

## **Inhibition of the Pro-Oxidant Activity of Copper by Primary Antioxidants in Lard**

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### *ABSTRACT*

*The pro-oxidant effect of copper in lard, as judged by the development of peroxide values and the shortening of induction periods, can be counteracted by quercetin but not by BHT. On a molar basis, between 100 and 1000 times as much quercetin as copper is required for complete inhibition of the copper. This is evidence that a weak chelate is formed between quercetin and cupric ions in the oil medium.*

### INTRODUCTION

The pro-oxidant effect of certain multivalent metal ions, primarily copper and iron, on edible oils is well known and is a constant cause of practical problems. The effect can be mitigated to some extent by conversion of the free metal ions to complexes. In industrial deodorisation, for example, citric acid is added, whilst the finished oil is cooling, to chelate, and thus deactivate, traces of multivalent metals that may be present (Gunstone & Norris, 1983). Such additives must not be regarded as antioxidants *per se* but, because they supplement and enhance the effect of primary antioxidants, they are called synergists.

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In our earlier studies on the naturally occurring polyhydroxy flavonoids (Hudson & Mahgoub, 1980, 1981; Hudson & Lewis, 1983) we became particularly interested in the rôle of quercetin as a stabiliser for edible oils. In this connection we have explored the interaction between quercetin and cupric ions, which we now report.

## MATERIALS AND METHODS

Lard, freshly rendered and dried, was kindly supplied by Messrs Scot Bowyers (Trowbridge), quercetin (Q) was from Koch Light and both butylated hydroxy toluene (BHT) and copper in the form of the oil-soluble salt (copper cyclohexyl butyrate) from BDH.

The copper content of the lard was determined by atomic absorption spectrophotometry (Perkin Elmer Model 306). The stability of the model systems towards oxidation was assessed by the determination of peroxide values (PV) and FIRA-ASTELL induction periods (IP) as described by Meara & Weir (1976).

## RESULTS

The effect on the stability of lard of progressively adding Cu II in the form of copper cyclohexyl butyrate is shown in Table 1. The original lard was found to contain 0.009 mg/kg of Cu and 0.3 mg/kg of Fe.

The results were as expected. From them it was concluded that addition

**TABLE 1**  
Effect of Cu II Concentration on the Stability of Lard at 100°C

<i>Added Cu II (mg/kg)</i>	<i>Molarity</i>	<i>PV (meq/kg) after heating at 100°C for</i>				<i>IP (h)</i>
		<i>0 h</i>	<i>4 h</i>	<i>6 h</i>	<i>8 h</i>	
—	—	5.5	8.4	10	14	4.0
0.07	$1.1 \times 10^{-6} \text{M}$	5.4	16	25	65	1.5
1.30	$2.1 \times 10^{-5} \text{M}$	5.6	27	125	235	0.5
1.6	$2.5 \times 10^{-5} \text{M}$	5.3	65	312	190	0.0
4.8	$7.6 \times 10^{-5} \text{M}$	5.4	124	323	207	0.0

**TABLE 2**  
Effect of the Antioxidants Q and BHT in Counteracting the Pro-oxidant Effect of Copper at 0.02 mg/kg ( $3 \times 10^{-7}$  M)

Additives to lard	PV (meq/kg) after heating at 100°C for			IP (h)
	0 h	4 h	8 h	
None	5.5	8.8	15	3.5
Cu	5.6	12	41	1.0
$10^{-4}$ M Q	5.5	6.3	7.1	37
$10^{-4}$ M Q + Cu	5.9	6.7	7.4	27
$10^{-5}$ M Q	5.5	6.5	8.2	31
$10^{-5}$ M Q + Cu	5.6	6.6	9.1	22
$10^{-5}$ M BHT	5.4	5.2	7.4	62
$10^{-5}$ M BHT + Cu	5.4	11	33	3.0

of Cu II at a level of 0.02 mg/kg ( $3 \times 10^{-7}$  M) would be suitable for the investigation of the effect of antioxidants on the rate of oxidation.

The effects of adding copper at the chosen level on lard stabilised by the incorporation of quercetin (Q) and BHT are shown in Table 2. The added Cu has little effect on the stabilising action of the Q, even when the level of the latter is as low as  $10^{-5}$  M (3 mg/kg). On the other hand, at the same molar concentration, BHT has almost no effect in counteracting the pro-oxidant capability of simultaneously added Cu.

**TABLE 3**  
Quantitative Aspects of the Interaction Between the Pro-oxidant Cu and the Antioxidant Q

Additives to lard	PV (meq/kg) after heating at 100°C for		IP (h)	PF <sup>a</sup>
	4 h	8 h		
None (Control)	8.8	14	4.8	—
$10^{-9}$ M Cu	17	64	0.0	—
$10^{-9}$ M Cu + $10^{-8}$ M Q	15	64	0.0	—
$10^{-9}$ M Cu + $10^{-7}$ M Q	13	40	0.0	—
$10^{-9}$ M Cu + $10^{-6}$ M Q	7.2	11	9.0	2.3
$10^{-9}$ M Cu + $10^{-5}$ M Q	6.8	10	11	2.8
$10^{-9}$ M Cu + $10^{-4}$ M Q	6.2	8.4	16	4.0

<sup>a</sup> PF = Protective factor (IP of test sample/IP of control).

To explore further the quantitative aspects of the interaction between Cu and Q, a series of more dilute systems were studied. The results are reported in Table 3, copper concentrations being reduced to  $10^{-9}$  M and Q concentrations varying from  $10^{-8}$  M to  $10^{-4}$  M.

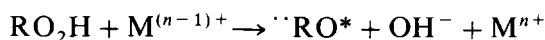
## DISCUSSION

It has already been shown (Hudson & Lewis, 1983) that Q and Cu interact in solution to form a complex characterised by the appearance of a new UV maximum at 434 nm, and that similar complexes (involving bathochromic shifts) are formed by other flavones and by the polyhydroxy chalcone, butein. Letan (1966), using different methods from ours for the evaluation of antioxidant activity, concluded that the metal-complexing capacity of polyhydroxy flavones is favoured especially by the presence, in the central ring of the molecule, of the 3-OH, 4-CO system with a 2,3-double bond.

The metal-complexing activity of polyhydroxy flavones and related compounds must be clearly distinguished from their activity as primary antioxidants. The latter depends on the formation of resonance-stabilised free radical hybrids, whereas the metal (M) free-radical initiating activity depends on reactions such as:



and



Such reactions can only be inhibited, in the presence of  $M^{n+}$ , by deactivating the metal ion by chelation. The chemical configuration of quercetin is such that it can fulfil this rôle, as well as its rôle as a primary antioxidant, whereas BHT, though it is a good primary antioxidant, lacks chelating capability and is therefore ineffective in the presence of  $Cu^{2+}$ .

The chelating capacity of quercetin, though significant, is weak since, even at 100 times the molar ratio with respect to copper, it is only just beginning to take effect and a molar ratio of the order of 1000 is required to completely counteract the pro-oxidant effect of copper. However, the suspected bimodal action of quercetin and related flavonoids (Pratt, 1976) is confirmed by the present studies.

## REFERENCES

- Gunstone, F. D. & Norris, F. A. (1983). *Lipids in foods. Chemistry, biochemistry and technology*. Pergamon Press, Oxford and New York, p. 164.
- Hudson, B. J. F. & Lewis, J. I. (1983). Polyhydroxy flavonoid antioxidants for edible oils. Structural criteria for activity. *Food Chem.*, **10**, 47–55.
- Hudson, B. J. F. & Mahgoub, S. E. O. (1980). Naturally occurring antioxidants in leaf lipids. *J. Sci. Fd Agric.*, **31**, 646–50.
- Hudson, B. J. F. & Mahgoub, S. E. O. (1981). Synergism between phospholipids and naturally occurring antioxidants in leaf lipids. *J. Sci. Fd Agric.*, **32**, 208–10.
- Letan, A. (1966). The relation of structure to antioxidant activity of quercetin and some of its derivatives. II. Secondary (metal-complexing) activity. *J. Fd Sci.*, **31**, 395–9.
- Meara, M. L. & Weir, G. S. D. (1976). The effect of  $\beta$ -carotene on the stability of palm oil. *Rev. Ital. Sost. grasse*, **53**, 178.
- Pratt, D. E. (1976). Rôle of flavones and related compounds in retarding lipid-oxidative flavour changes in foods. In: *Phenolic, sulphur and nitrogen compounds in food flavours* (Charalambous, G. and Katz, I. (Eds)). ACS Symposium No. 26, Washington, DC, USA, pp. 1–13.