

Antioxidant activity of BHA, BHT and TBHQ examined with Miller's test

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RESUMEN

Actividad antioxidante de BHA, BHT y TBHQ examinada con el test de Miller.

En experimentos modelos usando el test de Miller, a 5ml de emulsión se añadió 25, 12.5, 6.25 y 3.13 μ g de BHA, BHT y TBHQ. Las relaciones inhibitorias (IR) para el BHA y BHT fueron similares. IR para TBHQ fue más baja que para BHA y BHT con niveles de adición del 6.25 y 3.13. Esto sugiere que en estudios modelos usando el test de Miller 25 μ g de BHA, BHT, o TBHQ deben ser añadidos a 5 ml de muestra control.

PALABRAS-CLAVE: Actividad antioxidante – Butil hidroquinona terciaria (TBHQ) – Butil hidroxianisol (BHA) – Butilhidroxitolueno (BHT) – Test de Miller.

SUMMARY

Antioxidant activity of BHA, BHT and TBHQ examined with Miller's test.

In model experiments with the use of Miller's test to 5 ml emulsion 25, 12.5, 6.25 and 3.13 μ g BHA, BHT and TBHQ was added. Inhibitory ratios (IR) for BHA and BHT were similar. At addition level of 6.25 and 3.13 IR for TBHQ was lower than for BHA and BHT. It is suggested that in the model studies with the use of Miller's test 25 μ g BHA, BHT or TBHQ should be added to 5 ml of control sample.

KEY-WORDS: Antioxidant activity – Butylated hydroxyanisole (BHA) – Butylated hydroxytoluene (BHT) – Tertiary butyl hydroquinone (TBHQ) – Miller's test.

1. INTRODUCTION

Combined oxidation of β -carotene and linoleic acid was applied by Miller (8) in a test for determining activity of antioxidants. The test has been widely applied in the studies on antioxidant activity of natural compounds isolated from plant materials (1) (2) (3) (11) (12). Antioxidative activity of plant extracts, their fractions or pure compounds is often compared to the activity of synthetic antioxidants used in food technology. However, authors rarely report the concentration of these compounds in the samples tested or use various concentrations.

The aim of the study was to compare antioxidative properties of butylated hydroxyanisole (BHA), butylated toluene (BHT) and butylated hydroquinone (TBHQ) (Fig. 1) in a test with β -carotene and linolenic acid and proposing addition of the antioxidants mentioned above to be applied as a standard to which activity of natural compounds of plant origin could be related.

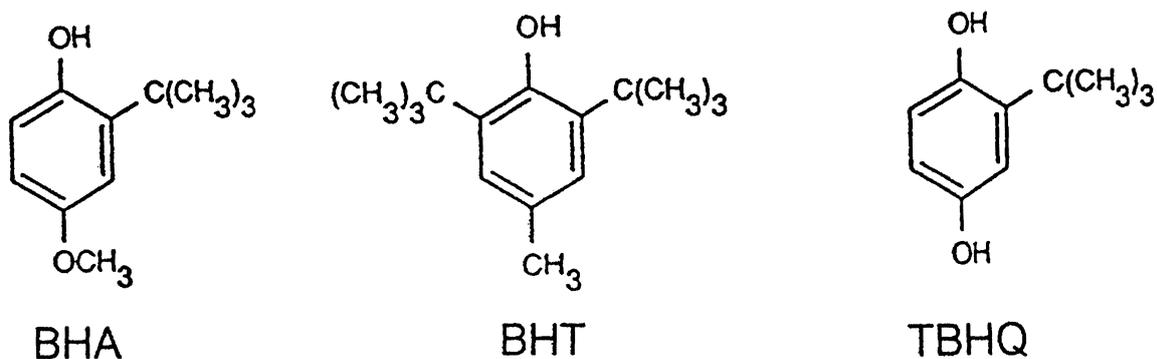


Figure 1
Chemical structure of BHA, BHT and TBHQ

2. MATERIALS AND METHODS

Model emulsion was prepared from linoleic acid, β -carotene and Tween 40 according to Miller (8). To a series of tubes containing 5 ml emulsion 0,2 ml methanol with dissolved 25, 12.5, 6.25, and 3.13 μg BHA, BHT and TBHQ (all reagents by «Sigma») was added. Thus prepared samples were heated for 2 h in a water bath at 50°C measuring absorbance at 470 nm every 15 min. Control sample contained 0.2 ml pure methanol.

Antioxidative activity was expressed by inhibitory ratio (IR) adapted from a study by Kajimoto (7):

$$\text{Inhibitory ratio} = \frac{A_t - A_{ct}}{A_o - A_{ct}} \times 100\%$$

A_o - absorbance of the sample before heating.

A_t - absorbance of sample with addition of antioxidant heated for t min,

A_{ct} - absorbance of control sample heated for t min.

In all experiments, samples were analysed in triplicate and mean value \pm standard deviation were recorded. Significant differences between samples incubated with different addition of antioxidant were determined at 95% level of probability using a t-Student test (5).

3. RESULTS

The results obtained are presented on Figs 2-4. After 15 min and 30 min heating at addition level of 25 and 12.5 μg IR for BHA and BHT was similar. Later, however, IR drop for BHA was faster. At addition of 6.25 and 3.13 μg , at the beginning of heating BHA inhibited oxidation process stronger than BHT. As heating proceeded, IR diagrams approximated each other, and after 2 h of heating IR values were almost identical.

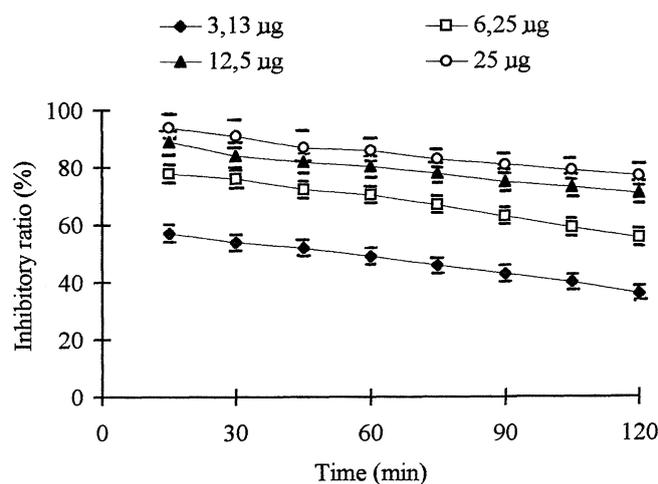


Figure 2
Inhibitory ratio of BHA in Miller's test

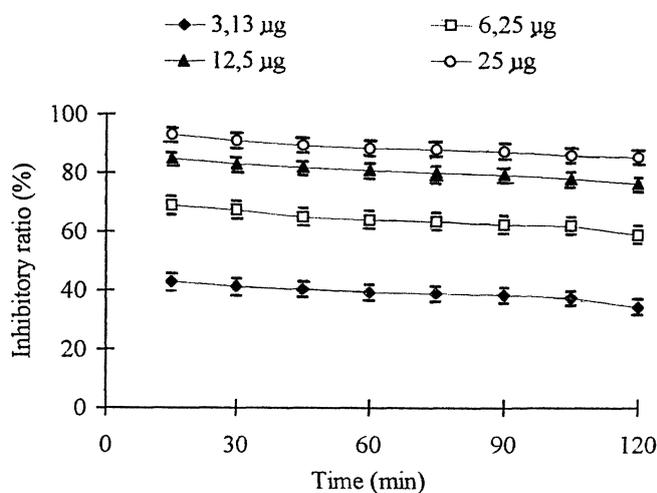


Figure 3
Inhibitory ratio of BHT in Miller's test

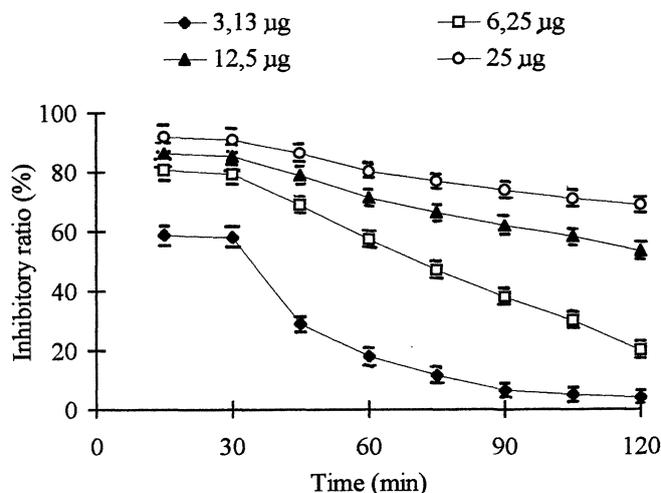


Figure 4
Inhibitory ratio of TBHQ in Miller's test

After 15 and 30 min of heating samples with addition of 25 and 12.5 µg TBHQ IR values were similar as for BHA and BHT. After 45 min of heating IR values for TBHQ diminished more than for BHA and BHT. Especially drastic drop in IR value was observed at addition of 6.25 and 3.13 µg TBHQ. After 90 min of heating values at addition of 3.13 µg TBHQ dropped below 10%.

Differences in IR between the samples with 25 and 12.5 µg and 12.5 and 6.55 µg of added TBHQ were not significant only during first 30 min of experimentation (Fig. 4). The plot of RI for 12.5 µg of BHA was situated below that for 25 µg of BHA (Fig. 2). However, differences for these RI values during the whole period of incubation were statistically not significant. In all other cases differences in RI values for used additions of antioxidants were significant.

From literature data it follows that antioxidative activity of BHA, BHT and TBHQ depends on the method used for model studies. Higher antioxidative activity of BHA than of BHT and manyfold lower for TBHQ was demonstrated in thin layer model system of methyl linoleate on cellulose (6). Berner *et al.* (4) reported antioxidative index in lard emulsion 30-fold higher for BHA than for TBHQ. Yet, in active oxygen method, antioxidant index for TBHQ was 3-fold higher than for BHA and BHT. Higher antioxidative activity of TBHQ compared with BHA and BHT in model studies with soybean oil and chicken fat in emulsion versus dry oil tests was reported by Cort *et al.* (6). Relative effectiveness of antioxidants-linoleic acid monolayer on silica gel for TBHQ was almost the same as for BHT, while for BHA almost 3-fold higher (9). Relative effectiveness of red blood cell ghosts perfusion uptake for BHA was 2-fold higher than that for TBHQ (10).

To conclude, in model studies of natural antioxidative compounds or extracts with antioxidative properties using Miller's test, it is suggested to compare the results obtained to a sample containing 25 µg BHA, BHT or TBHQ in 5 ml model emulsion.

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