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Cottonseeds oil conservation test by using *Ocimum basilicum* essential oil as natural antioxidant

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ABSTRACT

Ocimum basilicum essential oil, whose main constituents were linalool (48.73%) and eugenol (27.46%), was used as natural antioxidant in preventing the oxidation of a lipid: cottonseed oil. The study of the accelerated oxidation of cottonseed oil has been done in an illuminated oven at 60°C during 30 days. The results show that essential oil of Ocimum basilicum offered good possibility to control the oxidation rate of cottonseed oil. Essential oil was tested at different doses: 0, 200, 500 and 1000 ppm. It allowed the conservation of cotton oil during 5, 10 and 25 days for the respective doses of 200, 500 and 1000 ppm, where conservation time is the time period between experiments starting and when the peroxide index of oil reaches a value of 10 meq of O_2/kg of cotton oil. These results were compared to those of BHT and showed that O. basilicum essential oil could be an alternative to the use of synthetic antioxidants.

Keywords*: Ocimum basilicum*, essential oil, antioxidant activity, cottonseed oil, peroxide index.

INTRODUCTION

All fats during their use or their conservation undergo oxidative damage [1]. The primary products of oxidation are peroxides which, after their decomposition, change into low molecular weight compounds (aldehydes, ketones, acids, etc.) responsible for the quality deterioration of fats [1].

Cottonseed oil is an important component of diets in Burkina Faso. This vegetable oil may be subject to oxidative alterations due to the conditions of storage and especially when it is exposed to the sun (relatively high temperature) during the sale. In these conditions, the oil oxidation may have several impacts: nutritional and organoleptic impacts (degradation of fat-soluble vitamins and essential fatty acids, color change, smell, etc.), health impact (the primary compounds of oxidation - peroxides - have cytotoxic, mutagenic or carcinogenic effects) [2, 3], and economic impact (loss of value after oxidation which devalues the quality of the product) [4].

The oil industry has to pay special attention in this context, as oils, fats and fatty foods suffer from stability problems. To improve the stability of oils and fats, synthetic antioxidants such butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ter-butylhydroxyquinone (TBHQ) have long been used. But recent reports reveal that the synthetic antioxidants may be implicated in many health risks, including cancer and carcinogenesis. [5]. It was then necessary to limit the use of these synthetic antioxidants.

In order to reduce or to find an alternative of these synthetic antioxidants, there is an increasing trend among food scientists to replace them with natural ones, which are supposed to be safer [6-8]. Among the available natural

antioxidants, essential oils (EO) are beginning to have much interest as a potential source of natural antioxidant molecules; they are the subject of study for their possible use as an alternative for the protection of the fat and thus food containing against oxidation [9-10]. Indeed, several trials of stabilization of vegetable oils (rapeseed oil, sunflower oil, cottonseed oil, borage oil, etc.) by essential oils [11] or natural extracts [12] have already shown encouraging results. However, *O. basilicum* essential oil has not yet been subject to similar studies even if some works regarding the effectiveness of different extracts with solvents to conserve sunflower and rapeseed oil have been reported [7, 13].

The aim of this study is to evaluate the antioxidant efficacy of essential oil of *O. basilicum* (with linalool as major component) in the conservation of cottonseed oil produced and marketed in Burkina Faso. In this work we report the results of an experiment on the accelerated oxidation of cottonseed oil and its preservation by essential oil of *O. basilicum* as natural antioxidant by using Shaal method.

MATERIALS AND METHODS

Materials

Cottonseed oil is a product of the SN Citec, an industry of Burkina Faso for the production of edible oils. All products and chemical reagents used are of type Fluka or E. Merck. The BHT used is Sigma type. The natural antioxidant used is the essential oil obtained from the fresh leaves of *O. basilicum* by steam distillation using a Clevenger apparatus. The chemical composition of essential oil has already been reported in our previous work [14]. A CIBA - CORNING UV - VIS spectrophotometer was used for the measurement of antioxidant activities. Laboratory glassware (pipettes, burette, Erlenmeyer flask, beaker, etc.) was necessary for the different assays.

Screening of essential oil antioxidant activity

The screening of the antioxidant activity of essential oil was carried out by the diphenyl pycril hydrazine method (DPPH) [15]. 5, 10, 15,..., 100 µL of pure essential oil are successively mixed with 2 mL of DPPH ethanolic solution at a concentration of 10^{-4} M. 30 minutes after mixing, the absorbance is measured at 517 nm with a CIBA - CORNING UV - VIS spectrophotometer. In each case the percentage of reduction (or percent inhibition) was calculated by the relation:

$$\% \, inhibition = (1 - \frac{A}{Ao}) \times 100$$

A_0: absorbance of the solution of DPPH without essential oil
A: absorbance of DPPH solution with essential oil and after 30 min.

Cottonseed oil accelerated oxidation study and its preservation by *O. basilicum* essential oil

This study was conducted using the oven test (or Shaal method) [16]. The experiment is conducted in 150 ml glass jars. In each jar the necessary quantity of essential oil (0, 20, 50, 100 mg) is introduced, then 100 g of cottonseed oil were added. After homogenizing, the jars are placed in an oven at 60 ° C, lighted with an 18 watt light bulb. This device has the advantage to be closer to the real conditions of storage in Ouagadougou (case of transparent bottles containing oil and kept in daylight at room temperature).

The cotton seed oil oxidation inhibition has been tested by using essential oil at different doses:
- Dose 0. $T_0 = 0$: blank (cottonseed oil without antioxidant)
- Dose 1. $T_1 = 200$ mg/kg (200 mg of EO per Kg of cottonseed oil, or 200 ppm)
- Dose 2. $T_2 = 500$ mg/kg (500 mg of EO per Kg of cottonseed oil, or 500 ppm)
- Dose 3. $T_3 = 1000$ mg/kg (1000 mg of EO per Kg of cottonseed oil, or 1000 ppm)

BHT, the positive control, has been tested in equal doses. The tests are carried out in dupliquate. Before starting experiment, cottonseed oil's peroxide index (PI) has been determined. After the start of the experiment, peroxide index of each sample is determined every 24 hours for one month (30 days). Acid index (AI) and acidity are determined at the beginning and the end of the experiment. These three indexes (peroxide index, acid index and acidity) have been determined according to the methods of the IUPAC [17].

RESULTS AND DISCUSSION

Screening of antioxidant properties of *O. basilicum* essential oil

The values of percentage of reduction of *O. basilicum* essential oil are presented in table 1.

Table 1: *Ocimum basilicum* essential oil's antioxidant activity screening

EO (µL)	0	5	10	15	20	25	30	50	100
inhibition (%)	0	94.84	95.35	95.55	99.99	99.9999	99.9999	99.9999	99.9999

This preliminary work showed that the essential oil of *O. basilicum* has interesting inhibitory activity against DPPH radical. Indeed, 5 µL of the essential oil reduce more than 90% of free radicals in 2 ml of a solution of DPPH 10^{-4} M and 20 µL of the oil can reduce about 100% of free radicals of the same amount of DPPH solution.

The essential oil *of O. basilicum* showed good inhibitory activity and has been therefore used to stabilize food cottonseed oil. The study of this stabilization test was to follow over time, under conditions of accelerated aging, the evolution of the peroxide index, the index of acid and the acidity of the oil in the presence of essential oil by comparison with the control (without essential oil).

Evolution of the peroxide index, acid index and acidity in the presence of EO at different doses during accelerated oxidation of cottonseed oil

The results of this experiment showed that the peroxide index (PI) increased slowly in the samples contained essential oil comparing to the control (figure 1).The PI values were higher in the case of the blank (T=0) than the other samples that contained the essential oil at different concentrations. Indeed, in the control (without antioxidant), the PI increased quickly at the beginning (10 days), peaked around the fifteenth day, then decreased, and varied very slowly to thirtieth day. But this index did not increase exactly in the same way than the other samples.

In samples with essential oil at 200 and 500 ppm, the PI is changed similarly as in the case of the blank but this index increased again after the 20th day for the first and the 25th day for the second.

For the sample that contained the essential oil at 1000 ppm, the PI increased slightly the first five days and became virtually constant from the 5th to the 25th day, and increased again between the 25th and 30th day.

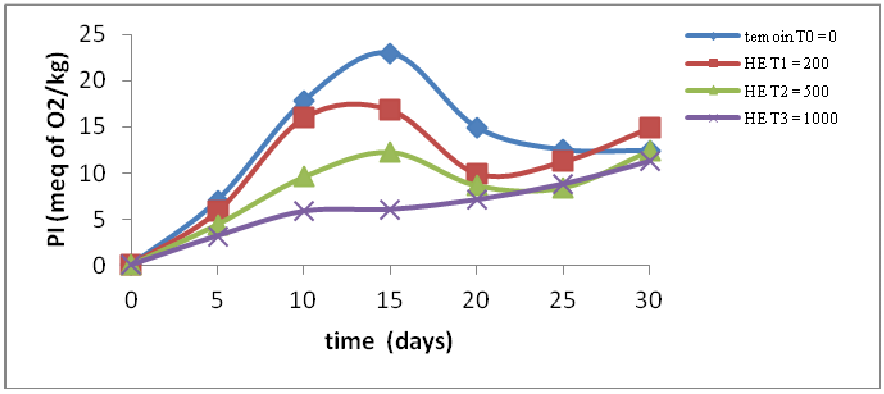

Figure 1: Evolution of peroxide index, with essential oil applied at different doses, compared to the witness (without antioxidant).

These observations were agreement consistent with [18] reported data. According to those data, during oxidative rancidity, various compounds appear over time: peroxides level will first increases significantly and then, decrease when the secondary products will appear. So in our case, the evolution of PI might correspond to a phase where the peroxides are highly formed; at the stage where the peroxides change into secondary more stable compounds (aldehydes, ketones, acids, etc.) the PI decreased. This is consistent with the works of Shahidi F. and al. [19] and Shahid Iqbal and al. [20] on the stabilization of canola oil and sunflower oil by natural antioxidant extracts. According to these authors, the decrease of the peroxide index might be due to the decomposition of peroxide formed in the early stages of oxidation in volatile secondary compounds.

For samples that contained the essential oil, the fact that the PI increased again after 25 days of experiment could be explained by the fact that the antioxidant effect of EO is over; may be the totality of the oil was consumed before the

end of the experiment, or the EO is denatured under the effect of heat, thus reducing its antioxidant power. The first hypothesis is consistent with reported data of Laandrault and al. [21] where the authors showed that all antioxidants (especially natural ones) are effective for a short period of time and beyond this period, their effectiveness decreased and they eventually became ineffective. Anwar and al. [22] abound in the same way by specifying that the natural antioxidant molecules inhibit lipid peroxidation and damaging themselves over time; the antioxidant effect stops therefore when all these molecules have been destroyed.

Also, the literature indicates that vegetable refined oil (such as cottonseed oil) is considered as edible if its PI is less than or equal to 10 meq of O2/kg, its acid value is less than or equal to 4 mg/kg, and its acidity is less than 1% [17]. For the control and the sample that contained the EO at 200 ppm, the PI is higher than the threshold value of 10 meq of O2/kg after the first 5 days. However, in the sample containing the EO at 500 ppm, the PI remains less than 10 meq of O2/kg during the first 10 days. For the sample containing EO at 1000 ppm, peroxide index has remained below 10 meq O2/kg until the 25th day and secondary oxidation products seem not to form in significant quantity (the PI did not decrease throughout the period of the experiment). These observations suggest that:

- After 5 days of conservation, control sample and the sample containing the EO at 200 ppm were not edibles;
- The sample containing the essential oil at 500 ppm was not edibles after 10 days of conservation;
- The sample containing the essential oil at 1000 ppm was consumable up to 25 days of conservation.

However, the acid index and acidity value (table 2) showed that only the control (without antioxidant) was not consumable at the end of the experiment, with acidity of 1.16% (thus superior to the standard). All other samples were supposed to be consumables. These results and those of the PI, seemingly contradictory, are complementary. Indeed, to the consideration of the values of the acid and the acidity index, it can be noticed that secondary oxidation products in particular acids did not yet form significantly. Thus, despite the high values of the PI found in some samples, they may not present a flavor and taste of rancid.

It is the place to remind that a fat can have a high value of the PI without any character of rancidity. The PI is in related to the peroxidation but not to the rancidity as the latter is highlighted by the determination of the secondary oxidation products [23].

Table 2: Index of acid and acidity values at the beginning and end of the experiment

Doses (mg/kg)	samples	beginning		end	
		Index of acid	Acidity (%)	Index of acid	Acidity (%)
$T_0 = 0$	blank	1.05 ± 0.07	0.52 ± 0.3	2.31 ± 0.07	1.16 ± 0,035
$T_1 = 200$	EO	1.05 ± 0.07	0.52 ± 0.3	1.82 ± 0.14	0.91 ± 0.070
	BHT	1.05 ± 0.07	0.52 ± 0.3	1.40 ± 0.00	0.70 ± 0.00
$T_2 = 500$	EO	1.05 ± 0.07	0.52 ± 0.3	1.40 ± 0.00	0.70 ± 0.00
	BHT	1.05 ± 0.07	0.52 ± 0.3	1.33 ± 0.07	0.66 ± 0.035
$T_3 = 1000$	EO	1.05 ± 0.07	0.52 ± 0.3	1.33 ± 0.07	0.66 ± 0.035
	BHT	1.05 ± 0.07	0.52 ± 0.3	1.33 ± 0.07	0.66 ± 0.035

In this experiment, EO of *O. basilicum* helped to prevent the formation of peroxides; therefore, it was effective for stabilizing cottonseed oil. This antioxidant effectiveness of EO is related to its chemical composition, as we reported in our previous works [14].

III.3. Comparison of essential and BHT effects on cottonseed oil

Figures 2, 3 and 4 illustrate the evolution of the cottonseed oil PI in the presence of the EO and the BHT.

We noticed that all the curves of BHT are below those of the EO. This means that at equal doses, BHT is more effective than EO. It is reported that at equal doses, synthetic antioxidants are generally more effective than the natural antioxidants [24]. However, it is noticed that:

- At dose of 1000 ppm, EO has an activity comparable to that of BHT at the dose of 500 ppm, and this for 15 days of conservation (figure 5);
- Always at a dose of 1000 ppm, EO is more effective than BHT at the dose of 200 ppm, and that, during 20 days of conservation (Figure 6).

Moreover, a similar experience, conduced to evaluate the antioxidant effectiveness of several antioxidants, acting alone or in synergy, to conserve lard oil, showed comparable time of conservation [25].

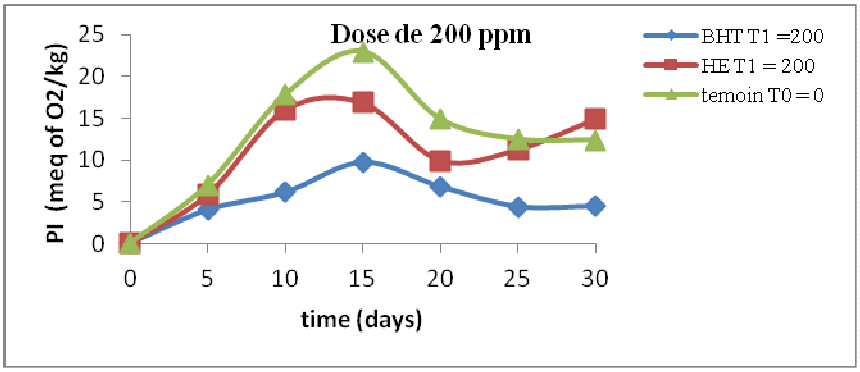

Figure 2: Evolution of the PI of the cotton oil treated by EO and BHT in equal doses of 200 ppm

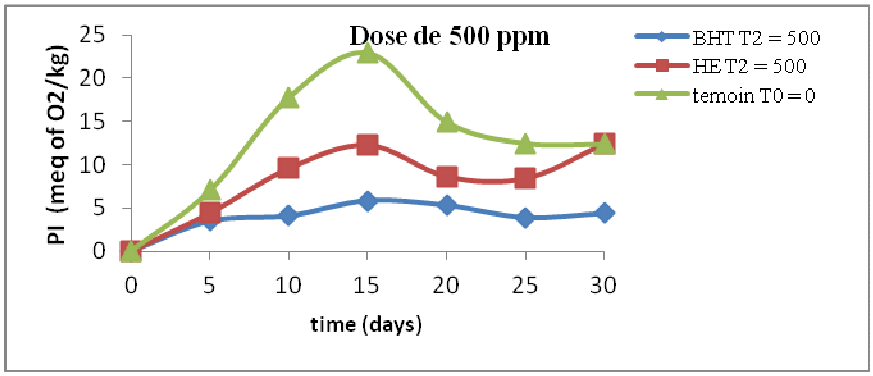

Figure 3: Evolution of the PI of the cotton oil treated by EO and BHT in equal doses of 500 ppm

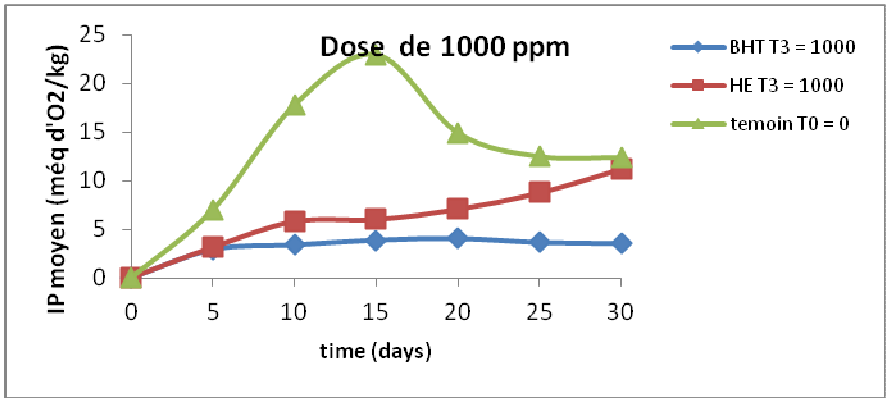

Figure 4: Evolution of the PI of the cotton oil treated with EO and BHT in equal doses of 1000 ppm

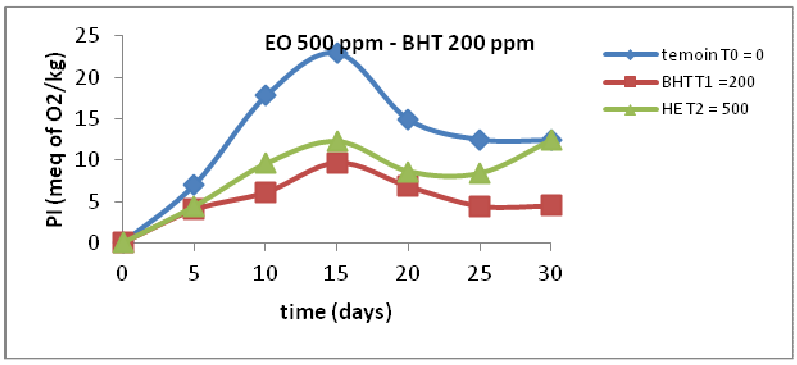

Figure 5: Evolution of the PI of cottonseed oil treated with EO at 500 ppm and BHT at 200 ppm

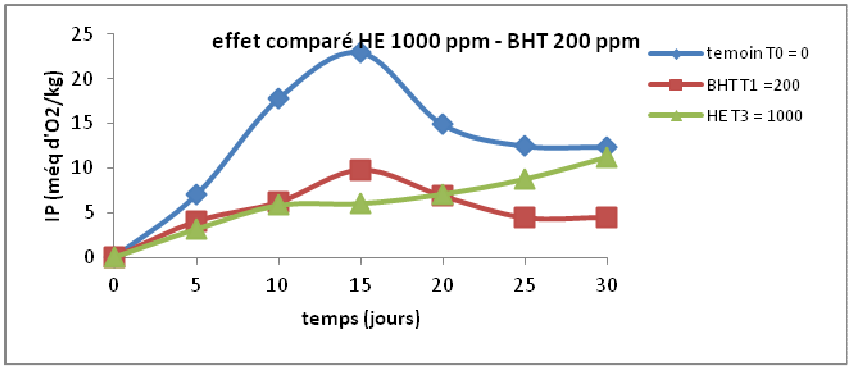

Figure 6: Evolution of the PI of cottonseed oil treated with EO at 1000 ppm and BHT at 200 ppm

CONCLUSION

The study of the accelerated aging of edible cottonseed oil in the presence of essential oil has shown that EO can delay degradation of this fat.

At the same concentration, BHT has an antioxidant activity higher than essential oil. Nevertheless, at 500 ppm, EO presented a comparable activity than BHT at 200 ppm; and at a 1000 ppm, EO is more effective than BHT at 200 ppm over an interval of 20 days of conservation in the oven. These results suggest that *O basilicum* EO has interesting antioxidant activity. Toxicity studies have already shown that the essential oil is slightly toxic (LD50 > 3000 mg/kg). This oil can be used in agro-food as a natural antioxidant to conserve fats or in all formulations containing fats. It could be also used by the small scale producers of edible oils (Shea butter, soybean oil, etc.) for oil stabilization.

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