DETERMINING ECONOMICAL TBHQ DOSES FOR CORN OIL STABILITY[1]

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SUMMARY

Corn oil obtained from a Brazilian industry, free from antioxidants and citric acid, added of different tertiary butylhydroquinone (TBHQ) concentrations, was submitted to accelerated oxidation in the Schaal oven test at 63°C for 120 hours and for 168 hours in a photooxidation chamber. Peroxide and absorptivity values at 232nm and 270nm were determined for this oil. From the Schaal oven test results, the best and the economical TBHQ doses were determined to this oil. Afterwards, a shelf life experiment was conducted and confirmed 115mg.kg^{-1} TBHQ as the best and economical dose for that oil.

Keywords: oxidative stability; TBHQ; corn oil; shelf life; economical dose.

RESUMO

DOSE ECONÔMICA DE TBHQ PARA A ESTABILIDADE DE ÓLEO DE MILHO. Óleo de milho obtido de uma indústria brasileira isento de antioxidantes e de ácido cítrico foi adicionado de diferentes concentrações de TBHQ e submetido a testes acelerados de oxidação em estufa a 63ºC por 120 horas e em câmara de fotoxidação por 168 horas. Foram analisados o índice de peróxido e as absortividades em 232nm e 270nm deste óleo. A partir dos resultados do teste em estufa, calculou-se a melhor dose e a dose econômica do antioxidante TBHQ. Em seguida foi conduzido, então ensaio ao ambiente, que confirmou a concentração de 115mg.kg^{-1} TBHQ como a dosagem econômica e melhor dose para o óleo sob estudo.

Palavras-chave: estabilidade oxidativa; TBHQ; óleo de milho; vida-útil; dosagem econômica.

1 – INTRODUCTION

Vegetable oils contain polyunsaturated fatty acids (PUFAs) in their composition which is considered to be good for health and its consumption has been increasing in the last few years. As PUFAs present double bonds, the oil becomes susceptible to oxidative reactions, decreasing its shelf life.

The use of PET packaging (polyethyleneterephtalate) has also increased in the oil industry. However this packaging does not give a total protection against the ultraviolet rays as the metallic packaging does, so it is necessary to use an antioxidant to assure this protection against light, oxygen and heat.

TBHQ (tertiary butylhydroquinone) is a phenolic antioxidant, approved by Brazilian food legislation and by the FDA (Food and Drug Administration), in the United States. Its mechanism of action and health implications have been studied for more than twenty years. Many studies have showed its efficiency when compared to other existing antioxidants. An economical dose, lower than the maximum allowed, can be established to be in agreement with the product needs and the market's economical competitivity.

Lipid oxidation can be triggered by endogenous species (H_2O_2, ROOH) and radicals ($^{\cdot}O_2$, $ROO^{\cdot}$,.OH, $GS^{\cdot}$) or by exogenous species ($^1O_2$, O_3), radicals (NO_x, SO_3^-), and agents, as UV rays, ionizing radiation and heat [26]. Metals are known to be pro-oxidants even in small amounts; heat is also a powerful oxidation accelerator, especially above 60ºC, when the reaction rate doubles every 15ºC increase [25].

On the free radical route, the fatty acid double bond adjacent to the methyl group is activated, turning it into a vulnerable site for the oxygen insertion. Thus, a free radical is formed and consequently a fatty acid hydroperoxyde is also formed, because one hydrogen was suppressed from an intact molecule, producing another peroxyde radical. The resonance effect on the molecule double bonds turns the free radical stable [15, 20], since it lowers the molecules energy [1] and the non-paired electron can interact with the other atoms or groups.

Photooxidation involves the interaction between the double bond and the singlet oxygen, produced by the light (especially the UV light) on the triplet oxygen, in presence of sensitizers such as chlorophylls [11, 25]. This is not a chain reaction, there is no induction period and it is faster than the autoxidation. Singlet suppressors such as carotene can inhibit it [11, 20]. The singlet oxygen is formed by the light energy transference (absorption) to the oxygen molecule, by a pigment (photosensitizer), generating excitated singlet state oxygen molecules that attack the oil [18, 30]. Singlet oxygen molecules can react at room temperature or below, during the oil storage and commercialization [15].

Packaging main function is to protect the food, to increase the shelf life and to be a barrier to oxygen, moisture and light radiation [9]. This protection depends on the light transmission characteristics and its chemical composition or molecular structure. PET films absorb about 80% of UV rays under 300nm [8].

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TBHQ was introduced in the 70's and approved as food antioxidant in 1972. It is a white crystalline and bright powder, with a light odor and minimum melting point around 125-126^{0}C. It is moderately soluble in oils and fats, it does not form complexes with iron or cupper, and it is a good antioxidant for frying oils [16, 24].

The antioxidant efficiency on the oxidative oil deterioration control has been observed in several studies. TBHQ proved to be more efficient than BHA (butyl hydroxyanisol), Di-BHA (dibutyl hydroxyanisol) and PG (propyl gallate). Citric acid did not prove to be a synergic agent to TBHQ [7].

TBHQ maximum allowed concentration according to the Brazilian legislation is 0.02g/100g of oil [5].

TBHQ superiority was observed when compared to BHT (butyl hydroxytoluene) in soybean, sunflower, canola and palm oil through the oxidative stability index (OSI). TBHQ showed to be more economical than the others [17].

TBHQ showed to be more efficient than BHA and BHT to prevent Brazil nut crude oil oxidation, and the economical dose was 82.37mg.kg^{-1} [12]. It was more efficient than BHA and BHT, under accelerated photooxidation of soybean, corn and canola oils [27]. TBHQ superiority was also confirmed on soybean oil against rosemary and oregano ethanolic extracts, or a mixture of BHA+BHT, after 5 and 7 days in Schaal oven tests at 63^{0}C. The economical dose was determinated to be 126.09mg.kg^{-1} [2].

The stability of oils added of antioxidants can be evaluated by storing them under room conditions or by exposing them to accelerated tests, which can be monitored by observation of chemical, physical and organoleptical changes [21].

Primary changes are evaluated by PUFAs losses, incorporated oxygen, weight gain, hydroperoxydes amounts and conjugated dienes content. The secondary changes can be followed by the formation of carbonyl compounds, malonaldehyde and other aldehydes, hydrocarbons and fluorescent products [13, 22].

Peroxide value is used to quantify the hydroperoxydes formed [21]; however these are intermediate products in the formation of carbonyls and hydroxy-compounds [13].

PUFAs oxidation can be analyzed by the absorptivity increase in the UV spectrum. During the oxidation process, lipids that have dienes and polienes showed a change in the double bonds position, due to isomerization and conjugation in the molecule. Dienes and trienes formation is proportional to peroxide formation on the oxidation initial steps and they show intense absorption at 234nm and 268nm, respectively [21, 23, 29].

In Brazil nut crude oil 200mg.kg^{-1} TBHQ was more efficient than 200mg.kg^{-1} BHA and 100mg.kg^{-1} BHT, during a Schaal oven test. The addition of this concentration retarded the oxidative degradation in this oil packed in glass flasks and stored for 180 days [19].

Soybean oil exposed to fluorescent light (8,370 Lux), in a rectangular box of 80cm length x 35cm height x 60cm width, provided with six of 20W fluorescent light bulbs, displayed 3 above and 3 under the samples, presented increasing peroxide value and absorptivities at 232 and 270nm with time [2].

A TBHQ economical dose was determined through the Schaal oven test for corn oil and the oil stability evaluation added of this economical dose under ambient conditions comparing to the maximum legislation limit and a control without TBHQ were the targets of this work.

2 – MATERIAL AND METHODS

The oil used in this experiment was refined and without antioxidant and citric acid, obtained from a Brazilian refinery industry. This oil was analyzed and packed in 500mL PET flasks.

Schaal oven tests were performed, with 20g corn oil samples, in triplicate 50mL beakers, added of increasing doses of TBHQ, from 0 to 200mg.kg^{-1}, stored in an oven at 63 ±1^{0}C, for 120 hours. Absorptivities at 232 and 270nm and peroxide values were analyzed in the oils.

In the photooxidation chamber test, 20g of oil in triplicate 50mL beakers was added of increasing doses of TBHQ, from 0 to 200mg.kg^{-1}, arranged in a rectangular box of 80cm length x 35cm height x 60cm width, provided with six of 20W fluorescent light bulbs, displayed 3 above and 3 under the samples, so that the light could equally reach all the samples. The chamber light intensity was 2,800 Lux. This assay was conducted for a period of 168 hours; the oil was submitted to the same analyses as in Schaal oven test.

The free fatty acids and peroxide value were determined according to the AOCS-Ca 5a-40 and AOCS-Cd 8-53 official methods [3], respectively, the iodine value according to the AOCS-Cd 1b-87 official method [3], and the absorptivities in 232nm e 270nm, were determined in Shimadzu spectrophotometer, UV 1203 model, according to the NDG C-40 [28] or IUPAC II.D.23 method [14].

A polynomial regression curve was obtained from the plot of peroxide values reached after 120 hours heating at 63^{0}C in the Schaal oven test and the increasing TBHQ doses. From this the best and the economical doses could be determined.

$$Y = ax^2 + bx + c \quad (1)$$

Where: y = peroxide value (meq O_2/1000g of oil)

x = antioxidant dose (mg.kg^{-1})

By deriving equation (1), the best dose of TBHQ was calculated.

The economical dose was calculated as following:

$$D.E.= [(\text{oil price}/\text{TBHQ price}) - b] / 2a \quad (2)$$

Where a and b are found in the quadratic polynomial equation [10].

The assay was conducted at room temperature for six months with 500mL of corn oil in PET bottles added of TBHQ economical dose, the maximum allowed dose 200mg.kg^{-1} and without any antioxidant (control), in triplicate. Absorptivities at 232 and 270nm and peroxide values were determined every month; iodine and acid values, every 3 months.

The Tukey test was applied, considering 5% as significance level, in the Sanest program [31].

3 – RESULTS AND DISCUSSION

Corn oil was analyzed at the beginning of the experiment and its characteristics presented on *Table 1* are according to the Brazilian legislation (Res. 482/99) [6], that states corn oil should present iodine value should be from 103 to 128mg I/100mg of oil, peroxide value be lower than 10meq/kg of oil and free fatty acids lower than 0.30g of oleic acid/100g of oil.

TABLE 1. Characterization of the refined corn oil

Analysis	Results
Free fatty acid (goleic acid/100g)	0.14
Peroxide value (meq O_2/kg)	0.07
Iodine value (mg I/100mg)	112
Absorptivity at 232nm	2.73
Absorptivity at 270nm	1.27

Schaal oven test was conducted for 5 days in order to study the antioxidant efficiency. Previous results indicate this period to be the minimum length required to observe differences in antioxidant activities in soybean oil peroxide values added of rosemary, oregano, BHA/BHT and TBHQ [2]. Results are presented in *Table 2*.

TABLE 2. Peroxide value of the corn oil added of different concentrations of TBHQ in the Schaal oven test for 5 days at 63°C.

Oil added of mg.kg^{-1} of TBHQ	Peroxide value (meq O_2/kg of oil) CV = 4.65%
0	2.32^{a}
25	2.02^{acd}
50	1.96^{acd}
75	1.70^{bcd}
100	1.96^{acd}
125	1.50^{b}
150	1.67^{bd}
175	1.56^{b}
200	1.86^{acd}

CV = variation coefficient.
The data are mean values of three analysis; when followed by the same letter, in the columns, they do not differ significantly by the test of Tukey at 0.05 level of significance.

As can be seen in *Table 2*, after 120 hours in an oven at 63°C corn oil presented decreasing peroxide values with increasing TBHQ concentrations until 125mg.kg^{-1} which presented the minimum peroxide value in this test, 1.5meq O_2/kg of oil. For higher than 125mg.kg^{-1} antioxidant doses increasing peroxide values were found, indicating that an antioxidant concentration lower than the maximum allowed (200mg/kg^{-1}), which gave a peroxide value of 1.86meq O_2/kg of oil, could be even more efficient in corn oil and at the same time present significant savings for the industry and less additives present in this food ingredient. This behavior had been previously observed in soybean and Brazil nut crude oils after 5 days in the Schaal oven test [2, 12,19].

Citric acid was not used as synergistic agent because it did not showed any effect in preliminary tests [21].

Figure 1 shows results of absorptivities at 232nm and 270nm of corn oil added of different TBHQ concentrations and confirms tendency observed on *Table 2*. Lower TBHQ concentrations than maximum allowed may be more efficient.

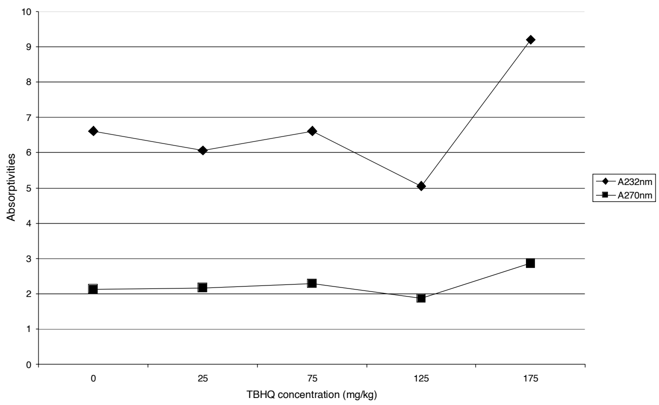

FIGURE 1. Absorptivities at 232nm and 270nm of corn oil added of different TBHQ concentrations and heated in Schaal oven test for 5 days at 63°C.

In the light induced oxidation experiment, higher peroxide and absoptivity values were obtained when compared to the Schaal oven test. TBHQ protection could also be observed, although concentrations below maximum were not as efficient (*Table 3*).

TABLE 3. Peroxide values (meq O_2/kg of oil), absorptivities in 232nm and 270nm of corn oil added of different TBHQ concentrations in a photooxidation chamber for 7 days.

Oil added of mg.kg^{-1} TBHQ	PV CV = 17.96%	$A_{232 nm}$ CV = 37.10%	$A_{270 nm}$ CV = 26.55%
0	33.08^{a}	21.73^{a}	5.16^{ab}
50	15.81^{bc}	15.25^{a}	3.66^{ab}
100	15.38^{bc}	12.49^{a}	3.38^{ab}
150	16.03^{bc}	11.72^{a}	2.94^{b}
200	11.29^{c}	11.97^{a}	3.28^{b}

CV = variation coefficient.
The data are mean values of three analysis; when followed by the same letter, in the columns, they do not differ significantly by the test of Tukey at 0.05 level of significance.

On the light induced oxidation test for soybean oil, an inverse correlation between the TBHQ doses and the peroxide value (PV) was obtained after 5 days [2]. When comparing with soybean oil photooxidative stability, corn oil was more stable, probably due to the absence of photosensitizers, like chlorophylls, and to a less unsatured fatty acid composition, besides the presence of tocotrienols, potent natural antioxidants [27].

The best and the economical doses were determined as described.

The following equations were obtained:

$$y = 0.00005\,x^2 - 0.0119\,x + 2.3313 \qquad (3)$$

So, $dy/dx = 0 \Rightarrow 0{,}0001\,x - 0{,}0119 = 0$, and the best dose was $119 mg.kg^{-1}$.

The economical dose (E.D.) was calculated considering US$ 701.47/ton as the refined corn oil price and US$ 30.00/kg as the TBHQ price (september/2001).

$$E.D. = [(0.70147 / 30{,}00) - 0{,}0119] / 2 \times 0.00005 \qquad (4)$$

115 $mg.kg^{-1}$ of TBHQ value was obtained as the E.D. for corn oil.

As the best dose and the E.D. were very close, 115$mg.kg^{-1}$ was employed in the shelf life test.

For soybean oil, after 7 days in the Schaal oven test at 63^{0}C, the best dose was 128.85$mg.kg^{-1}$ and 126.09 $mg.kg^{-1}$ was the E.D. [2].

TABLE 4. Characterization of refined corn oil.

Analysis	Results
Free fatty acid (% oleic acid/100g)	0.12
Peroxide Value (meq O_2/kg)	0.95
Iodine Value (mg I/100mg)	110
Absorptivity in 232nm	2.40
Absorptivity in 270nm	1.07

TABLE 5. Peroxide value (meq O_2/kg of oil) of corn oil added of different TBHQ concentrations, stored in PET bottles under ambient conditions.

Treatments	Time (months)						
	0	1	2	3	4	5	6
Control	0.95^{aE}	1.41^{aE}	2.90^{aD}	3.40^{aD}	4.11^{aC}	6.78^{aB}	8.31^{aA}
115$mg.kg^{-1}$	0.95^{aD}	1.20^{aD}	2.34^{abC}	2.73^{bC}	3.96^{aB}	3.96^{bB}	4.88^{bA}
200$mg.kg^{-1}$	0.95^{aD}	1.16^{aD}	2.70^{bC}	2.74^{bC}	3.67^{aB}	3.83^{bB}	4.77^{bA}

CV = 8,21%.
The data constitute means of three repetitions; the mean values followed by the same small letter, in the rows, and by capital letters, in the lines do not differ significantly by the test of Tukey in 0.05 level of significance.

A storage or shelf life experiment was conducted with a new corn oil whose initial characteristics are presented in *Table 4*. *Table 5* presents peroxide values obtained during stored. They increase with time, although TBHQ effect can be clearly observed. A very similar behaviour is observed for the economical and maximum TBHQ concentrations, which was expected, supporting our hypothesis that lower antioxidant concentrations. In despite of this, corn oil without TBHQ kept its quality within the legislation limit. A similar behavior was also observed by SIQUEIRA [27].

Significant differences in the absorptivity in 232nm values were observed in first four months (*Table 6*). From the fifth month on, significant differences between the control and the other treatments were observed, indicating TBHQ long term effect.

Absorptivities in 270nm did not detect differences between the treatments (*Table 7*). Since volatile and conjugated trienes are the detectable compounds, only volatiles would be detected because there are no relevant amounts of triunsaturated fatty acids. However odour compounds that absorb in this wavelength can be lost through the air [27]. The oil was analyzed *per se* and therefore no detectable difference in antioxidant activity towards the control could be seen.

TABLE 6. Absorptivity in 232nm of corn oil added of different TBHQ concentrations, stored in PET bottles under ambient conditions.

Treatments	Time (months)						
	0	1	2	3	4	5	6
Control	2.40^{aBC}	2.44^{aBC}	2.35^{aC}	2.34^{aC}	2.65^{aB}	3.20^{aA}	3.14^{aA}
115$mg.kg^{-1}$	2.40^{aAB}	2.41^{aAB}	2.27^{aB}	2.25^{aB}	2.65^{aA}	2.61^{bA}	2.62^{bA}
200$mg.kg^{-1}$	2.40^{aAB}	2.44^{aAB}	2.34^{aB}	2.39^{aAB}	2.61^{aA}	2.64^{bA}	2.57^{bAB}

CV = 4.09%.
The data constitute means of three repetitions; the mean values followed by the same small letter, in the rows, and by capital letters, in the lines do not differ significantly by the test of Tukey in 0.05 level of significance.

TABLE 7. Absorptivity in 270nm of corn oil added of different TBHQ concentrations, stored in PET bottles under ambient conditions.

Treatments	Time (months)						
	0	1	2	3	4	5	6
Control	1.07^{aB}	1.05^{aB}	1.04^{aBC}	1.04^{abBC}	1.10^{aA}	1.01^{bC}	1.11^{aA}
115$mg.kg^{-1}$	1.07^{aBC}	1.04^{aCD}	1.01^{aDE}	1.00^{bE}	1.10^{aAB}	1.03^{aDE}	1.12^{aA}
200$mg.kg^{-1}$	1.07^{aB}	1.05^{aB}	1.04^{aB}	1.06^{aB}	1.07^{bB}	1.05^{aB}	1.14^{aA}

CV = 2,41%.
The data constitute means of three repetitions; the mean values followed by the same small letter, in the rows, and by capital letters, in the lines do not differ significantly by the test of Tukey in 0.05 level of significance.

Acid values did not differ among the treatments for each period but, although very slightly, did differ statistically during storage time from their initial values. However, no differences were detected among the treatments with time (*Table 8*). As antioxidants play no role on hydrolysis, no significant difference was detected among the treatments. These results indicate the adequate prevailing ambient during the storage conditions. Iodine values decreased with the storage (*Table 9*), as was expected due to the conjugation of the double bonds resulting from oxidation which make them somewhat inaccessible for iodine to be reduced during the iodine value determination [4]. SIQUEIRA [27] observed the same behavior. *Table 9* results also indicate that lower antioxidant concentration added to the oil has a better effect as can be observed from the treatment added of 115mg/kg^{-1}.

TABLE 8. Free fatty acids (% oleic acid/100g of oil) of corn oil added of different TBHQ concentrations, in PET bottles stored under ambient conditions.

Treatments	Time (months)		
	0	3	6
Control	0.12^{aA}	0.11^{aB}	0.11^{aB}
115mg.kg^{-1}	0.12^{aA}	0.11^{aB}	0.11^{aB}
200mg.kg^{-1}	0.12^{aA}	0.11^{aB}	0.11^{aB}

CV = 0.03%.
The data constitute means of three repetitions; the mean values followed by the same small letter, in the rows, and by capital letters, in the lines do not differ significantly by the test of Tukey in 0.05 level of significance.

TABLE 9. Iodine Value (mg/100mg of oil) of corn oil added of different TBHQ concentrations, in PET bottles stored under ambient conditions.

Treatments	Time (months)		
	0	3	6
Control	110.31^{aA}	107.92^{aB}	103.80^{bC}
115mg.kg^{-1}	110.31^{aA}	108.34^{aB}	105.11^{aC}
200mg.kg^{-1}	110.31^{aA}	107.65^{aB}	103.92^{bC}

CV = 0.58%.
The data constitute means of three repetitions; the mean values followed by the same small letter, in the rows, and by capital letters, in the lines do not differ significantly by the test of Tukey in 0.05 level of significance.

Based on the research group experience, the authors reccomend that Schaal oven test is performed for every oil to be stored if a lower antioxidant concentration is to be used.

4 – CONCLUSIONS

TBHQ demonstrated to have protective effect in corn oil against oxidation at a dose lower than the maximum established by the Brazilian legislation. 115mg.kg^{-1} showed to be efficient for corn oil used in the experiment.

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