Inhibition of the Oxidation of Acetophenone by Aqueous Extracts of Edible Fruits and Vegetables

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Abstract

A body of research has identified some fruits and vegetables to have anti-oxidant potential in biological systems. However limited literature reports exist for the inhibition of chemical oxidation reactions by fruits and vegetables. The research reported here examined the effect of plants, fruits, and vegetables on the oxidation reaction of acetophenone by bleach and if the bleach oxidation reaction of acetophenone is chlorine-induced. To examine the ability of plants to inhibit the oxidation of acetophenone, the reactions were carried out in the presence and absence (control) of plant materials and Vitamin E. Results suggest that the reactions without the plant materials or Vitamin E produce 1.53 g of benzoic acid, an oxidative product, while reactions in the presence of plant materials or Vitamin E were inhibited and produced a varying amount of oxidative product ranging from 0-0.9 g. Similar reactions of acetophenone with chlorine free bleach in the absence of plant materials did not produce any oxidative product. The order of inhibition of oxidation by fruit and vegetable extracts which is inversely related to the amount of oxidative product formed is carrot~tomatoes~spinach~bitter leaf (0.0 g) > yellow bell pepper (0.008 g) > mustard green (0.01 g) > turnip green (0.03 g) > broccoli (0.27 g) > red bell pepper (0.17 g) > green bell pepper (0.31 g) > cucumber (0.59 g) > red lettuce (0.68 g) > rosemary (0.76 g) > green onions (0.82 g) > green lettuce (0.84 g) > romaine lettuce (0.91 g). These results suggest that (i) fruits and vegetables have varying degrees of antioxidant ability and (ii) the bleach oxidation of acetophenone to benzoic acid is chlorine induced.

Keywords: Antioxidant; Vegetables; Bitter leaf; Vernonia amygalalina; Oxidation reaction; Bleach; Acetophenone

Introduction

Several recent research has focused on fruits and vegetables as potential sources of natural antioxidants [1-8]. The phytochemicals in fruits and vegetables have been reported to have beneficial health effects against coronary disease [9,10], cancer [11], and degenerative diseases [12]. Normal metabolic processes in the body produce radicals as by-products. These radicals cause lipid and protein oxidation. Vitamins C, E, and carotene are known antioxidants that scavenge or destroy these free radicals and inhibit lipid and protein oxidation. Thus, the research reported here evaluates the ability of various plants, fruits and vegetables to inhibit the oxidation of acetophenone by oxidizing agents such as sodium hypochlorite a major component of commercial bleach.

Materials and Methods

Regular chlorinated bleach and chlorine free-bleach (Chlorox 2) were bought from a local market. Acetophenone and dl-alpha tocopherol (Vitamin E) were bought from Aldrich Chemicals. All reactions were stirred for 45 min at room temperature. The level of inhibition of oxidation by each plant material is inversely related to the amount of oxidative product formed.

Preparation of plant materials (extracts)

Fresh plants (100 g of the following plants: Fruity Vegetables: Capsicum annum (red, green, and yellow bell pepper), Dauscar carota (carrot), Lycopersicon esculentum (tomatoes).

Leafy Vegetables: Spinacia oleracea (spinach), Brassica juncea (Mustard Green), Brassica oleracea, Brassica rapa var rapifera (turnip Green), Vernonia amygalalina (bitter leaf), Lettuce sativa (red, green, and Romaine lettuce), Cucumis sativus (Cucumber), Allium fistulosum, and Rosemarinus officinalis (Rose Mary herb); Fruits: Vitis vinifera (red and green grapes), Malus domestica (red and green apple), Citrus sinensis (orange), Ananas comosus (pineapple) and Citrus latifolia (lime) bought from a local market were washed with deionized water and patted dry with kimwipes. Each vegetable or fruit was pureed in a regular kitchen blender using 200 ml of de-ionized water. The puree was filtered using a white handkerchief bought from a local Wal-Mart store. The filtrate was put in 4-50 ml centrifuge tubes. The tubes and their contents were centrifuged for 10 minutes at 3000 rpm. The supernatants were collected and placed in a clean beaker.

Reactions of acetophenone

Reaction of acetophenone and chlorinated bleach in water: Acetophenone (5 ml) was added to a mixture of 70 ml of regular bleach (purchased from local market) and 70 ml of water (in triplicates). The resulting mixture was stirred for 45 minutes at room temperature. Ether (70 ml) was added to the mixture and stirred for 5 minutes. The resulting mixture was poured into a 250 ml separatory funnel. The layers were separated and the aqueous layer was extracted with another 70 ml of ether. The resulting two layers were separated and to the aqueous layer, was added concentrated Hydrochloric acid (drop wise) until the pH of the solution was 1. The solid product that formed was filtered, dried, and weighed.

Reaction of acetophenone and non-chlorinated bleach in water: Acetophenone (5 ml) was added to a mixture of 70 ml of non chlorinated bleach (purchased from local market) and 70 ml of water (in triplicates). The resulting mixture was stirred for 45 minutes at room temperature. Ether (70 ml) was added to the mixture and stirred for 5 minutes. The resulting mixture was poured into a 250 ml separatory funnel. The layers were separated and the aqueous layer was extracted with another 70 ml of ether. The resulting layers were separated and
to the aqueous layer, was added concentrated Hydrochloric acid (drop wise). No precipitate was observed even after excess acid was added to make the solution very acidic at pH 1.

**Reaction of acetophenone and chlorinated bleach in the presence of each fruit or vegetable material:** Acetophenone (5 ml) was added to a mixture of 70 ml of regular bleach and 70 ml of a vegetable or fruit extract (in triplicates), respectively. The resulting mixture was stirred for 45 minutes at room temperature. Either (70 ml) was added to the mixture and stirred for 5 minutes. The resulting mixture was poured into a 250 ml separatory funnel. The layers were separated and the aqueous layer was extracted with another 70 ml of ether. The resulting layers were separated and to the aqueous layer was added concentrated Hydrochloric acid (drop wise) until the solution has a pH of 1. If any solid product is formed, the mixture was filtered and the solid that formed is dried and weighed on an analytical balance.

**Reaction of acetophenone and chlorinated bleach in the presence of vitamin E (dl alpha tocopherol):** Acetophenone (2 ml) was mixed with 1 ml of dl alpha-tocopherol purchased from Aldrich chemicals and 70 ml of regular chlorinated bleach (purchased from local market) was added. The resulting mixture was stirred for 45 minutes at room temperature. Ether (30 ml) was added to the mixture and stirred for 5 minutes. The resulting mixture was poured into a 250 ml separatory funnel. The layers were separated and the aqueous layer was extracted with another 30 ml of ether. The layers were separated and to the resulting aqueous layer, was added concentrated Hydrochloric acid (drop wise). No precipitate was observed even after excess acid was added to make the solution very acidic at pH 1.

**Effect of heating the fruit and vegetable extracts on their ability to inhibit the oxidation of acetophenone**

**Reaction of Acetophenone and Chlorinated Bleach in the presence of heated green and yellow bell pepper, broccoli, carrot, and lime extracts:** The extracts of green bell pepper, yellow bell pepper, broccoli, carrot and lime prepared above were heated to a boil for five minutes and then allowed to cool. Acetophenone (5 ml) was added to a mixture of 70 ml of the chlorinated bleach and 70 ml of the heated vegetable or fruit extract (in triplicates), respectively. Each mixture was stirred at room temperature for 45 minutes. Ether (70 ml) was added to each mixture and stirred for 5 minutes. Each of the resulting mixture was poured into a 250 ml separatory funnel. The layers were separated and the aqueous layer was extracted with another 70 ml of ether. The resulting layers were separated and to the aqueous layer was added concentrated Hydrochloric acid (drop wise) until the solution has a pH of 1. The mixture was filtered and the solid that formed (when applicable) was obtained, dried, and weighed.

**Results**

The control reaction of benzophenone with chlorinated bleach in water produced on average 1.53 g of oxidative product while similar reaction with chlorine free bleach failed to produce any oxidative product. Reaction of acetophenone with chlorinated bleach in the presence of vitamin E did not produce any oxidative product. The order of inhibition of oxidation by fruity vegetables is carrot = tomatoes (0.0 g) > yellow bell pepper (0.008 g) > red bell pepper (0.17 g) > green bell pepper (0.31 g) > cucumber (0.59 g). With the leafy vegetables, the order of inhibition of oxidation is spinach = bitter leaf (0.0 g) > mustard green (0.01 g) > turnip green (0.03 g) > broccoli (0.27 g) > red lettuce (0.68 g) > rosemary (0.76 g) > green onions (0.82 g) > green lettuce (0.84 g) > romaine lettuce (0.91 g). All the fruits (red and green grapes, red and green apples, pineapple and lime) completely inhibited the oxidation reaction except for the reaction in the presence of orange which gave 0.9 g of oxidative product.

Figures 1-3 below show the variation in the inhibition of the oxidation reaction of acetophenone with regular bleach in the presence of various fruit and vegetable extracts. Furthermore, it compares the inhibition potential of leafy vegetables, fruity vegetables, and fruits.

Figure 4 compared unheated and heated vegetable and fruit extracts in inhibiting the oxidation reaction of acetophenone with bleach.

The results demonstrate that heating the vegetable extracts greatly reduced their ability to inhibit the oxidation reaction.

In related but different reactions, the oxidation of benzyl alcohol with potassium permanganate to produce benzoic acid was inhibited in the presence of plant materials while the reaction in the absence of plant material gave oxidative product (benzoic acid). Similarly, cyclohexene reacted with KMnO₄ in water to give adipic acid. However, similar reaction in the presence of vitamin E or plant materials did not produce any adipic acid (Figures 1-4).

**Discussion**

On whether fruit and vegetable extracts can inhibit the oxidation of acetophenone by bleach, the results showed that plants inhibited the oxidation reaction of acetophenone by bleach.
content of each plant material to a greater extent influences the anti-
oxidant potential. There is a varying degree of inhibition of oxidation
antioxidant potential between various fruits and vegetables used in this
study. The inhibition of the oxidation reaction does not correlate with
the vitamin C content of the fruit or vegetable. This is in agreement with
the finding by Wang who found that the high antioxidant activities of
some common fruits could not be accounted by their vitamin C content
[13]. However, the order of inhibition of oxidation reported in this
project is closely in line with the antioxidant activities of these fruits
and vegetables reported by Cao [14]. Thus, the order of inhibition of
acetophenone oxidation reported here could be related to the phenolic
or other phytochemical content of the fruits or vegetables. Furthermore,
our data suggest that fruits inhibited the oxidation of acetophenone
better than the vegetables. The fact that heating vegetable extracts
(bell pepper, yellow bell pepper, and broccoli) drastically reduced
their oxidation inhibitory effect when compared to the heated carrot
and Lime that did not show any effect suggests that probably some of
the phytochemicals in the vegetable extracts could have evaporated,
decomposed, or denatured as the temperature of the extracts is
increased from 25°C to 90°C or higher. Agwaramgbo [15] reported
similar observation in the phytoremediation of lead contaminated
water using heated and unheated plant extracts.

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