

# Cereal Grain Alk(en)ylresorcinols Protect Lipids against Ferrous Ions-Induced Peroxidation

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Resorcinolic lipids, significant constituents of cereal grains are long chain (C13–C27) derivatives of 1,3-dihydroxy-5-alk(en)ylbenzene. It was shown that due to their phenolic nature resorcinolic lipids are effective in protection against fatty acids peroxidation. The phospholipid bilayers appeared to be also protected in the presence of these compounds. The maximal inhibition of peroxidation was 50–90% with  $IE_{50}$  values in the range of 0.85–3.6 mol% of resorcinolic lipids.

## Introduction

Peroxidation of lipids in biological membranes is a complex process in which rearrangement and destruction of double bonds in lipid molecules occurs through propagation of free lipid radicals formed at the beginning of the process [1–3]. Hydroperoxides formed affect the structure of the membrane [4–6] and inhibit membrane-bound enzymes [7–9].

Besides effects upon the biological membranes lipid oxidation products and products of degradation of free radicals are of great importance in cancer biogenesis and development [e.g. 10–12]. At present there is an increasing evidence that many strong carcinogens are spontaneously formed during food processing. These compounds are supposed to be responsible for colon and breast cancer [13, 14].

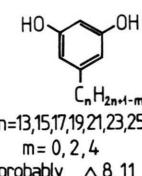
Most natural and synthetic compounds acting as antioxidants, *i.e.* protecting lipids against non-enzymatic oxidation *in vitro* as well as *in vivo* are of phenolic nature *e.g.* tocopherols, ubiquinols, BHA, BHT, TBHQ and gallic acid derivatives. Many natural phenolic compounds of plant origin also exhibit antioxidant properties [15].

The phenolic nature of resorcinolic lipid molecules (**I**), which are significant constituents of cereal grains and the similarity of these compounds to tocopherol suggested that they might also exhibit

**Abbreviations:** BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; TBHQ, 2-*tert*-butylhydroquinone.

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antioxidant properties. In this report results supporting this hypothesis are presented. 5-n-Heptadec(en)ylresorcinol, one of the main homologues of resorcinolic lipids present in cereal grains, shows the ability to reduce 50–90% of  $Fe^{+2}$ -induced peroxidation of fatty acids as well as of phospholipids in bilayers.

## Materials and Methods

5-n-Heptadecyl- and 5-n-heptadecenylresorcinols were isolated chromatographically from acetone extracts of rye grains as described earlier [16]. Phosphatidylcholine was isolated from egg yolks according to the procedure of Van Deenen and De Haas [17]. Linolenic acid (18:3) (Merck) was used without further purification.

For studying fatty acid peroxidation a stock (550  $\mu$ M) emulsion obtained by injection of an alcoholic solution of fatty acid into 25 ml of 0.15 M NaCl in 20 mM Tris-HCl pH 7.4 was used. Phospholipid vesicles were prepared as small unilamellar vesicles (SUV). Hand-shaken liposomes were subjected to sonication (3  $\times$  15 min) at a 40  $\mu$ m amplitude and a power setting of 0.8 kW in an ultrasonic disintegrator UMD 10 (Techpan) followed by high-speed centrifugation for removal of metal particles and large liposomes. Vesicles made



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of pure phosphatidylcholine and prepared from phospholipid-resorcinolic lipid mixtures were used. In the latter case resorcinolic lipid constituted 1–15 mol% of total liposome membrane lipids. When alk(en)ylresorcinols were to be added directly to the samples, microliter volumes of ethanolic 5 mM stock solution were used (final alcohol concentration was below 0.7%).

Lipid peroxidation was initiated by addition of freshly prepared aqueous ferrous sulphate (final concentration of 50  $\mu$ M) to the sample solutions. The samples were incubated for 60 min at 37 °C and the amount of peroxidation products formed was determined colorimetrically with thiobarbituric acid [18].

## Results and Discussion

The extent of peroxidation was expressed in nanomoles of thiobarbiturate reacting substances (TBRS) and in per cent of inhibition in relation to peroxidation of the control samples containing no resorcinolic lipids. Because the partition coefficient of resorcinolic lipids between buffer and lipid is small (about  $1 \times 10^{-5}$ ) [19] it was assumed that the number of alk(en)ylresorcinol molecules remaining in the aqueous phase in relation to the number of molecules incorporated into the lipid phase will be small enough so that they would have no significant effect on the result, therefore it could be omitted. This assumption allowed us to present the amount of resorcinolic lipid in the lipid phase as the per cent mole fraction in relation to total amount of lipids in the sample. Therefore the effect of absolute concentrations of the compounds in the sample on the peroxidation extent was avoided.

In Fig. 1 the effect of increasing amounts of 5-*n*-heptadecyl- and 5-*n*-heptadecenylresorcinols upon  $\text{Fe}^{+2}$ -induced peroxidation of linolenic acid. Both homologues inhibited fatty acid peroxidation. At 20 mol% resorcinolic lipid these compounds inhibited peroxidation up to about 90%. The mol fractions at which half maximal effect were observed ( $\text{IE}_{50}$ ) were of 2.6–3.6 mol%. Somewhat higher suppression of peroxidation was observed for saturated-chain homologue, although this difference seems to be nonsignificant.

Peroxidation of fatty acid incubated with a constant amount of resorcinolic lipid and ferrous ions

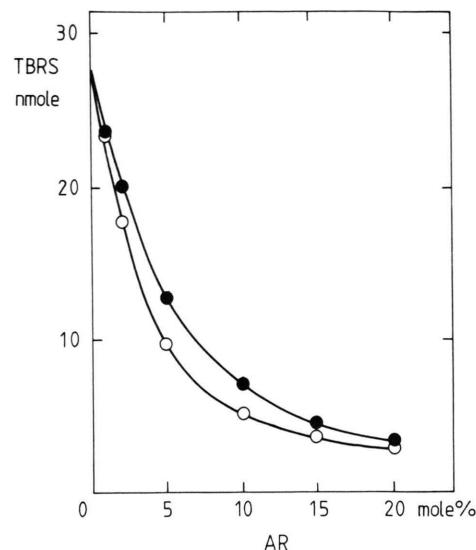


Fig. 1. The effect of 5-*n*-heptadecyl- and 5-*n*-heptadecenylresorcinols on  $\text{Fe}^{+2}$ -induced peroxidation of an aqueous emulsion of linolenic acid. Peroxidation was estimated as the amount of thiobarbituric acid-reacting substances formed during 60 min of incubation at 37 °C. Concentration of the ferrous ions in the sample was 50  $\mu$ M. ○ 17:0 AR\*, ● 17:1 AR, \*AR – alk(en)ylresorcinol.

is time dependent. The effect at short times (below 10 min) of incubations is somewhat stronger than at longer times (Fig. 2). Possibly with extended incubation time resorcinolic lipids might directly interact with  $\text{Fe}^{+2}$  ions or their effective concentration on the surface of the emulsion droplets might have been decreased as a result of penetration of the alk(en)ylresorcinol molecules into the interior of the droplet.

Resorcinolic lipids also protect phospholipid membranes against peroxidation (Fig. 3). Addition of these compounds into the liposomal suspension (they should be located mainly at the outer half of the membrane as their flip/flop is limited [24]) resulted in the inhibition of the peroxidation process by 80–87% at 4 mol% of alk(en)ylresorcinol, depending on the homologue studied. The values of  $\text{IE}_{50}$  estimated for this system (0.85–0.90 mol%) are about four times lower than those obtained from previous experiments. This decrease of the proportion of the agent necessary for a similar effect may be due to the different localization of antioxidant molecules in the systems studied. In the liposome system the site of localiza-

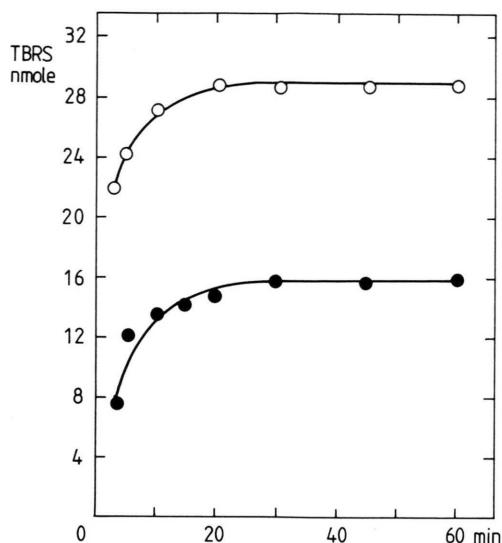


Fig. 2. Time dependence of the amount of TBRS formed in a linolenic acid emulsion incubated in the presence (●) and absence (○) of 4.6 mol% of 5-n-heptadecylresorcinol and ferrous ions (50  $\mu$ M) at 37 °C.

tion and action of alk(en)ylresorcinols is defined – the bilayer, whereas in fatty acid emulsions a substantial part of the antioxidant could be located inside the fatty acid droplet. Therefore, the effective concentration of antioxidant at the site of peroxidation is higher for bilayers than for emulsions. The lack of significant differences between the activity of saturated and unsaturated homologues indicates the key role of 1,3-dihydroxybenzene ring in the antioxidative properties of resorcinolic lipids.

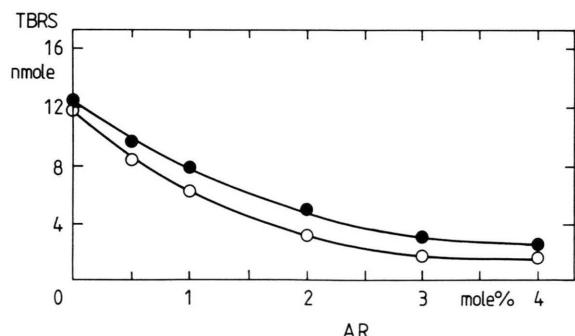


Fig. 3. The effect of resorcinolic lipids on ferrous ions-induced peroxidation of phosphatidylcholine liposomes. Conditions as in the legend for Fig. 1. ○ 17:0 AR, ● 17:1 AR.

It was shown that the protective action of the compounds studied is proportional to the incubation time. The stronger effect of the saturated homologues (Fig. 4) suggests that the localization of the resorcinolic lipid in the membrane bilayer is important for antioxidant properties. Resorcinolic lipids might be considered as antioxidants acting in close proximity to the double bonds of acyl chains. Smaller increase in time and lower antioxidant activity of the unsaturated homologues may suggest a different final localization of the homologues in the membrane after sufficiently long incubation (equilibration) times. The experiment in which alkenylresorcinol was incorporated into the liposome membrane during its formation might support this suggestion. As shown in Fig. 5 the  $IE_{50}$  increased to 3 mol% and the maximal inhibi-

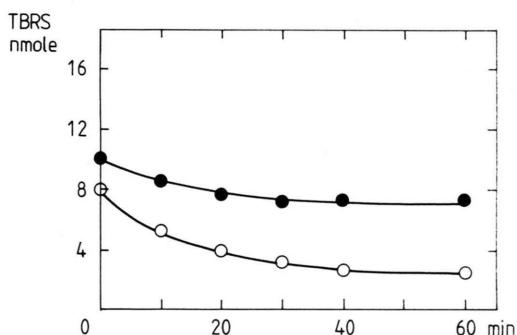


Fig. 4. Dependence of the amount of TBRS formed in phosphatidylcholine liposomal bilayers incubated for various time with resorcinolic lipids and subsequently with  $Fe^{+2}$  ions for 60 min at 37 °C. ○ 17:0 AR, ● 17:1 AR.

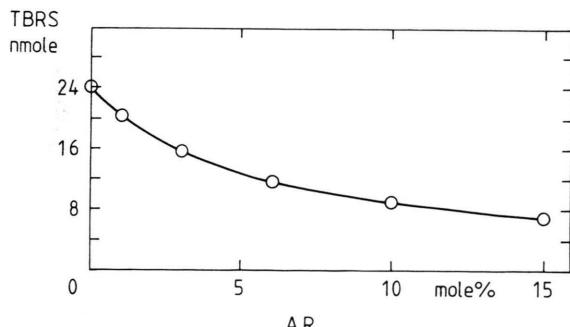


Fig. 5. The effect of various amounts of 5-n-heptadecylresorcinol incorporated into phosphatidylcholine liposomal membrane during its formation (preincorporated) upon the level of TBRS after 60 min incubation of liposomes in the presence of 50  $\mu$ M  $Fe^{+2}$  at 37 °C.

tion decreased to 70%. Assuming an equal distribution of resorcinolic lipids between both monolayers during liposome formation only a two-fold increase of the  $IE_{50}$  value would be expected. The three-fold increase of this value suggests the possibility of an asymmetrical localization of the unsaturated homologue in the bilayer and a preferential localization in the inner monolayer of the liposomal membrane. According to the shape concept [20] this would indicate that the shape of unsaturated resorcinolic lipids is reversed-conical, similar to phosphatidylethanolamine. This supposition is in accordance with the previously observed [21] ability of unsaturated resorcinolic lipids for induction of nonlamellar structures (reversed micellar or hexagonal  $H_{II}$ ) in membranes. Saturated homologues induced only micellar phases at much higher concentrations in the membrane.

Based on the results presented in this paper and the results concerning the effect of resorcinolic lipids on the fluidity of biological membranes [22] it

may be assumed that a membrane-stabilizing effect of resorcinolic lipids may also participate in their antioxidant activity, particularly at low concentrations. The decrease of the mobility of molecules in the membrane would restrict not only the accessibility of peroxidants to the membrane interior but it would also inhibit contacts between radicals and double bonds that stimulate propagation of the peroxidation process. In this respect the effect of resorcinolic lipids would be similar to the membrane-stabilizing effect of  $\alpha$ -tocopherol, a well known natural antioxidant compound [23].

Results presented in this paper indicate that the antioxidant properties displayed by resorcinolic lipids, which are compounds localized in cereal grain materials, might be of importance for processing and storage of certain cereal products.

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