Pro-oxidative Activity in Some Thai Spices

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Abstract
Twenty methanolic extracts from Thai spice powder (anise, cardamom, chilli, Chinese key, cinnamon, clove, coriander, cumin, deeplee, dill, fennel, galangal, kencur, lemongrass, ginger, nutmeg, oregano, pepper, safflower, and turmeric) were screened for pro-oxidative activity using a lecithin-liposome model system. The oxidative stress was induced by four systems: (I) non-metal induction; (II) FeCl3/H2O2/EDTA; (III) FeSO4/ascorbate; and (IV) CuSO4/H2O2. Of these spices, safflower, lemongrass, and nutmeg extracts showed the pro-oxidative activity observed by thiobarbituric acid reactive substance elevation. Since pro-oxidation were metal dependent, hydroxyl radical formation was investigated by monitoring fluorescence products from benzoate hydroxylation. The formation of hydroxyl radical was confirmed with the fluorescence detected and the suppression due to addition of mannitol, a hydroxyl radical scavenger.

INTRODUCTION
Recent studies showed that many vegetables, fruits, tea, herbs, and spices exerted an antioxidant action (Bray, 2000). Although the antioxidant capacity of many plants has been investigated, little is known about the pro-oxidative activity and its mechanisms. Experimental evidences showed that various teas cause the oxidative damage of deoxyribose, DNA and DNA base in vitro (Yen et al., 1997). Furthermore, some antioxidants such as ascorbic acid, green tea extracts (Gutteridge, 1984), gallic acid and its derivatives (Aruoma et al., 1993), and carotenoids, are found to act as pro-oxidants depending on the reduction-oxidation potential and the biological environment (Bagnati et al., 1999). The aim of this work was to evaluate the pro-oxidative activity of powdered spices, to facilitate greater understanding about pro-oxidative activity in these spices.

MATERIALS AND METHODS
Screening of the Pro-oxidative Spices
Ten grams of 20 powdered spices, purchased from a local market in Chiang Mai, Thailand: cardamom (Elettaria cardamomum), clove (Eugenia caryophyllata) cinnamon (Cinnamomum cassia), chilli (Capsicum annuum), Chinese key (Boesenbergia pandurata), pepper (Piper nigrum), coriander (Coriandrum sativum), deeplee (Capsicum frutescens), ginger (Zingiber officinale), lemongrass (Cymbopogon citratus), cumin (Cuminum cuminum), turmeric (Curcuma domestica), nutmeg (Myristica fragrans), galangal (Alpinia galanga), anise (Pimpinella anisum), dill (Anethum graveolens), kencur (Kaemferia galanga), fennel (Foeniculum vulgare), safflower (Carthamus tinctorius), and oregano (Origanum vulgare) were extracted twice with 75 mL of methanol and evaporated (Donovan et al., 1998).

Preparation of Lecithin-liposome
Twenty grams egg yolk lecithin was dissolved in 100 mL of chloroform and then evaporated in round-bottomed flask with a rotary evaporator. The resulting thin film was re-suspended in 20 mM phosphate buffer (pH 7.4) to 15 mg/mL lecithin, then vortexed
and sonicated to produce multilamellar vesicles (Nakayama et al., 1992).

**Monitoring of Liposome Oxidation (TBAR Measurement)**

Liposome suspension was incubated with four different systems: (i) non-metal induced system, (ii) 50 μM FeCl₃/3 mM H₂O₂/100 μM EDTA (Yen et al., 1997), (iii) 50 μM FeSO₄/50 μM ascorbic acid (Donovan et al., 1998), and (iv) 100 μM CuSO₄/3 mM H₂O₂ (Donovan et al., 1998) at 37°C in the presence or absence of 1 mg/mL spice extracts for 1 h. Butylated hydroxytoluene was added to stop the oxidation. The reaction mixture was measured for thiobarbituric acid reactive substances (TBARS) as the indicator for lipid oxidation (Burge et al., 1978).

**Determination of Hydroxyl Radical Formation**

Reaction mixtures containing 1 mM benzoate and safflower, lemongrass or nutmeg extracts (0.1, 0.25, 0.5, 1, 2.5, or 5 mg) in three oxidation systems: FeCl₃/H₂O₂/EDTA, FeSO₄/ascorbic acid, and CuSO₄/H₂O₂ were incubated at 37°C for 1 h. Hydroxyl radical formation was detected by monitoring the fluorescence products of benzoate (Ex 305 nm, Em 407 nm; Gutteridge, 1987). The effect of mannitol as hydroxyl radical scavenger in metal-induced hydroxyl radical formation was also investigated.

**RESULTS AND DISCUSSION**

**TBAR Formation Lecithin-liposome Oxidation**

The pro-oxidative activity of methanolic extracts of 20 spices in metal-induced lecithin-liposome oxidation was investigated. In this experiment, the lecithin-liposome vesicles were oxidized and degraded to form malonaldehyde. As a result, the spices i.e. safflower, lemongrass, and nutmeg at 1 mg could increase the TBAR formation (Fig. 1). In non-metal induced system, pro-oxidative spice extracts possibly enhanced lipid oxidation by the process of one electron transfer, which was catalyzed by trace metal ion in the reaction mixture. If molecular oxygen received one electron, then it would be converted into superoxide anion (O₂⁻), and conceivably initiate the lipid oxidation. Along with the progress of lipid oxidation, other reactive oxygen species such as hydroxyl radical (OH·), as well as alkyl radical would be formed.

The pro-oxidative spices could also enhance the hydroxyl radical formation in metal induced oxidation system by reducing Fe³⁺ or Cu²⁺ to Fe²⁺ and Cu¹, respectively. These ions then could have further reacted with hydrogen peroxide or ascorbic acid, resulting in hydroxyl radical formation.

**Effect of Safflower, Lemongrass, and Nutmeg Extract Concentrations on Hydroxyl Radical Formation**

The hydroxyl radical formation in the reaction mixtures was detected by benzoate hydroxylation method. Maximum OH· formation was observed in 1 mg extracts (Fig. 2). Among the metal-induced systems tested, Cu²⁺/H₂O₂ system was the strongest to provoke the benzoate hydroxylation. As this hydroxyl radical production could be inhibited by the addition of mannitol, these results confirmed that the hydroxyl radical was produced in all systems tested (data not shown).

**CONCLUSIONS**

Among the 20 spices examined, safflower, lemongrass, and nutmeg extracts exhibited the pro-oxidative activity in lecithin-liposome model. The pro-oxidative activity of these extracts was influenced by the presence of metal ion. Spices that are pro-oxidative would enhance the free radical formation, i.e. hydroxyl radical or superoxide anion.

**Literature Cited**

Fig. 1. TBAR formation of the reaction mixtures containing 1 mg of spice extract and liposome incubated at 37°C for 1 h. (a) non-metal induced system; (b) FeCl₃/H₂O₂/EDTA; (c) FeSO₄/ascorbic acid; and (d) CuSO₄/H₂O₂ (control: no addition of the spice extracts).
Fig. 2. Benzoate hydroxylation due to (a) FeCl$_3$/EDTA/H$_2$O$_2$; (b) FeSO$_4$/ascorbic acid; (c) CuSO$_4$/H$_2$O$_2$ systems with safflower, lemongrass, or nutmeg extracts. (■ safflower; ▲ lemongrass; ● nutmeg)