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Highlights

▶ A concentrated shot of beetroot juice has a high total antioxidant capacity (TAC) and polyphenol (TP) content. ▶ TAC and TP are increased significantly ($P < 0.01$) following in vitro digestion. ▶ The beetroot shot (70 mL) provides a convenient method of increasing polyphenol consumption. ▶ Antioxidants from the beetroot shot are more bioaccessible than those from other vegetable juices.

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Short communications

A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants

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ABSTRACT

The total antioxidant capacity (TAC) and total polyphenol (TP) content of a beetroot juice shot (70 mL) was examined following in vitro digestion. TAC was assessed using the ferric reducing antioxidant power (FRAP) assay and TP content was measured using the Folin Ciocalteu (FC) method (measured as gallic acid equivalents (GAE) before and after an in vitro digestion procedure with simulated gastric (GAS) and duodenal (DUO) phases. The beetroot shot had a high TAC (697.9 ± 1.6 μmol/70 mL) and TP content (68.4 ± 0.3 mg GAE/70 mL). FRAP values increased approximately 3-fold after GAS (2361.2 ± 20.9 μmol/70 mL) and remained high following DUO (1740.3 ± 21.1 μmol/70 mL). TP content increased 5-fold following GAS (341.6 ± 4.8 mg GAE/70 mL) and remained 3.3-fold higher following DUO (223.2 ± 5.4 mg GAE/70 mL). The beetroot shot delivers a high amount of bioaccessible antioxidants and may be a cost effective and convenient method of increasing antioxidant status.

1. Introduction

The balance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), collectively termed 'RONS', and the protective mechanisms against them is considered important in preserving good health (Valko et al., 2007). Excess RONS production, termed 'oxidative stress', is implicated in the pathology of a number of serious disease states such as cardiovascular disease (Heitzer, Schlinzig, Krohn, Meinertz, & Munzel, 2001), cancer (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006), and neurological decline (Jomova, Vondrakova, Lawson, & Valko, 2010) and can affect cell signalling (Hensley, Robinson, Gabbita, Salsman, & Floyd, 2000). Consumption of natural produce which is rich in antioxidant compounds may help to redress the balance between RONS production and endogenous protection when the body is under oxidative stress. A number of biological RONS such as superoxide anion ($O_2^{-\cdot}$), hydroxyl radical ($^{\cdot}OH$), nitric oxide (NO) and the peroxyl radical ($ONOO^-$) have been shown to mediate redox-dependant signalling pathways (Hensley et al., 2000).

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Abbreviations: FRAP, ferric reducing antioxidant power; FC, Folin Ciocalteu; GAS, gastric phase of in vitro digestion; DUO, duodenal phase of in vitro digestion; RONS, reactive oxygen and nitrogen species; GAE, gallic acid equivalents; TAC, total antioxidant capacity; TP, total polyphenols

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Antioxidants, both endogenous and exogenous are responsible for regulating these actions by 'turning off' this pathway when it is not beneficial.

Additionally, RONS can cause damage to lipid membranes (through lipid peroxidation), proteins (through S-nitrosylation) and DNA (through nucleotide oxidation). Antioxidants can directly inhibit the damage caused by these proliferative reactions by donating the necessary electron to stabilise RONS without becoming reactive in turn (Valko et al., 2007). In some disease states (e.g., cancer), where the production of endogenous antioxidants including superoxide dismutase (SOD) is disrupted, exogenous antioxidant consumption may become more important in oxidative stress regulation (Valko, Izakovic, Mazur, Rhodes, & Telser, 2004). Thus the balance between the required RONS production for normal cell signalling, and undesirable excess production leading to substrate damage, is critical.

Beetroot juice contains a high level of biologically accessible antioxidants (Wootton-Beard, Moran, & Ryan, 2011) as well as many other health promoting compounds such as potassium, magnesium, folic acid, iron, zinc, calcium, phosphorus, sodium, niacin, biotin, B6 and soluble fibre. Additionally, drinking beetroot juice provides a more convenient alternative to consuming the whole vegetable. The specific interest in beetroot juice has arisen because it is a rich source of a number of polyphenolic compounds (Kaur & Kapoor, 2002; Pitalua, Jimenez, Vernon-Carter, & Beristain, 2010).

Beetroot predominately contains pigments called betalains, a class of betalamic acid derivatives which are composed of betacyanins and betaxanthins (Pitalua et al., 2010), and a number of phenolic compounds. The betalain and phenolic composition of red beetroot has been studied in detail by Kujala, Loponen, Klika, and Pihlaja (2000) and Kujala, Vienola, Klika, Loponen, and Pihlaja (2002). The first of these studies measured the effects of cold storage on the two main betacyanins; betanin and isobetanin, and the ferulic acid ester; β-d-fructofuranosyl-α-d-(6-O-(*E*)-feruloylglucopyranoside) (Kujala et al., 2000). The structures of these compounds are shown in Fig. 1. A further study by this research group isolated a number of other compounds in four beetroot cultivars using high-performance liquid chromatography- electrospray ionisation-mass spectrometry (HPLC–ESI–MS); Table 1 shows the predominant betalain and phenolic constituents. Betanin was shown to be the most abundant betalain in the flesh and peel of each cultivar whilst 5,5′,6,6′-tetrahydroxy-3,3′-biindolyl, feruloylglucose and β-d-fructofuranosyl-α-d-(6-O-(*E*)-feruloylglucopyranoside) were all present in higher quantities in the peel compared to the flesh. Vulgaxanthin I (shown in Fig. 1) and II were the predominant betaxanthins (Kujala et al., 2002). There are also other varieties of beetroot where the ratio of betacyanins to betaxanthins changes which is reflected in the colour of the root. Betanin, together with its aglycone betanidin have been independently shown to have high antioxidant activity (Butera et al., 2002; Tesoriere, Butera,

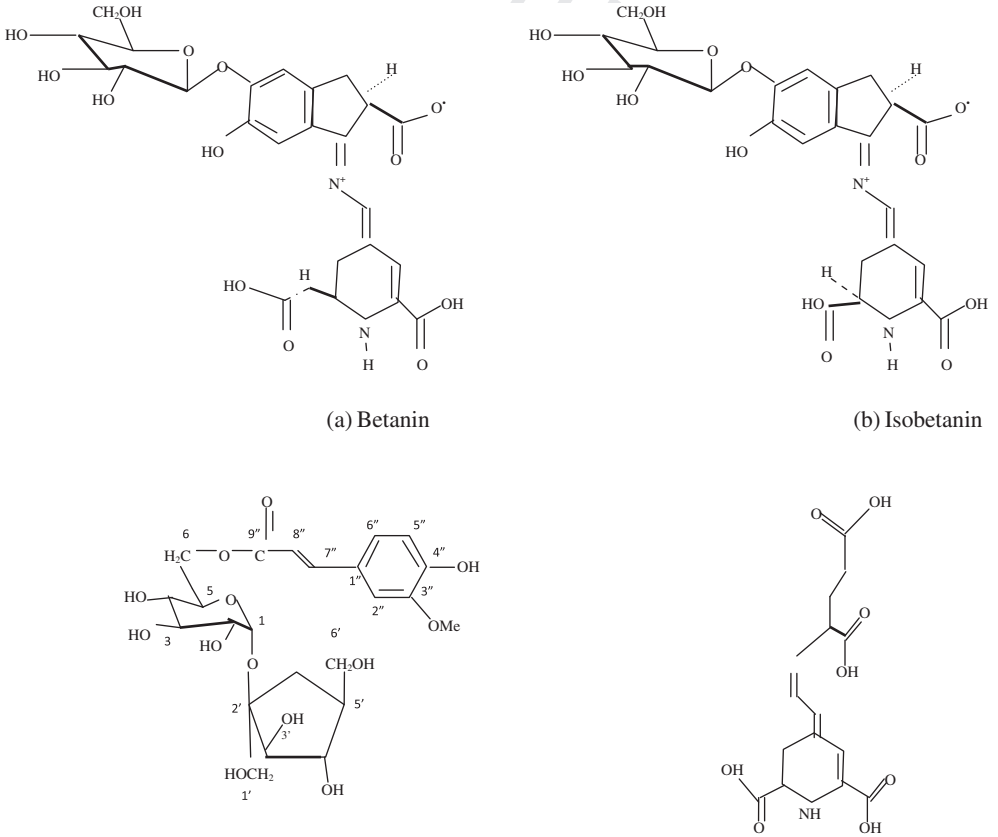

Fig. 1 – Structures of Betanin, Isobetanin, β-d-fructofuranosyl-α-d-(6-O-(*E*)-feruloylglucopyranoside) and Vulgaxanthin I.

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Table 1 – Reported phytochemical composition of red beetroot, adapted from Kujala et al. (2002).

Classification	Compound
Betalains	
Betaxanthins	Vulgaxanthin I
	Vulgaxanthin II
Betacyanins	Betanin
	Isobetanin
Phenolics	
Ferulic acid conjugates	5,5′,6,6′-tetrahydroxy-3,3′-biindolyl
	Feruloylglucose
	β-d-fructofuranosyl-α-d-(6-O-(*E*)-feruloylglucopyranoside)
Phenolic amides	*N-trans*-Feruloyltyramine
	N-trans-Feruloylhomovanillylamine
Flavonoids	Betagarin
	Betavulgarin
	Cochliophilin A
	Dihydroisorhamnetin

Pintaudi, Allegra, & Livrea, 2004). Beetroot also contains smaller amounts of other compounds, such as carotenoids and ascorbic acid which may further increase its total antioxidant capacity.

Convenience is considered a considerable marketing tool in the food industry (Drewnowski & Darmon, 2005), and conscientious food manufacturers are looking for ways to make healthy food, particularly fruit and vegetables, more convenient to consume in order that public health may be improved. There has been a significant increase in the number of fruit based beverages which are available in UK commercial outlets over the past decade and they have become an important method of fruit intake, particularly for children. By examining the shelves in local supermarkets it is clear to see that vegetables juices are beginning to expand in the same way. Fruit and vegetable blends in particular appear to have become more popular, given the emergence of brands such as V8 (Campbell Foods, Belgium), Cawston Press (Wokingham, UK) and Sunraysia (Sunraysia UK Ltd, London, UK). Vegetable juices are considered to be a viable method of bridging the gap between actual and observed vegetable intake (Shenoy et al., 2010). Recent research in our laboratory has aimed to identify the antioxidant content of a number of these juices in order that their contribution to dietary intake can be quantified (Wootton-Beard et al., 2011). Following this work, beetroot juice was found to be a particularly rich source of antioxidants and polyphenols as measured using a variety of biochemical methods before and after in vitro digestion. However, beetroot juice consumption may not be as popular as other fruit and vegetable juices such as tomato, carrot, apple or mango, perhaps due to perceived issues of taste, texture and urinary colouration. In reality beetroot juice has a relatively pleasant taste in comparison with other vegetable juices due to its relatively high sugar content (Thakur & Das Gupta, 2006). A small increase in the habitual consumption of polyphenol-rich beverages such as beetroot juice may have a significant positive effect on public health. Recently, a number of more convenient vegetable juice products have been created to try to increase consumption, including popular brands of mixed vegetable juice which are available in cans and small bottles (V8, Campbell Foods, Belgium). Additionally a beetroot juice 'shot' (70 mL) has been developed (James White Drinks, Ipswich, UK). This particular product offers an opportunity for beetroot juice to be consumed with ease and convenience by the general public and may contribute positively toward increasing consumption of polyphenol rich produce.

2. Materials and methods

2.1. Materials and sample preparation

All chemicals were of analytical grade and were purchased from Sigma Aldrich (Poole, UK). Beetroot juice shots were provided by James White Drinks Ltd, Ipswich, UK. The beetroot shot was analysed in its original form. In all experiments, the beverages were prepared using a standard protocol. Amber bottles were used throughout to prevent the photodecomposition of antioxidants and efforts were made to exclude oxygen contact with the samples. All experiments were carried out on a minimum of three separate occasions. Samples were analysed in triplicate for each experiment.

2.2. Determination of total antioxidant capacity and total polyphenols

The total antioxidant capacity of the samples was determined using a modification of the FRAP assay of Benzie and Strain (1996). Briefly FRAP reagent was prepared from 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM 4,6-tripryridyl-s-triazine (TPTZ) made up in 40 mM HCl. All three solutions were mixed together in the ratio 10:1:1. The FRAP assay was performed by warming 1 mL of dH_2O to 37 °C before adding 25 μL of sample and 1 mL of reagent and incubating at 37 °C for 4 min. Absorbance at 593 nm was determined relative to a reagent blank also incubated at 37 °C. The total antioxidant capacity of samples was determined against a standard of known FRAP value, ferrous sulphate (1000 μM). Total polyphenol content was determined using the Folin Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). The beetroot juice aliquot (0.2 mL) was added to 1.5 mL of freshly prepared Folin Ciocalteu reagent (1:10, v/v, with water). The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 mL of 60 g/L sodium carbonate solution. After incubation at room temperature for 90 min, the absorbance of the mixture was read at 725 nm using the respective solvent as blank. The results were expressed as mg of gallic acid equivalents (mg GAE).

2.2.1. In vitro digestion procedure

Analysis was repeated following in vitro digestion. The in vitro digestion model was adapted from Ryan, O'Connell, O'Sullivan, Aherne, and O'Brien (2008). Beetroot juice samples were transferred to clean amber bottles and mixed with Hanks' balanced salt solution (with $NaCO_3$, without phenol

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Table 2 – Total antioxidant capacity and total polyphenol content of the beetroot juice shot and other beverages.

Product	FRAP (μmol)		FRAP post digestion (μmol)		TP (mg GAE)		TP post digestion (mg GAE)	
	Per L	Per serving	Per L	Per serving	Per L	Per serving	Per L	Per serving
James White Beetroot Shot	9971 ± 22	697.9 ± 1.6	24,862 ± 300*	1740.3 ± 21.1*	977.2 ± 5.2	68.4 ± 0.3	3189.1 ± 77.3*	223.2 ± 5.4*
James White Beetroot Juice[a]	8354 ± 84	584.8 ± 5.9	12,152 ± 336	850.6 ± 23.5	1450.3 ± 42.1	101.5 ± 2.9	1527.1 ± 18.0	106.9 ± 1.3
V8 Vegetable Juice[a]	2573 ± 48	180.1 ± 3.4	3488 ± 223	244.2 ± 15.6	617.8 ± 15.6	43.2 ± 1.1	1053.9 ± 6.4	74.7 ± 0.5
V8 Fruit and Vegetable Juice[a]	2060 ± 49	144.8 ± 3.4	4986 ± 202	349.0 ± 10.3	537.8 ± 17.9	37.6 ± 1.3	1097.4 ± 7.5	76.8 ± 0.5
Del Monte Tomato Juice[a]	2202 ± 39	154.1 ± 2.8	3071 ± 50	215.0 ± 3.6	695.3 ± 19.3	48.7 ± 1.4	1120.1 ± 12.2	78.4 ± 0.9
Eden Organic Carrot Juice[a]	1516 ± 11	107.3 ± 0.78	2731 ± 27	191.9 ± 1.5	473.7 ± 7.7	33.2 ± 0.5	1022.2 ± 4.8	71.6 ± 0.3

* Significantly increased compared to the juice prior to digestion, $P < 0.01$. FRAP = Ferric reducing antioxidant power. TP = Total polyphenols using Folin Ciocalteu method. GAE = Gallic acid equivalents. One serving represents a volume of 70 mL, equal to the total volume of the beetroot juice shot.

a Data adapted from Wootton-Beard et al. (2011), values are given for comparative purposes and are not included in the statistical analysis for this experiment. Results are expressed as mean ± SEM of three experiments each performed in triplicate. 'Post digestion' refers to the levels following the duodenal phase of the in vitro digestion procedure (Section 2.3).

red, sterile filtered, Sigma Aldrich, Poole, UK) to create a final volume of 20 mL. The samples were acidified to pH 2.0 with 1 mL of a porcine pepsin preparation (0.04 g pepsin in 1 mL 0.1 M HCl) and incubated at 37 °C in a shaking water bath at 95 rpm for 1 h. After gastric digestion, a 500 μl aliquot of each sample was stored at −20 °C. The pH was then increased to 5.3 with 0.9 M sodium bicarbonate followed by the addition of 200 μL of bile salts glycodeoxycholate (0.04 g in 1 mL saline), taurodeoxycholate (0.025 g in 1 mL saline), taurocholate (0.04 g in 1 mL saline) and 100 μL of pancreatin (0.04 g in 500 μL saline). The pH of each sample was increased to 7.4 with 1 M NaOH. Samples were incubated in a shaking water bath (95 rpm) at 37 °C for 2 h to complete the intestinal phase of the in vitro digestion process. After the intestinal phase, a 500 μL aliquot of each sample was stored at −20 °C, samples were analysed within 48 h.

2.3. *Data analysis*

All data are presented as means (±SEM) of at least three independent experiments ($n = 3$), each experiment had a minimum of three replicates of each sample. For comparisons between samples, data was analysed by ANOVA and Tukey's multiple comparison test (SPSS, version 17). Statistical significance was set at $P < 0.01$.

3. Results and discussion

The beetroot juice shot delivers a significant amount of antioxidants in a small, convenient volume as measured by the FRAP assay (697.9 ± 1.6 μmol/70 mL) (Table 2). The TAC of the shot increased significantly ($P < 0.01$) following the in vitro digestion procedure (1740.3 ± 21.1 μmol/70 mL) despite a decrease in TAC between the gastric (2361.2 ± 20.9 μmol/70 mL) and duodenal phases. The differences observed between the gastric and duodenal phase of digestion were in agreement with previous studies (Bermudez-Soto, Tomas-Barberan, & Garcia-Conesa, 2007; Wootton-Beard et al., 2011). In both of these studies the greatest increase in FRAP was seen following the gastric phase with a marked decrease after the duodenal phase, which may result from biotransformation of antioxidants caused by interaction with the enzymes present in the system (Ryan & Prescott, 2010). The increase in FRAP observed for the beetroot juice shot, following the individual phases of digestion, was proportionally much higher than that of other vegetable juices, most notably the other beetroot juice product produced at the same location, suggesting differences in the processing conditions between the two products. Both beetroot juice products started at relatively similar values prior to digestion and despite significant increases in both, the shot produced a particularly large increase in TAC following both the gastric and duodenal phases, around 2-fold higher than the standard product (Table 2). These results clearly demonstrate that antioxidants contained in this particular product become more accessible following digestion than those in other vegetable juice products.

The in vitro digestion model gives an indication as to the bioaccessibility of vegetable juice antioxidants in a biological system, since the model is designed to simulate in vivo digestion. Bioaccessibility specifically refers to the amount of antioxidants which are potentially presented to the intestinal brush border for absorption. It differs significantly from bioavailability which refers to the amount of antioxidants which pass this intestinal border and are available for use. Furthermore the model omits the colonic phase of the digestive process. Bioaccessibility can however, be used to suggest the rank order of products, and infers that if more antioxidants are presented to the intestinal brush border it is likely that more will be absorbed.

Additionally, Beetroot juice compares favourably with other well accepted high-antioxidant products such as pomegranate and cranberry juices, particularly in terms of bioaccessibility. Research from our laboratory has shown that pomegranate and cranberry juice (both fresh and long life), display FRAP values of 16,789–20,063 μmol/L and 8419–8570 μmol/L respectively, after the same in vitro digestion procedure (Ryan & Prescott, 2010). This is significantly lower

than the value observed for the beetroot juice shot following digestion (24,862 ± 300 μmol/L).

The high TAC of the beetroot juice shot can be largely attributed to its high polyphenol content. The shot was a concentrated source of dietary polyphenols as measured by the FC method (68.4 ± 0.3 mg GAE/70 mL) (Table 2). In similarity with the trends observed in the FRAP assay, the TP content increased 5-fold following the gastric phase of the in vitro digestion to 341.6 ± 4.8 mg GAE/70 mL. Following the duodenal phase the TP content was still 3.3-fold higher than the level prior to digestion (223.2 ± 5.4 mg GAE/70 mL). The term polyphenol refers to a particularly wide range of compounds found almost ubiquitously amongst plant foods, in order to be classified as a polyphenol, a compound must simply contain one or more aromatic rings and at least two hydroxyl groups according to a recent review by Sies (2010). Despite such a broad classification, Sies (2010) comments that any compound matching these criteria can be considered biologically interesting. With criteria such as these it becomes ever more important to quantify the total polyphenolic content of food products, since all polyphenolic compounds contained in a product may contribute to health. In beetroot, both its pigment and its polyphenol content are related to betanin and indicaxanthin concentrations.

Opinions had once focussed on polyphenols primarily as antioxidants with a direct action against RONS, and to a degree this may well be the case. However, due to the relatively low concentrations of free circulating polyphenols found in the body amongst other factors such as biotransformation and a lack of clinical data from intervention trials, researchers have begun to focus on other mechanisms to define the health benefits inferred by increasing fruit and vegetable consumption. In recent years polyphenols, have been shown to inhibit alpha-glucosidase/maltase, as well as potentially stimulating insulin secretion which may reduce the absorption of glucose into the blood stream, having profound implications for diabetes management (McDougall & Stewart, 2005). Additionally, they have been shown to display important indirect functions, acting as a 'switch' in redox-dependant signalling pathways and affecting the pro-inflammatory effects of RONS through such actions as the modulation of nuclear factor-kappa B activation (Rahman, Biswas, & Kirkham, 2006).

4. Conclusion

Given the potential for the multiple health benefits of polyphenol consumption, beverages containing high levels can be considered a positive addition to the diet. Additionally, both sensory characteristics and convenience would appear to be important factors in obtaining an increased level of consumption amongst the general public. Beetroot juice provides a significant source of dietary polyphenols and in particular, a shot can provide a significant quantity of these bioactive components together with a convenient method for consumption.

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