# Whisky – An ESR and Antioxidant Study

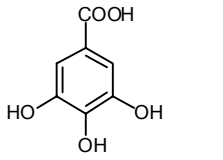
Irwin Cheah,[a] Jim Kelly,[b] Steven J. Langford[a] and Gordon Troup[c]

*[a]School of Chemistry, Monash University, Victoria 3800, [b]Angoves Pty Ltd, and [c]School of Physics and Material Science, Monash University, Victoria, 3800.*

Three single malt Scotch and two USA whiskies were investigated by EPR spectrometry and their antioxidant activity evaluated. The EPR spectra obtained for the five samples showed the presence of a $Cu^{2+}$ species that was unique to each whisky as well as an expected free radical signal. The results of the antioxidant assay show that whisky (either neat or as its extract) confers excellent antioxidant activity and that these properties relate to the "non-phenolic" constituents of the extract.

## 1. Introduction

The antioxidant action of Scotch Whiskies was first reported in 1996 [1]. It was measured by an EPR technique observing the decay of superoxide radicals: however, no EPR spectra of the whiskies were reported. Since the antioxidant action was presumably due to gallates and other similar (phenolic) compounds extracted from toasted hard oak barrels previously used to age sherry, a stable free radical signal should be detectable by EPR spectrometry. The research work described in this paper aimed at characterising five whisky samples by EPR spectrometry and also to evaluate quantitatively the antioxidant activity reported by McPhail *et al*.[1] As part of a research program aimed at finding preventative methods for diseases relating to neurodegenerative conditions, we developed a simple assay to determine the efficiency of our drug candidates based on previous literature.[2,3] This assay has been used here to determine the antioxidant efficacy of the whiskies against the know antioxidants, vitamin C (ascorbic acid) and vitamin E (α-tocopherol).

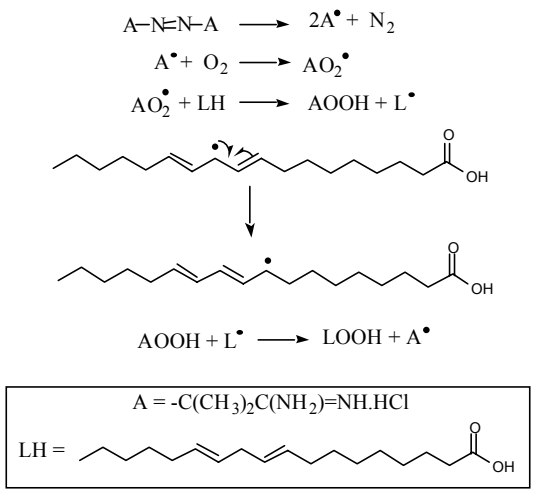


Gallic acid

## 2. Sample preparation

For the EPR work, each sample of 150 ml was concentrated to 10 ml on a rotary evaporator (25-30°C). Samples containing the solid and supernatant were placed in standard quartz tubes and frozen to avoid microwave polar water losses. A Varian E-12 EPR spectrometer, operating at 9.1 GHz was used to collect the spectra. There was little difference between the shape spectra of the liquid and solid portions of the heterogenous samples.

A convenient test to determine the efficiency of antioxidants in aqueous systems was setup using 2,2`-azobis(2-amidinopropane).2HCl (AAPH) as a free radical initiator (Scheme 1). The production of conjugated diene hydroperoxide (LOOH) generated through the oxidation of linoleic acid in an aqueous system at 37ºC is monitored at 234nm for 15 minutes using a Cary 100 UV-Vis spectrophotometer. The efficiency of the antioxidant is measured by its ability to quench free radicals and hence slow or stop oxidation of linoleic acid. Prior to testing the efficiency of the five




**Scheme 1**

whisky samples (supplied by *Angoves Pty Ltd*) at a volume of 10 μL, a number of well known antioxidants including vitamin E and ascorbic acid were tested (0.01 M solutions), giving similar results to those presented in literature.[2,3]

The efficiency is determined according to a standard, that is the generation of free radicals in the absence of an antioxidant. Efficiency is then calculated using the following equation:

$$\text{Efficiency (\%)} = 1 - [K_2 / K_1] \times 100$$

Where:

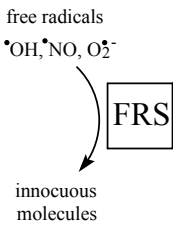
$K_1$ = rate of oxidation of standard (no antioxidant) = [difference in absorbance]/ [time (sec)]

$K_2$ = rate of oxidation with antioxidant = [difference in absorbance]/ [time (sec)]

## 3. Results

### 3.1 Antioxidant Activity

Lipid oxidation can usually be prevented by the addition free radical scavengers (FRS), more commonly known as antioxidants. Their role is to turn very reactive and harmful molecules such as hydroxy, nitroxy and superoxide radicals into innocuous molecules that can be passed or converted by biological processes. One of the main pathways that lead to apoptosis in cells is oxidative stress. This event is a product of the action of these harmful radicals on the cell walls and membranes, leading to their breakdown. In terms of the antioxidant evaluation (Scheme 1), AAPH was utilised as a free radical initiator generating superoxide, and the efficiency was calculated based on the formation of a conjugated diene hydroperoxide generated through the oxidation of linoleic acid in the presence and absence of a free radical scavenger. The conjugated diene hydroperoxide mimics the products of cellular oxidative stress imposed on the cell wall. The antioxidant can act at any stage of the reaction to stop or slow down the formation of the conjugated diene by reacting with the appropriate radicals (Scheme 1).



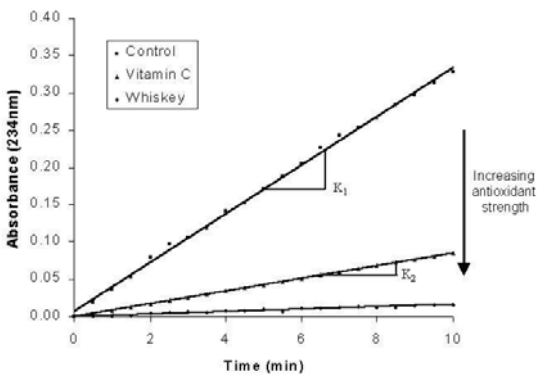

The results of the assay are shown in Figure 1 and summarised in Table 1. Quite clearly, all five whiskies conferred excellent antioxidant action (77-95% efficiency) under the reaction conditions. Alcoholic solutions (40% v/v ethanol) were ineffective suggesting that the antioxidant action is a result of the extract. Interestingly, the removal of phenolic compounds by passage of the whisky through polyvinylpolypyrrolidone (PVPP) still conferred excellent activity. This contradicts the proposal of McPhail *et al.* [1] and suggests that other components are responsible for the action.

Table 1. A comparison of the efficiency of the 5 whiskies, vitamin C and vitamin E to stop the formation of the conjugated diene hydroperoxide.

| Sample | Efficiency[a] |
|---|---|
| α-tocopherol (vitamin E) | 93% |
| Ascorbic Acid (vitamin C) | 74% |
| Malt Whisky 1 | 95% |
| Malt Whisky 1 (no phenolics) | 83% |
| Malt Whiskey 2 | 79% |
| Malt Whisky 3 | 77% |
| USA Whisky 1 | 89% |
| USA Whisky 2 | 89% |

[a] Error ± 3%.

Fig. 1. Schematic representation of thespectrophotometric results at 234 nm obtained upon the addition of antioxidant (10 μL) to linoleic acid in the presence of AAPH.



3.2 EPR Analysis

The spectra of the highest quality Scotch Whisky and the lowest quality USA are shown in Figure 1. Note the free radical signals and the differing $Cu^{2+}$ spectra. In fact, the observed $Cu^{2+}$ spectrum is unique for each whisky and is presumably a result of leaching from the copper within the stills, the different $Cu^{2+}$spectra a result of different ligation which is most likely a result of the different fermentation methods employed. Whisky samples treated with polyvinylpolypyrrolidone (PPVP) to remove phenolics prior to cold evaporation showed reduced free radical signals, but the reduction was not as dramatic as in red wines (90%). The antioxidant efficiency achieved by the treated whiskies was approx. 10% of that of the untreated, showing that the removable phenolics did not contribute significantly.

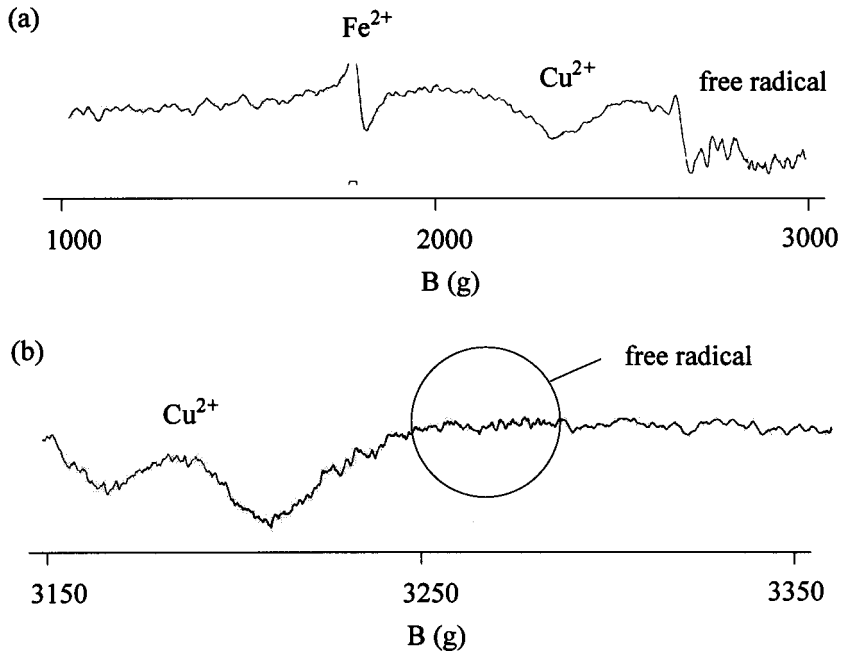



Fig. 2. EPR spectra of concentrated samples of (a) USA Whiskey 1 and (b) Malt Whisky 1 at 77K.

In conclusion, the results indicate that whisky acts as an antioxidant with a similar efficiency to α-tocopherol and greater efficiency than ascorbic acid. Given these results, a shot of whisky will give the equivalent "antioxidant potential" to the recommended daily intake of vitamin C! We are at present investigating the source of the antioxidant action with a view to utilizing it for neurodegenerative conditions.

**Acknowledgments**

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**References**

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