A Comparison of *In Vitro* Bioavailability of Total Antioxidant Capacity of Selected Organic and Conventional Vegetables

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Abstract

The objective of the study is to know if there is a difference in *in vitro* bioavailability (also known as *in vitro* digestibility) of organically and conventionally cultivated vegetables with respect to antioxidant capacity. A method that simulates human digestive system was used to study the *in vitro* bioavailability of antioxidants present in organically grown and conventionally grown vegetables. Four commonly consumed vegetables namely spinach, tomato, beetroot and carrot were selected for the study. Institute of Marketlogy Organic Certification (I.M.O) certified organic vegetables and conventional vegetables grown in the same soil and agro climatic condition were selected for the study. The vegetables were freeze dried using Lyodryer and stored at 4°C±2 in dark. Four different solvents were used for extraction to identify the best solvent for each vegetable. Total antioxidant capacity was estimated by Ferric Reducing Antioxidant Power (FRAP) method as described by Benzie and Strain (1996). *In vitro* bioavailability was determined according to a method described by Luten *et al* (1996). The percent recovery was studied based on the difference between antioxidant capacity of vegetables before and after *in vitro* digestion. The results indicate that among the vegetables studied, antioxidants in conventional beetroot had the highest recovery after digestion. The recovery of antioxidant capacity after digestion is slightly better for conventional vegetables compared to organic vegetables in case of spinach, beetroot and tomato whereas there was no significant difference in the *in vitro* bioavailability of organic and conventional carrot.

Keywords: Freeze dry**,** solvent optimization**;** FRAP method**,** percent recovery of antioxidants,

1. Introduction

The term organic food refers to food that is produced to encourage and enhance biological cycles within the farming system, to maintain and increase long-term fertility of soils, to minimize all forms of pollution, to avoid the use of synthetic fertilizers and pesticides, to maintain genetic diversity of the production system, to consider the wider social and ecological impact of the food production and processing system, and to produce food of high quality in sufficient quantity (International Federation of Organic Agricultural Movements,1998). In short, organic foods are produced without the use of synthetic chemicals during production, storage and processing. In contrast to this conventional farming uses nearly 400 different chemicals at various stages of food production.

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According to Carl and Sarah (2006) there are two major hypotheses which have tried to explain the possible increase in antioxidants in organic compared to conventional foods. In conventional agriculture, the use of fertilizers promotes primary growth, at the cost of secondary growth. Secondary growth results in the production of secondary plant metabolites or phytonutrients many of which have antioxidant properties. Organic crops are not protected by pesticides and antioxidant plant metabolites are generated by a plant when attacked by pests (Asami *et. al.*, 2003). Consumers also have a strong perception that organic foods are superior to conventional foods. But there is very limited scientifically validated research work to prove or disprove this claim.

The term total antioxidant capacity refers to the combined ability of all antioxidants in a given food to neutralize the free radicals. Factors influencing total antioxidant capacity of a food include soil type and chemistry, plant nutrients, climatic conditions, pest pressure etc. Phytonutrients, with known beneficial (often antioxidant) effects on human health, are expected to be higher in organic produce for various reasons. In conventional agriculture the use of fertilizers promote primary growth, at the cost of secondary growth. Organic crops are not protected by pesticides and antioxidant plant metabolites are presumed to be generated by a plant when attacked by pests. Organic foods if proved to contain more antioxidants can increase antioxidant intake without altering the calorie intake. The extent to which these potentially important antioxidants can be absorbed is not very clear.

The bioavailability of various nutrients present in a diet ultimately decides the nutritional status of a person. It is time consuming, expensive and complicated to study the bioavailability by *in vivo* methods particularly using human being. So, *in vitro* methods, which simulate the human digestive system, are used for studying the bioavailability of nutrients. The purpose of the present study is to compare the *in vitro* bioavailability of total antioxidant capacity of selected organic and conventional vegetables.

2. Materials and methods

2.1 Selection of sample

Organic vegetables were procured from Era Organic foods, Bangalore (I.M.O Certified). Same varieties of conventional vegetables were obtained from Yeshwanthpur market, Bangalore after confirming that they were grown in same area as organic vegetables. All other chemicals were of the analytical grade; Sigma, Himedia, Qualigens, Boisar, Ranbaxy.

2.2 Preparation of vegetable extract

Four vegetables *viz*. tomato, spinach, carrot, beetroot were selected for this study. The vegetables for the entire period of study were freeze dried using a Lyodryer LT5B ISI lyophilisation System INC USA and the freeze dryed samples were kept in polythethylene (4 inch × 6 inch) size bags and sealed using manual sealing machine and the polythene bags were kept in a dessicator which was sealed with vacuum grease and covered with black wrapping paper and stored in a cold room at 4°C ± 2. A flow chart describing the preparation of vegetable extract is given in Figure 1. Four different solvents were used for extraction viz 1) water 2) 75% ethanol (v/v) 3) Methanol : Ethanol : Water : HCl (69 : 20 : 10 : 1 v/v/v/v) 4) Methanol : Ethanol : Water : HCl (69 : 20 : 10 : 1 v/v/v/v). All the solvent extractions were extracted as shown in Figure 1. First three solvent extractions were used immediately for analysis whereas the fourth solvent extract was stored for 24 h in dark and then analysed for antioxidant assay.

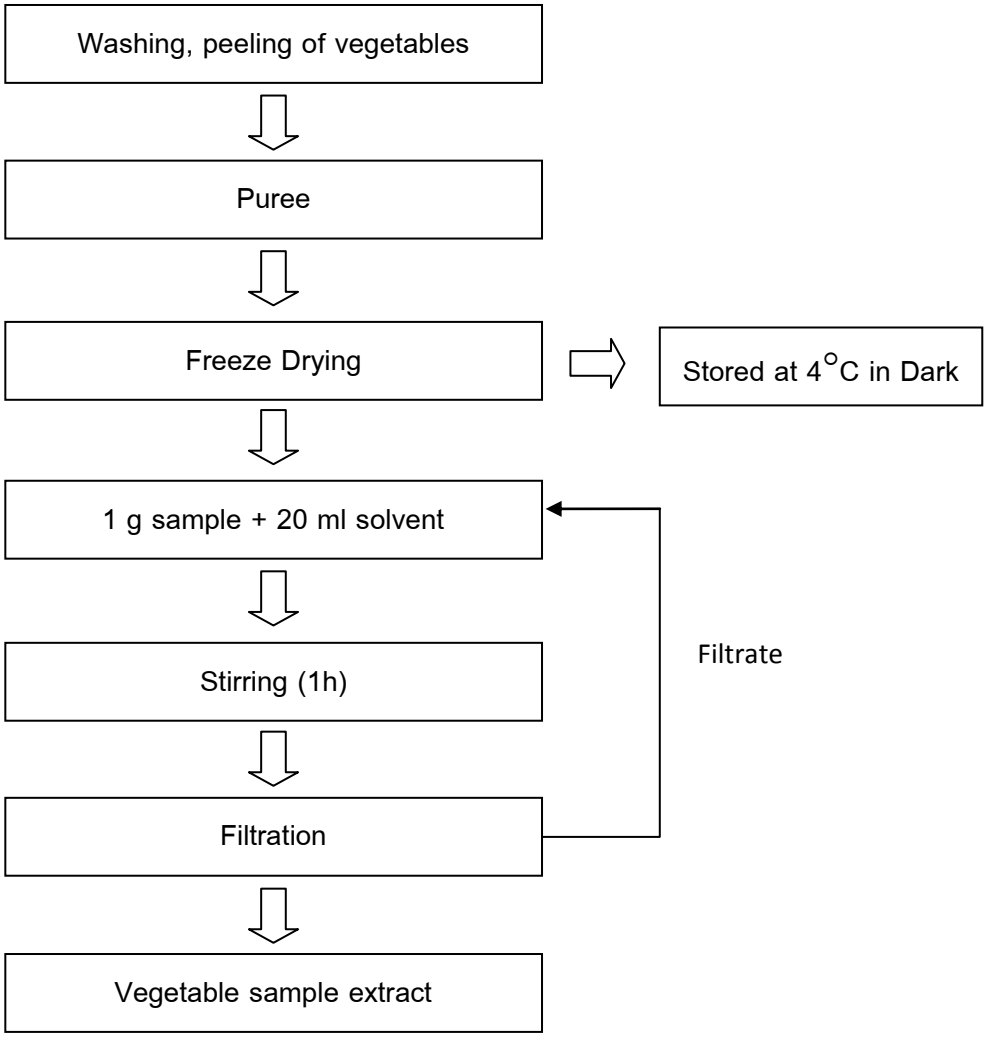

Figure 1 Flow chart for the preparation of vegetable extract

2.3 Solvent optimization

The total antioxidant capacity is contributed by a number of antioxidant components. Some of these may be water soluble whereas others may be fat soluble. Since the vegetable samples used for this study contain both fat soluble and water soluble compounds antioxidant activity was determined in 4 different solvents. The best solvent for each vegetable was identified based on the antioxidant capacity by FRAP method and is given in Table 1.

Table 1 Solvent optimization for the preparation of vegetable extracts

Vegetable	Solvent
Spinach	Methanol+Ethanol+Water+ HCl kept in dark for 24 h
Tomato	Methanol+Ethanol+Water+ HCl kept in dark for 24 h
Beetroot	75% ethanol
Carrot	Methanol+Ethanol+Water+ HCl

2.4 Antioxidant capacity estimation

Antioxidant activity was estimated by Ferric Reducing Antioxidant Power (FRAP) method. Ferric-reducing antioxidant power was measured following the procedure originally described by Benzie and Strain (1996), in which Fe^{3+} to Fe^{2+} ion reduction, at low pH, causes the formation of a coloured ferrous-TPTZ(2,4,6 tripyridyl-s-triazine) complex, resulting in an increase in absorbance at 593 nm. Samples in the range of 2 to 10 µl were added to 900 µl of FRAP reagent. FRAP reagent consists of the following

1) 0.3 M acetate buffer, pH 3.6
2) 10 mM TPTZ in 40mM hydrochloric acid
3) 20 mM ferric chloride in 40 mM hydrochloric acid.

All the above were mixed in the ratio of 10:1:1 (v/v/v) to obtain FRAP reagent. The reagent was preheated to 38°C±2 and the initial absorbance was measured using acetate buffer blank. The reaction mixture was shaken vigorously for 15 sec and incubated at 27°C±2 for 90 min. The absorbance was measured at 593 nm at the end of 90 min. Control experiments without the sample or TPTZ were carried out to exclude the effect of the added test compounds. Higher absorbance indicates higher ferric reducing power. The results are expressed as Trolox equivalent reducing power.

2.5 *In vitro* bioavailability assay

In vitro bioavailability of organic and conventional vegetables was determined according to a method described by Luten *et al.* (1996).

2.5.1 Reagents

Pepsin 1.6 mg of pepsin dissolved in 0.1M HCl and volume made up to 10 ml. Pancreatin bile extract mixture: 160 mg of pancreatin and 1 g of bile extract dissolved in 0.1 N $NaHCO_3$ and made up to 40 mL.

2.5.2 Procedure

20 g of the sample was taken, to which150 ml water was added and pH was adjusted to 2.0 using 6 M HCl. 3 ml of pepsin solution was added and made up to 300 ml using triple distilled water. It was incubated in water for 2 h in shaking water bath at 120 rpm at 37°C±2. Then 20 g each of the sample was taken in 4 conical flasks. Three conical flasks were kept in freezer(-4°C±2) and to one conical flask; 5ml of pancreatin bile extract mixture was added and titrated with 0.5 M NaOH till pH 7.5 is reached. Using this titre value the amount of $NaHCO_3$ needed for dialysis was calculated (titre value x 0.5 x 84.01). The calculated amount of $NaHCO_3$ is dissolved in 25 ml of triple distilled water and added to an activated dialysis bag (Spectrum MWCO: 12–14,000, width 32+2 mm, diameter 20.4 mm). Dialysis bag was activated using EDTA and sodium bicarbonate and boiled for 2–3 min. Three such dialysis bags per sample were prepared and one dialysis bag was inserted in each conical flask in which 20 g of pepsin digested sample was put and kept in shaking water bath at 37°C±2, 120 rpm. Solution from dialysis bag was used for analysis.

2.5.3 Statistical Analysis

Results on continuous measurements have been presented on mean ±SD. Results on categorical measurements are presented in number (per cent). Significance is assessed at 5 per cent level of significance. Statistical software SPSS 17.0 was used for analysis. Graph pad prism has been used to generate graphs.

3. Results and discussion

3.1 Total Antioxidant Capacity before and after *in vitro* digestion

The percentage recovery was 39.33 for conventional spinach and 32.57 for organic spinach. So the loss of antioxidant activity was higher in case of organic spinach. It is to be noted that although the recovery of antioxidants after digestion was low in case of organic spinach, the antioxidant capacity was still higher after digestion compared to that of conventional spinach.

Percent recovery of antioxidants after *in vitro* digestion was 20, 30 and 6.25 per cent higher in conventional spinach, beetroot, and tomato respectively compared to their respective organic samples. There was no significant difference between organic and conventional carrot samples. There was a higher recovery of antioxidants from conventional samples than organic samples.

A study on *In vitro* gastrointestinal digestion of pomegranate juice has shown that 22% of anthocyanins, and 29% of phenolic compounds were recovered after digestion (Perez-Vicente, A. *et al*., 2002), whereas McDougall *et al* (2005) have found that the percent recovery of anthocyanins and phenols from red wine was 34.1% and 39.7% respectively. A study on *in vitro* bioaccessibility of carotenoids and tocopherols from fruits and vegetables (liquat, Orange and broccoli) has found that more than 70% carotenoids and tocopherols were recovered after digestion. (Granado-Lorencio *et al.,* 2007). Argyari *et al.* (2006) found that the co digestion of red wine (fortified with iron) with vitamin C and meat resulted in an increase and a decrease respectively in the antioxidant capacity and total phenol content.

It can be concluded that the difference between the digestibility of organic and conventional samples varies from one vegetable to another. It might depend on the individual components as well as the synergistic relation between various components that contribute to the total antioxidant capacity. This is an initial *in vitro* study used for estimation of total antioxidant capacity. An extensive *in vitro* bioavailability studies are required to know if there is a positive correlation between *in vitro* digestibility and *in vivo* absorption studies in case of total antioxidant capacity to draw any conclusion.

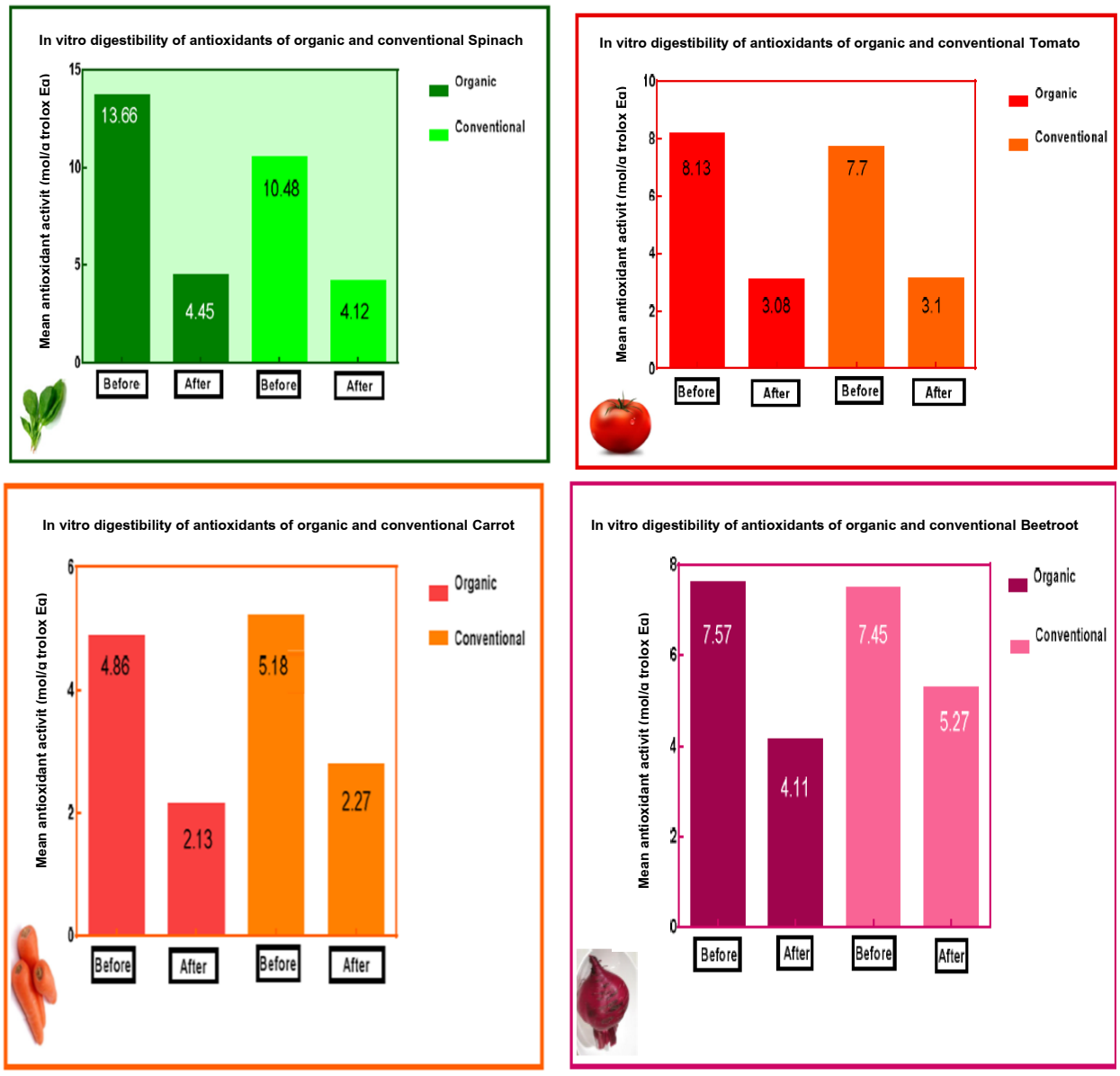

Figure 2 *In vitro* digestibility of selected organic and conventional vegetables

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