also evaluated in the treatment of diabetes, hypertension and hepatitis (Aida et al. 2009). However, few studies focus on the antioxidant activity of mushroom extracts against lipid oxidation.

Tocopherols are monophenol compounds existing in plants, mainly in oil seeds and leaves, which have an antioxidant activity and vitamin E. The nomenclature of these compounds gets the prefix of α , β , γ e δ , depending on the number and position of the methyl group attached to aromatic ring. The α -tocopherol is the compound that has greater activity of vitamin E (Mau et al. 2004; Yang et al. 2002).

Thus, the aim of this work was to evaluate the influence of the methanol extracts of *L. edodes* and *A. blazei* on the retention of tocopherols in soybean oil when subjected to an accelerated storage test.

A. C. da Silva · N. Jorge (✉)
Department of Engineering and Food Technology,
São Paulo State University,
2265, Cristóvão Colombo St., Jd. Nazareth, São José do Rio Preto,
São Paulo 15054-000, Brazil
e-mail: njorge@ibilce.unesp.br

Materials and methods

Mushrooms and soybean oil Approximately 4.0 kg of raw mushroom (*Lentinus edodes*), produced and marketed in the region of Salto, in the state of São Paulo, on April/2009, was used. It was packaged and cooled and sent directly to the Laboratory of Oils and Fats, at the Department of Engineering and Food Technology, at UNESP. The *Agaricus blazei* was acquired in the region of São José do Rio Preto, in the State of São Paulo, during the months of April, May and September/2009, totaling about 3.0 kg. Immediately after harvest, raw *A. blazei* was washed and sent to the Laboratory of Oils and Fats, Department of Engineering and Food Technology, at UNESP.

The samples were frozen at $-30\text{ }^{\circ}\text{C}$ for 24 h and lyophilized in lyophilizer brand Liotop model L101 and crushed in a grinding mill, brand Erbele, model 2508.001-2. The powder obtained was stored in dark plastic containers, sealed with screw caps and labeled for later analysis. The samples of *A. blazei* were homogenized in order to minimize possible variations between batches.

For analysis of oxidative stability, the refined soybean oil was used, without the addition of synthetic antioxidants (TBHQ and citric acid), Cargil Agrícola S/A brand, purchased in packs of 900 mL in the local market.

Extracts and antioxidants The methanol extracts of mushrooms were obtained by slow extraction (120 rpm, 3 h on shaker). The extract was obtained stirring 8 g of mushroom with 80 mL of methanol, in the dark and at room temperature. Then, the mixture was centrifuged at 3,000 rpm for 5 min and the supernatant was filtered with vacuum pump and subjected to rotary evaporator under reduced pressure at $40\text{ }^{\circ}\text{C}$. The dry extract was weighed and resuspended in methanol, yielding a stock solution containing 1.0 g of extract and 10 mL of methanol, used for direct application in soybean oil.

The synthetic antioxidants *tert* butyl hydroquinone (TBHQ) (100 mg/kg) and butylated hydroxytoluene (BHT) (100 mg/kg) were used in their commercial form, supplied by Danisco A/S.

Experimental testing The following treatments were subjected to an accelerated storage test in an oven at $60\text{ }^{\circ}\text{C}$ for 15 days: Control (soybean oil without antioxidants), TBHQ (soybean oil+100 mg/kg of TBHQ), BHT (soybean oil+100 mg/kg of BHT), *L. edodes* (soybean oil+3,500 mg/kg of *L. edodes* extract) and *A. blazei* (soybean oil+3,500 mg/kg of *A. blazei* extract), with 50 mL beakers containing 30 mL of sample with surface/volume ratio 0.4 cm^{-1} . All samples, at different time intervals (0, 3, 6, 9, 12 and 15 days), were collected and flushed with nitrogen gas and stored at approximately $-18\text{ }^{\circ}\text{C}$ until analysis of tocopherols naturally present in soybean oil added to

extracts of mushrooms. Simultaneously the test was conducted to mass gain, in which glass Petri dishes containing the oil samples were taken daily from the oven for weighing. For application of the mushroom extract in soybean oil, several extract concentrations were tested (0 to 3,500 mg/kg). By linear regression, it was observed that the highest antioxidant activity, measured by the oxidative stability, agreed with the highest concentration of shiitake and almond mushroom extracts, which is of 3.500 mg/kg.

Mass gain For mass gain analysis, 2.0 g of each sample were placed in glass Petri dishes, which were kept in an oven at $60\text{ }^{\circ}\text{C}$. The rate of oxidation, in terms of mass increase, was recorded at 24 h intervals up to 16 days. The time required for a 0.5% mass increase for oil was taken as the index of stability and the mass gain was calculated as percentage of the original weight (Iqbal and Bhanger 2007). The weighing machine used was Ohaus, model AS200 (number of digit 0,0001 g and precision 0,0025 g).

Tocopherols For the chromatographic analysis of tocopherols, performed using the method AOCS Ce 8–89 (1997), the high performance liquid chromatography was used, consisting of a Varian pro Star 210, with a fluorescence detector. Under the conditions of analysis, it was used silica column of $250\times 4.6\text{ mm}$ with pores of $5\text{ }\mu\text{m}$, flow 1.2 mL/min, wavelength of excitation at 290 nm and emission at 330 nm and as a mobile phase the mixture of 99.5% of *n*-hexane and 0.5% of isopropanol, all with purity for HPLC. The identification of tocopherols was made by comparison with the retention time of standards Sigma 95% purity. These were quantified by external standard and the levels of tocopherols expressed as mg/kg.

Statistical analysis The results obtained for tocopherols and mass gain, in duplicate, were submitted to analysis of variance to determine the influence of the factors (treatments and storage period) on the modification of oils subjected to the accelerated storage test. The 5×6 factorial experiment was performed in a completely randomized delineation (Gacula and Singh 1984). Both the analysis of variance and the Tukey test for the 5% average were obtained using the ESTAT (System for Statistical analysis, version 2.0).

Results and discussion

Mass gain Mass gain is usually employed to estimate the amount of oxygen added to the unsaturated content of lipid molecules and formation of hydroperoxides during oxidation. The mass gain was measured for all the samples at 24 h intervals for 16 days and results, in percentage, are shown in Fig. 1.

Fig. 1 Mass gain of treatments subjected to accelerated storage. Data are mean $\pm$ SD ($n=2$)

Figure 1 shows that the time required to reach a 0.5% increase in mass were 10, 13, 10, 11 and 15 days for the Control, TBHQ, BHT, *L. edodes* and *A. blazei*, respectively. According to Evans et al. (1973), each day of storage in an oven at 60 °C is equivalent to 1 month storage at room temperature. Initially, the mass gain was not significant, but

a sudden increase was observed in all samples followed by a decline for Control, TBHQ, BHT and *L. edodes* along the storage.

The decline of mass gain is due to the fact that peroxides, primary products of oxidation formed during accelerated storage, can be degraded into low molecular weight compounds including volatile substances. The Control remained stable until the 9th day, followed by a sudden increase until the 11th day, assuming the maximum 4.71% increase in mass gain followed by a sharp decrease until the end of the storage period.

The treatment BHT had similar behavior to Control, assuming greater mass gain in 11 days of storage, with 4.18%. The *L. edodes* was efficient against oxidation during the first 11 days of storage, and the maximum amount reported was 4.05% after 13 days of storage. The TBHQ showed no significant increase until the 13th day since *A. blazei* maintained this protection until the 15th day, followed by a sharp increase, becoming more efficient in protecting the oil against oxidation compared to other treatments.

Fig. 2 Residual content (%) of total tocopherols and their isomers in soybean oil with the addition of antioxidants. Data are mean $\pm$ SD ($n=2$)

Iqbal and Bhanger (2007) investigated the mass gain of sunflower oil with the addition of garlic extract (250, 500 and 1,000 mg/kg) subjected to an accelerated storage test in an oven at 65 °C for 14 days. The BHT (200 mg/kg) was used as a comparison. The authors found that the garlic extract at 1,000 mg/kg was more effective in protecting the oil, followed by BHT. The sunflower oil without antioxidants, unlike soybean oil, showed mass gain in the first days of storage. It shows that the sunflower oil is more susceptible to oxidation because its unsaturation (89.0%) is higher than that in soybean oil (83.4%).

Tocopherols The retention of tocopherols in soybean oil is one way to evaluate the efficiency of mushroom extracts added to oil during the accelerated storage and compare them with synthetic antioxidants.

It is observed that the levels of α -tocopherol remained constant in treatments TBHQ and *A. blazei*, while in the other treatments the levels of this isomer decreased with storage period. At the end of 15 days it was found that there was no significant difference either in the content of α -tocopherol for TBHQ and *A. blazei* treatments or for Control and BHT.

The only treatment that effectively protected the contents of β -tocopherol was the *A. blazei*. For the other treatments, there was a slight decrease in the quantity of the isomer,

especially at the end of the process of storage, the TBHQ and *A. blazei* were statistically equal to 15 days, the same as the Control, BHT and *L. edodes*.

The levels of γ -tocopherol were maintained throughout the storage for all treatments, except the *L. edodes* that showed a decrease at the end of storage. The same trend was observed for the δ -tocopherol isomer.

For contents of total tocopherols, it was found that the protective effect of Control and BHT were reduced during storage, showing 351.95 and 378.10 mg/kg of total tocopherols, respectively, at the end of 15 days of storage.

L. edodes was effective in protecting the total tocopherols from soybean oil until the 9th day of storage, with 363.05 mg/kg of tocopherols on the 15th day, statistically equal to Control and BHT. For treatments TBHQ and *A. blazei*, the levels of total tocopherols did not reduce during the storage time featuring an average of 446.07 and 476.73 mg/kg of total tocopherols, respectively.

Overall, it was observed that the effect of treatments on the content of tocopherols and their counterparts was more significant compared to the effect of storage period. This is because the test was conducted in mild temperature, that is, 60 °C. Studies on thermoxidation of oil, simulating frying processes, show that the most severe temperatures are applied to the oil reaching 180 °C. In these cases, the loss of the protective effect

Fig. 3 HPLC chromatograms of *A. blazei* at the 0 day **a** and 15 days **b** of storage, respectively. (1) α -tocopherol, (2) β -tocopherol, (3) γ -tocopherol, (4) δ -tocopherol

of tocopherols by antioxidants can be seen more explicitly (Angelo and Jorge 2008; Ramalho and Jorge 2008).

To better assess the protection of tocopherols naturally present in soybean oil, the residual content (%) of tocopherols during the storage period was calculated. These values are represented in Fig. 2. It was observed that the degradation of tocopherols was just significant, that is, below 50%, for treatments Control and BHT in α -tocopherol. With the exception of Control, all treatments protected the α -tocopherol of soybean oil up to the 6th day of storage. Starting on the 9th day it was observed a loss of α -tocopherol for BHT. At the end of the test, the antioxidant which was most effective in retaining α -tocopherol was TBHQ (95.18%), followed by *A. blazei* extract (95.17%), *L. edodes* extract (70.84%), BHT (28.18%) and Control (21.17%).

The *L. edodes* extract was the less effective treatment in the retention of β , γ and δ isomers in soybean oil at the end of storage. On average, it has 76.23% of residual tocopherols.

For total tocopherols, the most outstanding treatments were TBHQ and *A. blazei*, with 100 and 97.50% of residual content tocopherols, followed by Control (86.11%), BHT (80.02%) and *L. edodes* (76.64%).

The Fig. 3 represents the chromatograms of *A. blazei* at the 0 day (a) and 15 days (b) of storage, respectively.

Ramalho and Jorge (2008) found a retention factor of 95% for soybean oil added to rosemary extract (1,000 mg/kg) subjected to 10 h of thermoxidation and 70% for soybean oil naturally, showing good efficiency of the rosemary to protect the soybean oil. For the same period of thermoxidation, Angelo and Jorge (2008) determined 26.70% of retention factor of α -tocopherol for the sunflower oil, and at the end of 30 h, this figure dropped to 4.0%.

Conclusion

The extracts of *A. blazei* and the TBHQ were the most effective treatments on the retention of α -tocopherol naturally present in soybean oil. In all treatments, the isomers β , γ and δ -tocopherol were more resistant to storage period when compared to α -tocopherol, with high residual levels after 15 days of storage. Regarding the retention of total tocopherols content, the TBHQ and *A. blazei* extract were the most efficient, followed by BHT, Control and *L. edodes*. Therefore, natural extracts of mushrooms can be applied to vegetable oils as a way to reduce the degradation caused by lipid oxidation.

Acknowledgments The authors wish to thank Regina H. C. Garcia and Edalcy Ferrano (Estância Vilamar) who generously provide the *A. blazei* samples of and Denise Abackerli for the *L. edodes*.

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