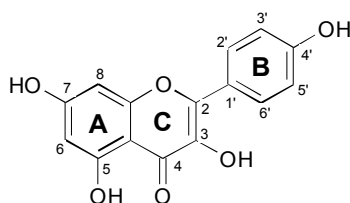


# THE INFLUENCE OF OXIDATION CONDITIONS ON FREE RADICAL FORMATION IN KAEMPFEROL

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Dietary flavonoids play important roles in disease prevention, by their ability to scavenge reactive oxygen species (ROS), which include the free radicals  $O_2^{\cdot-}$  and  $HO^{\cdot}$ . They are present in most plants, enriched in seeds, fruit skin or peel, bark, and flowers (Wollenweber, 1994). Their functions include, among others, helping to protect against predators and infectious agents as well as absorbing UV-B radiation (Bais et al., 2003; Woo, 2002). Oxidation of flavonoids leads to the formation of free radicals many of which have moderate stability because of their delocalised electronic structures. In the present work we have used EPR spectroscopy to study free radical formation in the flavonoid kaempferol (Fig. 1).



*Fig. 1.* Structure of kaempferol.

We investigated autoxidation and reactions of kaempferol with  $^{\cdot}OH$  and  $O_2^{\cdot-}$ . Autoxidation was carried out by mixing the flavonoid solution with an aerated solution of alkali, either externally or directly in a flat cell. Reactions investigated were with (a) products from horseradish peroxidase/ $H_2O_2$ , (b)  $^{\cdot}OH$  radicals from a simple Fenton system and (c)  $O_2^{\cdot-}$  using either xanthine/xanthine oxidase or potassium superoxide. All of these measurements were performed at physiologically relevant pH of 7.

Five different EPR spectra were observed. Except from one signal (a quintet) which resembles p-benzosemiquinone (Kuhnle, 1969), all other signals are unreported to our knowledge. The main site of oxidation is the 4'-OH group. Possible structures for the various radicals will be discussed along with their implications for understanding the reactions of various flavonoid molecules. It is clear, however, that these reaction pathways are neither simple nor straightforward and it is unreasonable to discuss the dietary behaviour of flavonoids solely in terms of scavengers of ROS.

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