Evaluation Of Ingredient Combination on The Antioxidant Capacity of Coffee Drinks

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Abstract
Coffee is a rich source of natural antioxidants (polyphenols). It is among the most widely consumed beverage worldwide, and thus it contributes majorly to the dietary intake of antioxidants. Processing of coffee and added ingredients like sugar and spices (to obtain a desirable flavor) often compromises its anti-oxidation property. In the present study, we assessed the radical scavenging activity, of different coffee brands, its preparations and commonly added spices and sweeteners, by DPPH assay. Based on the observations, we aimed to propose a preparation method for antioxidant rich beverage. Nescafe classic brand showed 16-40% more radical scavenging capacity as compared to Nescafe Sunrise, Bru Instant, Fresh and Pure Select and filter coffee. The black and spice coffee preparations of Nescafe classic particularly showed higher antioxidation property as compared to lemon, mint and milk coffee preparations. Among sweeteners and spices, stevia leaf powder, honey and cardamom were identified as good sources of anti-oxidants. Hence, the interaction of these components was evaluated by combining black coffee preparations of Nescafe classic with cardamom and stevia leaf powder/ honey. It was observed that the addition of honey and cardamom further increased the radical scavenging activity of black coffee by 20.63%. Thus, our study suggests that black coffee preparation of Nescafe classic containing cardamom and honey is the best form of antioxidant rich beverage.

Keywords: Antioxidants, DPPH, Coffee

Introduction
Free radicals are metabolic byproducts produced during cellular processes. They may also be induced on activation of defense mechanisms against various pollutants like ozone, cigarette smoke, radiations, pesticides and industrial chemicals [1]. In excess amount, however, the free radicals severely damage the cells due to oxidation of proteins, lipids and DNA. The progressive accumulation of these defects leads to oxidative stress and is linked with several inflammatory conditions, aging, cancer, neurological and immune disorders [2]. Antioxidants are compounds that protect the cells from damage caused by free radicals. Although dietary intake of common fruits and vegetables provides us with sufficient antioxidants, factors like urban lifestyle, stress, pollution and excess use of chemical fertilizers in agriculture has necessitated the need for intake of additional antioxidant supplements. Hence, marketing of synthetic antioxidants are increasingly becoming common in the marketplace. Unfortunately, it is only recently that the adverse effects of synthetic anti-oxidants have been recognized [3]. Literary evidence reflects the damages caused due to long term intake of synthetic antioxidant supplements on liver and kidneys [4].
Identification of dietary antioxidants has led to our understanding that berries and citrus fruits are among the good sources of antioxidants. However, their consumption is limited based on factors like availability, seasons, desirability and cost [5]. To this effect, coffee is an inexpensive and commonly consumed beverage worldwide that is available throughout the year. It is loaded with powerful antioxidants like polyphenols as well as other pharmacologically active compounds. In general, plant polyphenols play a key role in prevention of several chronic conditions like cancer, type 2 diabetes, and neurological and cardiovascular diseases [6]. Regular intake of coffee in low to moderate levels is associated with prevention of Parkinson disease, Alzheimers, dementia and age-related cognitive decline [7]. All isomers of chlorogenic acid-a polyphenol found in coffee are potent antioxidants and primarily regulate blood pressure [8]. Caffeine is another component of coffee that stimulates the central nervous system, heart and muscles. Moreover, coffee contains trigonelline, tryptophan, alkaloids, diterpenes and other secondary metabolites that provide many health benefits [9].

The antioxidant properties of coffee and its ingredients are well studied [10]. However, the interaction of sugars, spices and other additives with coffee and the resulting alteration in antioxidant property is rarely reported [11]. Hence, the current study was carried out with an objective to evaluate the effects of different coffee ingredients on its radical scavenging property, and propose a recipe for an anti-oxidant rich beverage.

Materials And Methods

Chemicals and other ingredients
All chemicals used in this study were of highest purity and analytical grade. Reagents like 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), methanol and standard L-ascorbic acid were procured from SD Fine Chemicals Ltd. All other ingredients like sweeteners (sugar, honey, stevia, jaggery), spices (cardamom, cinnamon, nutmeg, cloves), mint, lemon and milk were purchased from local market.

Brands of coffee evaluated for antioxidative activity
Most popular coffee brands like Nescafe Classic, Nescafe Sunrise, Bru Instant, Fresh and Pure Select and filter coffee were purchased from the local market to evaluate their antioxidation potential.

Preparation of coffee extracts
1. Black coffee:
Black coffee was prepared by mixing 120ml warm water with 1.5g of coffee powder.
2. Lemon coffee:
Prepared by mixing 120ml water, 1.5g coffee powder, 7g sugar and 10ml lemon juice.
3. Spice coffee:
Prepared by mixing 120ml milk, 1.5g coffee powder, 7g sugar and 0.5g spice mix (containing cloves, nutmeg, cardamom and cinnamon).
4. Mint coffee:
Prepared by mixing 120ml milk with 1.5 g coffee powder, 7g sugar and few crushed mint leaves.
5. Milk coffee: Prepared using 120 ml milk and adding 1.5 g coffee powder and 7 g sugar.
DPPH assay
The ability of coffee extracts to scavenge free radicals was measured using a modified method described by Miliauskas et al. [12]. The coffee extracts (0.1 ml) were mixed with 3 ml of methanolic DPPH solution and kept in dark for 30 mins. A control tube was prepared similarly by replacing coffee extracts with distilled water. The absorbance of these tubes was measured at 517.5 nm using a spectrometer. A standard graph of absorbance v/s concentration of ascorbic acid (5-10 µg/ml) was prepared for the assay and the radical scavenging capacity of coffee extracts was calculated using the equation:

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\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

RESULTS
Among the five coffee brands tested for their radical scavenging potential, Nescafe classic showed the highest activity with 50% inhibition of free radicals (Fig. 1). Also, since the same beverage is prepared in many ways depending on the taste preference of consumers we further tested the antioxidation activity of different preparations (Figure 2). Besides coffee preparations, the choice of sugar and spices also vary with consumer preference. Hence, the radical scavenging activity of common sweeteners (Figure 3) and spices (Figure 4) used in coffee preparations were also tested. Considering the significant difference between scavenging potential of Nescafe classic and other brands (Figure 1), it was selected for above studies.

Figure 1: Radical scavenging activity of common coffee brands
Figure 2: Radical scavenging activity of common preparations of Nescafe classic

Figure 3: Radical scavenging activity of common sweeteners used in coffee preparations
As indicated in Figure 2, black coffee (40%) preparation showed highest antioxidant activity followed by spice coffee (31.5%). The study of antioxidation activity of different sweeteners indicated the suitability of honey & stevia leaves in coffee preparations. Among the 4 common spices tested, only cardamom showed good antioxidation activity. Hence, these ingredients were used in all possible combinations for coffee preparations and their radical scavenging activity was tested to evaluate the interaction between individual components. Based on observed results, two preparations showed improved antioxidant activity as compared to black and spice coffee preparations of Nescafe classic. The coffee preparation that included water (120 ml) + coffee powder (1.5 g) + cardamom (0.5 g) + honey (7 g) showed radical scavenging activity of 60.63%. Another preparation that contained milk (120 ml) + coffee powder (1.5 g) + cardamom (0.5 g) + stevia (7 g) showed radical scavenging activity of 52.33%.

**DISCUSSION**

Coffee is the 3rd most commonly consumed beverages worldwide. The distinct flavor of coffee not only tickles the taste buds but also stimulates the nerves and relaxes the muscles [9]. Individual taste preferences have led to wide range of coffee preparations with or without sugars, milk and spices. It is well known that food additives interact with its ingredients resulting in increased or decreased potency and health benefits [13]. Hence, considering the need for natural source of antioxidant, the present study was carried out to evaluate the antioxidant capacity of different coffee preparations and suggest the most potent recipe for antioxidant rich beverage.

The observations in this study indicated that addition of cardamom (0.5 g) and honey (7 g) to black coffee highly complimented its radical scavenging activity which increased by 20.63%. Replacement of water with milk and honey with stevia leaves in above preparations negatively affected its radical scavenging activity and reduced it by 8.3% (i.e., 52.33%). However, it was still significantly higher than the antioxidation potential of black coffee (40%), and hence can be considered as a suitable antioxidant.
rich beverage for consumers who prefer sweet and flavored taste. Additionally, compared to original spice coffee preparation (that contained sugar and spice mix), the radical scavenging capacity of the above mixture increased by 20.83%. Hence, Nescafe classic coffee prepared with or without milk and containing cardamom and/or honey/stevia leaves can be the most suitable dietary source for daily dose of anti-oxidants.

In addition to coffee, the health benefits of cardamom, honey as well as stevia is well reported in literature [14-15]. Hence, the coffee preparation proposed in this study can favor much more health benefits in addition to the antioxidation potential linked to plant polyphenols and coffee components. Limited research has been carried out on the effect of sugar and flavor additives on antioxidation potential of coffee. However, similar to our findings, high antioxidation capacity of coffee brews, and instant black coffee is commonly reported [16].

An interesting observation of this study was the reduction in radical scavenging potential of coffee in presence of milk. Similar findings suggested that fats and proteins in milk interfere with the polyphenols and particularly reduce the bioavailability of chlorogenic acid. Thus, milk affects the antioxidation potential of coffee [17, 18]. However, contrasting results were reported in other studies that indicated enhanced scavenging activity of coffee by 10-20% on addition of milk [11, 19]. Due to limited research on the subject, evaluation of these findings and making concluding remarks on the efficacy or interference of milk is very difficult and more in depth research is required. Besides coffee, addition of milk, sugar and lemon juice is also reported to reduce the antioxidation capacity of tea infusions [20, 21].

In this study too, lemon coffee showed least antioxidation capacity, indicating the interference of ingredients in lemon juice with those of coffee that leads to compromised antioxidant activity. Spices are known to lose their antioxidation potential when cooked for a long time. Hence, it is advised to use powdered and slightly roasted spices to improve flavor as well as its health properties [22]. For this reason, use of ground spices is preferred in food and the same was used in this study. However, to the best of our knowledge, no published studies have reported the mechanism of enhanced antioxidation activity of beverages on addition of cardamom (as reported in our study) or other spices.

CONCLUSION

The present study demonstrates the efficacy of cardamom and honey in improving the antioxidation potential of black coffee. On the other hand, filter coffee and use of lemon juice, mint and spices like nutmeg, clove or cinnamon showed least antioxidation potential. Thus, our study demonstrates significant relation between coffee preparation method/ ingredients and radical scavenging activity of coffee. In order to completely understand the underlying mechanisms, the interaction between bioactive components of useful ingredients (that improve antioxidation potential) and coffee components can be studied in more detail. More valuable insights in to improvement of antioxidation potential can be gained by comparing the activity of differently processed coffee beans and their common preparations. Thus, the present study can prove extremely valuable in improvising a practical recipe for daily dietary intake of antioxidant rich beverage.
References


