Effect of in vitro digestion on antioxidant capacity of pomegranate co-products extracts

O.A. Fawole¹, U.L. Opara²

1- Department of Horticultural Science, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa
   Phone: (+27 21) 808 9243 – e-mail: (olaniyi@sun.ac.za)
2- Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Horticultural Science, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa
   Phone: (+27 21) 808 4064 – e-mail: (opara@sun.ac.za)

ABSTRACT –
From value-addition viewpoint, the aim of this study was to evaluate the stability of antioxidant capacity of pomegranate fruit co-products in different solvent extracts using the FRAP, DPPH and ABTS + assays during in vitro digestion. Antioxidant capacity in the investigated assays were in the order of peel > marc > juice throughout the in vitro digestion irrespective of the extraction solvents used. Peel extracts showed between 5 to 75-fold of antioxidant capacity than juice and marc extracts. Interestingly, contrary to the anti-radical activities measured by DPPH and ABTS assays, the FRAP values increased significantly in gastric phase of digestion for all the extracts followed by 10 to 26% decrease in the duodenal digests. Findings from this study also showed that pomegranate antioxidant during in vitro digestion may not reflect the pre-digested antioxidant capacity. Thus, it is important to provide biologically relevant information that reflect antioxidant activity after in vitro digestion.

KEYWORDS: Marc, Ethanol, polyphenols, pepsin, value-addition

1. INTRODUCTION

The antioxidative phytochemicals, especially phenolic compounds, found in pomegranate (Punica granatum L.) fruit have received increasing attention for their potential role in the prevention of human diseases (Gil et. al. 2000). The phenolic content of pomegranate is usually influenced not only by the cultivar, but also depends on the fruit fraction (Fisher et al., 2011). Phenolic compounds such as ellagitannins, punicalagin and punicalin are found in the juice however, most of the phenolic compounds are mainly located in the fruit peel and mesocarp (Fisher et al., 2011). Although very few industrial processing techniques allow the introduction of pomegranate peel extract into the juice, most juice extraction techniques involve direct disposal of co-products of commercial juicing as waste or for limited purposes such as cattle or pig feed (Qu et al., 2010). The co-products include fruit peel and the residual material (seed and aril membrane) called marc. Often times, disposal of these co-products represent a problem for management, contamination, and environmental issues. Interestingly, phenolic compounds such as punicalagins contained in pomegranate peel, when released into the juice, gives the outstanding antioxidant activity and strongly influence the nutritive value of the juice and are wholly or partially responsible for possible therapeutic effects observed in some commercial pomegranate juice (Gil et. al. 2000).

From the agro-industrial and health perspectives, the co-products obtained from pomegranate juice processing contain high levels of polyphenols with high added values. Therefore, the recovery of valuable compounds from these co-products are beneficial. There has been extensive research into the antioxidant capacity of different co-products of pomegranate fruit and co-products (Qu et al., 2010). While it may be useful to know the antioxidant capacity of pomegranate co-products relative to the juice

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before digestion for comparative purposes, this is not a true reflection of the potential health benefits. A more realistic view is the antioxidant capacity after a given sample has undergone a simulated in vitro digestion procedure, when the antioxidants potentially available for absorption can be measured (Ryan and Prescott, 2010).

The potential availability of antioxidants expressed as antioxidant capacity after digestion is an important initial measure. For instance, previous studies have shown that the bioavailability of certain phenolic compounds in pomegranate and orange juices is poor, resulting in limited effect on health (Gil-Izquierdo et al., 2001). Hence, the most important factors in determining the potential beneficial effects of polyphenols on the gut epithelial cells are their stability under gastro-intestinal conditions.

From value-addition viewpoint, the aim of this study was to assess changes in antioxidant capacity of pomegranate co-products using the FRAP, DPPH˙ and ABTS˙+ assays during simulated in vitro digestion.

2. MATERIALS AND METHODS

2.1. Fruit Procurement

Pomegranate fruit (cv. Kessari) were harvested from an orchard in Ladismith (33°29′S 21°16′E) Western Cape, South Africa. Harvested fruit at commercial maturity were transported to the postharvest laboratory at Stellenbosch University. In triplicates, ten healthy fruit were washed to remove sand or dirty particles. Peel fraction was obtained after manual peeling, juice from extracted arils using a blender (Mellerware, South Africa), and the resultant residue from juicing was collected as the marc. Fruit juice was kept in clean jars and stored at −20°C while peel and marc were freeze-dried. Dried samples were ground into powder and stored in airtight containers at 7°C in the dark until used.

2.2. In Vitro Digestion and Antioxidant Capacity

In vitro gastro-intestinal digestion model was adapted according to the procedure described by Ryan and Prescott (2001). The DPPH˙ assay was carried out in triplicate according to the method used by Karioti et al. (2004). ABTS˙+ radical scavenging activity of samples before and after the gastric and duodenal phases was analysed using the method as described by Thaipong et al. (2006) with minor modifications. The antioxidant power of samples at different digestion phases was carried out in triplicate according to the method of Benzie and Strain (1996).

3. RESULTS AND DISCUSSION

Prior to in vitro digestion, radical scavenging activity (RSA) reflected the levels of total phenolic concentrations in the investigated co-products, as evident by the order of peel > marc > juice (Fig.1). Overall, the result showed that irrespective of the extraction solvent all the extracts were effective in scavenging the DPPH free radical (Fig. 1). The RSA decreased by 7 – 10% in the gastric digests, with significant (p>0.05) decreases observed for water extracts (juice, marc and peel), 50% EtOH (peel) and EtOH (peel) (Fig. 1). However, in duodenal phase the ability of the extracts to scavenge the DPPH˙ radical increased significantly (p>0.05) compared to those observed in the gastric digests, ranging between 5 – 18% (Fig. 1). Interestingly, RSA was higher in extracts of duodenal digest in comparison to the radical scavenging activity exhibited by undigested extracts. Generally, radical cation scavenging activity (RCSA) reflected the order being peel > marc > juice for all undigested extracts (Fig. 2). Again, the highest RCSA was exhibited by 50% EtOH of undigested extracts and in vitro digests (Fig. 2). Amongst the co-products, peel extracts exhibited between 6 – 10-fold and 2.5 – 20-fold RCSA than
juice and marc extracts, respectively. Similar to radical scavenging activity in DPPH assay, RCSA decreased significantly (p>0.05) in the investigated extracts of gastric digests with the exception of peel 50% EtOH and EtOH extracts, which showed no significant decline. However, RCSA exhibited by extracts of duodenal digests was higher than those exhibited by undigested and gastric digests. The main highlight was observed in all marc extracts where between 5 and 75-fold radical cation scavenging activity was observed (Fig. 2). As observed in the anti-radical activities (DPPH˙ and ABTS˙+), the reducing antioxidant powers of the investigated extracts of pomegranate co-products were consistent with the total phenolic concentrations measured in undigested extracts (Fig. 3). Overall, 50% ethanol and water extracts showed higher reducing power than ethanol extracts (Fig. 3). Furthermore, peel extracts showed between 5 to 30-fold reducing power than juice and marc extracts, with the activity again in the order of peel > marc > juice. Interestingly, contrary to the anti-radical activities measured by DPPH and ABTS assays, the FRAP values increased significantly in gastric phase of digestion for all the extracts, perhaps as a result of the observed increase in phenolic concentration at this phase. However, the reducing powers decreased significantly (p>0.05) by 10 to 26% in the duodenal digests. Albeit, FRAP values remained relatively higher in duodenal phase compared to undigested extracts (Fig. 3). This study has demonstrated that bioaccessible phenolics were able to reduce or scavenge free radicals (in the DPPH and ABTS assays) during a simulated digestion. The radical scavenging activity in both gastric and duodenal phases of in vitro digestion were even higher than those exhibited before being subjected to a simulated digestion, presumably due to dependency of phenolic activity on pH of the digestion medium. For instance, high pH values (alkaline pH) have been reported to significantly increase phenolics scavenging ability (Tagliazucchi et al., 2010). It is believed that transition from acidic to alkaline environment enhances the antioxidant activity of phenolics by causing deprotonation of hydroxyl moieties present on their aromatic rings (Bouayed et al., 2011). This buttresses the report by Lee et al. (2003) that aglycones phenolics display an antioxidant power higher than their glycoside forms. Specifically, since radical scavenging activity is mainly dependent on the number and position of hydrogen-donating hydroxyl groups on the aromatic rings of the phenolic compounds (Tagliazucchi et al., 2010), it would thus be appropriate to evaluate antioxidant activity in the duodenal phase of in vitro digestion conducted in weak alkaline condition rather than in gastric acidic environment. The observed dynamics in the reducing power of the investigated extracts again could primarily be due to pH of the medium. The pH of a substance is known to affect racemization of molecules, possibly creating two chiral enantiomers with different reactivity (Wootton-Beard et al., 2011). As a result, some antioxidants could be rendered more reactive at acidic pH in the gastric phase and less reactive at alkaline pH during the duodenal phase of in vitro digestion (Wootton-Beard et al., 2011), a trend observed in this current study. In addition, polyphenols are highly sensitive to alkaline conditions and do transform into different structural forms with different chemical properties (Ryan and Prescott, 2001). Since this was observed consistently across the investigated pomegranate extracts, it would be reasonable to assume that the overall loss in reducing power (between gastric and duodenal phases) in this current study could be as a result of the above-mentioned reasons during the duodenal phase of in vitro digestion at pH 7.4 (weak alkaline). In addition, with respect to their stability, one could assume that the phenolic antioxidants in the investigated extracts responsible for ferric reduction in FRAP assay are fewer at the duodenal phase (compared to the gastric phase), transformed or impaired. Furthermore, according to Wootton-Beard et al. (2011), it could also be suggested that metabolites formed as a result of structural changes in the alkaline condition could have reacted differently in the FRAP assay.

4. Conclusion

There is a large variation in the phenolic concentration and antioxidant capacity of the investigated fruit co-products. These results suggest that pomegranate waste (peel and marc) could be considered as a source of great interest to obtain pomegranate phenolic extracts for nutraceutical and development of value-added products. Findings from this study also showed that the antioxidant...
capacity during in vitro gastro-intestinal digestion may not reflect the pre-digested antioxidant capacity. Thus, this study highlights the need to provide biologically relevant information on antioxidant activity by providing data reflecting antioxidant activity after in vitro digestion.

5. FIGURES

![Graph showing changes in radical scavenging activity (RSA) during in vitro digestion model of water, 50% ethanol and 100% ethanol extracts of pomegranate peel, marc and juice. Average values (±S.E) are presented. Bars with different letter(s), per co-product for each solvent extract, are statistically significant different (p< 0.05). AAE, ascorbic acid equivalent.]

Fig.1. Changes in radical scavenging activity (RSA) during in vitro digestion model of water, 50% ethanol and 100% ethanol extracts of pomegranate peel, marc and juice. Average values (±S.E) are presented. Bars with different letter(s), per co-product for each solvent extract, are statistically significant different (p< 0.05). AAE, ascorbic acid equivalent.
Fig. 2. Changes in radical cation scavenging activity (RCSA) during in vitro digestion model of water, 50% ethanol and 100% ethanol extracts of pomegranate peel, marc and juice. Average values (±S.E) are presented. Bars with different letter(s), per co-product for each solvent extract, are statistically significant different (p< 0.05). TE, trolox equivalent.
Fig. 3. Changes in ferric reducing antioxidant power (FRAP) during in vitro digestion model of water, 50% ethanol and 100% ethanol extracts of pomegranate peel, marc and juice. Average values (±S.E) are presented. Bars with different letter(s), per co-product for each solvent extract, are statistically significant different (p< 0.05). TE, trolox equivalent.

6. REFERENCES


