THE INFLUENCE OF SOME INHIBITORS OVER MINERAL OILS AUTOOXIDATION REACTION USING COLORIMETRIC METHODS

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Abstract: This paper presents a study about the influence of some inhibitors (β-carotene and tocopherol) over mineral oils autooxidation reaction, using colorimetric techniques.The principle of this method consists in determination of linoleate oxidation absorbtion measured at 234 nm at 25^0C in the presence of β-carotene and tocopherol as enzymatic inhibitor

1.INTRODUCTION

The oil's autooxidation reaction realised by the lipoxidase activity, produced through metabolic pathways in common microorganism in the presence of air and small quantities of water is undesirable in every industry [1]. So, they are used antioxidants to prevent these reactions [2, 3]. This paper presents the influence of β-carotene and tocopherol, over the mineral oils and technical lipids oxidation reaction with lipoxidase, using colorimetric methods.

Lipoxidase catalyzes the folowing reactions:

$$-CH=CH-CH_2-CH=CH- + O_2 \rightarrow -CH=CH-CH=CH-CH(OOH)-$$

Lipoxidase has a molecular weight about 108.000-150.000 u.a.m. an isoelectric point at pH 5,65 and an optimum pH at 6,5-7 . I has the great activity towardpolynesaturated acids possesing double bonds at sixth and ninth atoms from the methyl group. The presence of calcium ions broadenes the enzymatic specificity [1].

2. EXPERIMENTAL

The principle of this method consists in determination of linoleate oxidation absorbtion measured at 234 nm at 25^0C.

Reagents

Enzyme – it was prepared an stock solution containing 1mg/ml in 0,2m borate buffer, pH9. Immediately prior to use it was diluted with buffer to 10-20 microgame/ml.

Substrate- it was dissolved 0,1ml technical linoleic acid in 60 ml of 95%ethanol then it was diluted to 100 ml with water and shake thoroughly to disperse. For use it was diluted with 5 volumes of 0,2M borate buffer at pH 9.

Procedure

It was pipetted 2,0 ml of substrate into a 1cm cuvette and oxigenate for a few minutes and then added 1 ml of enzyme solution and 0,5 ml borate buffer determined the absorbance at 234 nm. This sample was considered to be the control.

To determined the influence of β-carotene and tocopherol over the lipoxidase activity it was substitute the buffer wit 0,5 ml of β-carotene (sample 1) and with 0,5ml tocopherol (sample 2) and determined the absorbance at 234 nm using a Perkin-Elmer spectrophotometer.

To transform the optical densities read for the tests and controls in moles peroxid it was made a standard curve in concordance with the data from table 1.

Table 1. Standard curve

Peroxid (ml)	0,1	0,3	0,5	0,7	1	-
Linoleic acid (ml)	0,9	0,7	0,5	0,3	-	1
Borate buffer pH 9 (ml)	1	1	1	1	1	1
Extinction	0,150	0,305	0,455	0,605	0,765	0,800

The experiment results were calculated as follow: the optical densities for the controls were subtracted from the test extinction and the results were expressed in μmoles peroxide enzymatic delivered/1ml/1min.

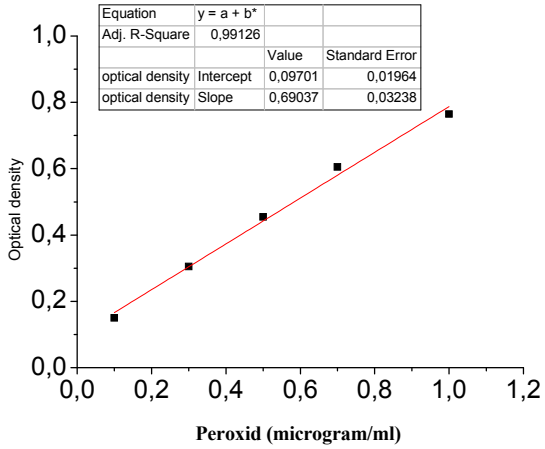

Figure 1. Standard curve

The experiments were made as shown in table 2.

Table 2. Lab techniques

	Control	**Sample 1**	**Sample 2**
Linoleic acid (ml)	2	2	2
Enzyme (ml)	1	1	1
Inhibitor β-carotene (ml)	-	0,5	-
Inhibitor tocopherol (ml)	-	-	0,5
Borate buffer pH 9(ml)	0,5	-	-

3. RESULTS AND DISCUSSIONS

The influence of the studied inhibitors on the oxidation reaction is presented in table 3 and in the figure 2.

Table 3. The influence of the β-carotene and tocopherol on the oxidation reaction of linoleate acid

No.crt.	**Sample**	**Optical Density**
1	Standard	0,395
2	β-carotene	0,105
3	tocopherol	0,170

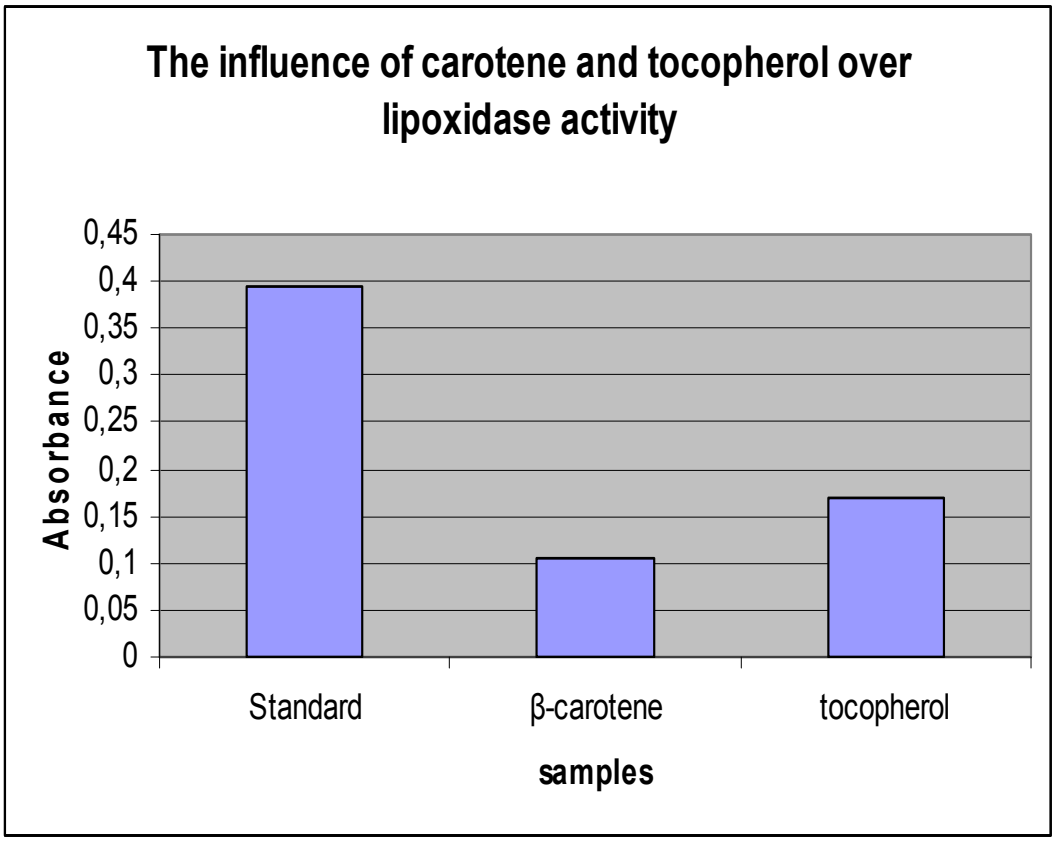

Figure 2. The influence of the β-carotene and tocopherol over autooxidation reaction of mineral oil with lipoxidase

4.CONCLUSIONS

β-carotene and tocopherols are antioxidants for lipoxidase activity from oils. They acted as inhibitors for the enzymatic autooxidation reaction and their presence in the mineral oils, preserved the chemical properties and the lifetime of the technical lipid materials and oils.

5.REFERENCES

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