

Inhibition of protein glycation by pomegranate (*Punica Granatum*)GREENSPAN Phillip¹, PEGG Ronald² & DORSEY Pamela¹¹*University of Georgia, College of Pharmacy, Athens, USA*²*University of Georgia, College of Agricultural and Environmental Sciences, Athens, USA*Corresponding Author:

Phillip Greenspan

University of Georgia, College of Pharmacy, Athens, GA 30602, USA

Greenspn@uga.edu**Abstract**

The non-enzymatic binding of reducing sugars to proteins is responsible for the formation of advanced glycation end products (AGEs). This study investigates the effect of a whole pomegranate fruit extract and various parts of pomegranate fruit on the *in vitro* fructose-mediated glycation of albumin. Compared to apple, whole pomegranate fruit exhibited a much higher total phenolics content and antioxidant potential. Pomegranate fruit decreased glycation by 80% when incubated at a phenolic concentration of 2.5 µg gallic acid equivalents (GAE)/ml; apple, at this phenolic concentration, inhibited protein glycation by only 20%. Pomegranate membrane exhibited the highest total phenolics content and antioxidant potential compared to the pomegranate aril and peel. At 2.5 µg GAE/ml, the membrane fraction decreased glycation by 85% compared to the aril (42%), and peel (75%). The inhibitory activity was concentrated in the pomegranate membrane and is attributed to the presence of ellagitannins, which are not found in whole apple.

Introduction

Complications stemming from diabetes mellitus are initiated by the accumulation of advanced glycation end products (AGEs). A wide variety of polyphenols have been shown to inhibit the formation of AGEs; the degree of inhibition of flavonoids has been correlated with their antioxidative properties (Wu *et al.*, 2005) while other phenolic compounds can trap the AGE precursors, glyoxal and methylglyoxal (Lo *et al.*, 2011). We previously showed that polyphenols found in pomegranate juice are superior inhibitors of fructose-mediated protein glycation when compared to commonly consumed juices (Dorsey *et al.*, 2014). This suggests that the major polyphenols contained within the pomegranate are unique inhibitors of the

glycation process. The major group of polyphenols, ellagitannins, is found in the peel and membrane of the fruit (Gil *et al.*, 2000). The current study examined the effect of extracts from whole pomegranates and its various parts (i.e., the peel, membrane, arils) on the glycation of albumin mediated by fructose. Apple, which does not contain ellagitannins, was employed as a control fruit.

Materials and methods***Chemicals and reagents***

Bovine serum albumin (essentially fatty acid free), D-(-)fructose, Folin & Ciocalteu's phenol reagent, TPTZ (2,4,6-tri[2-pyridyl]-S-triazine), and Amberlite XAD-16 were purchased from the

Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Pomegranates (POM Wonderful) and apples (Red Delicious) were purchased locally from a Publix Supermarket (Athens, GA, USA).

Extraction of pomegranate and its pericarp parts

Pomegranates were cut into quarters and the arils, membrane, and peels separated. After being macerated in a Super 5000 Vitamix (Cleveland, OH, USA) for one min, the resultant slurries were subjected to a hot water extraction at 95°C at a material to solvent ratio of 1:5 (w/v) in a shaking water bath for 2 h followed by Amberlite XAD-16 chromatography. After elution of simple sugars and organic acids with water, phenolic compounds were eluted using methanol. Methanol was removed and the aqueous residue was frozen and then lyophilized. All sample extracts were dissolved in 50% ethanol.

Assays

Total phenolic content of fruit extracts was determined by the Folin-Ciocalteu method (Slinkard *et al.*, 1977). The ferric reducing antioxidant potential (FRAP method) of all fruit extracts was determined (Benzie *et al.*, 1996). The glycation of albumin was determined fluorometrically (Farrar *et al.*, 2008) at an excitation/emission wavelength pair of 370/440 nm using a PerkinElmer LS 55 luminescence spectrometer (Waltham, MA). All experiments were performed in triplicate and expressed as mean \pm SEM. Data were analyzed utilizing a one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was set at $P < 0.05$.

Results and discussion

The phenolics fraction of fruits and the components of pomegranates were freeze dried and resuspended in 50% ethanol at a

concentration of 1 mg/ml. Whole pomegranate fruit had a higher content of total phenolics than whole apple (Table 1), which is in agreement with a study by Martin and coworkers (2009). Whole apple and pomegranate fruit were also analyzed for their antioxidant capacity as determined by the FRAP assay (Table 1); pomegranate exhibited an antioxidant capacity that was approximately four times greater than that of apple.

Table 1. The total phenolics content and FRAP values of fruit extracts

Fruit	Phenolics Content (mg GAE/ ml)	FRAP Units (mmol FeSO ₄ /l)
Apple	0.39 \pm 0.01	3.6 \pm 0.1
Pomegranate	0.60 \pm 0.01	13.8 \pm 0.1
Arils	0.39 \pm 0.01	5.0 \pm 0.1
Peel	0.79 \pm 0.02	14.9 \pm 0.1
Membrane	0.81 \pm 0.02	16.0 \pm 0.1

The aril extract had the lowest phenolics content among the pomegranate parts, which is in agreement with work by Tzulker *et al.* (2007). It is noteworthy to mention that the peel and membrane extracts exhibited an antioxidant capacity that was approximately three times greater than the aril extract (Table 1). Dorsey *et al.* (2014) previously documented that the phenolics in the pomegranate possessed much greater relative antioxidant capacity than apple phenolics. To examine the effect of pomegranates and apples on protein glycation, pomegranate and apple extracts (2.5 and 5 μ g GAE/ml) were incubated in a solution containing BSA (10 mg/ml) and fructose (250 mM). When the BSA/fructose mixture was incubated in the presence of whole pomegranate extract (2.5 μ g GAE/ ml), glycation was inhibited by approximately 80% (Fig. 1); however, the same concentration of apple phenolic compounds

resulted in only a 20% decrease in glycation. Apple phenolics, at 5 μg GAE/ml, did not produce the same degree of inhibition observed

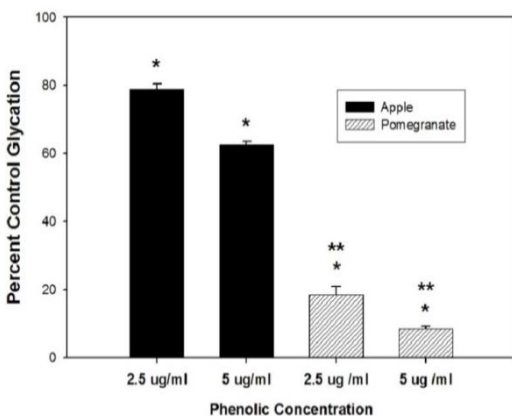


Figure 1. Inhibition of albumin glycation by whole pomegranate and apple at a concentration of 2.5 and 5 μg GAE/ml. Results represent the mean \pm SEM for triplicate determinations. * $P < 0.05$ when compared to control values; ** $P < 0.05$ when compared to apple extract

with pomegranate phenolics at 2.5 μg GAE/ml. The effect of extracts from various parts of the pomegranate pericarp (arils, peel, membrane) on protein glycation was also examined. In this study (Figure 2), the phenolics content for each component was normalized to 2.5 or 5 μg GAE/ml in the incubation mixture. All three extracts from the various components significantly inhibited protein glycation at both concentrations; yet, the membrane extract produced the greatest decrease in glycation followed by the peel extract (Figure 2). A similar inhibitory pattern was observed when

these components were incubated at the same antioxidant capacity in the glycation assay (data not shown).

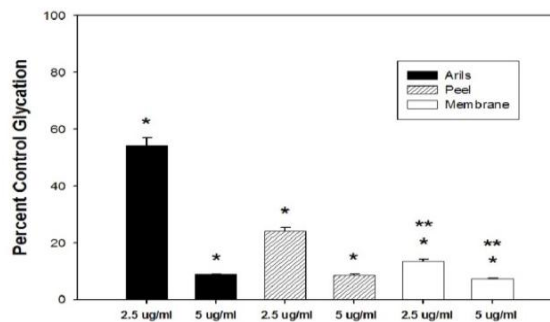


Figure 2. Inhibition of albumin glycation by components of the pomegranate pericarp (aril, peel, membrane) at a concentration of 2.5 and 5 μg GAE/ml. Results represent the mean \pm SEM for triplicate determinations. * $P < 0.05$ when compared to the control; ** $P < 0.05$ when compared to aril and peel extracts

The extract of whole pomegranate produced a greater inhibition of protein glycation when compared to a whole apple (Figure 1). The results agree with our previous research; apple juice was much less effective in inhibiting protein glycation when compared to pomegranate juice (Dorsey *et al.*, 2014). The greatest concentrations of ellagitannins are found in the membrane and peel of the pomegranate (Gil *et al.*, 2000) and these phenolic compounds afford the majority of the antioxidative properties of the fruit. Pomegranate has been extolled for many years due to its medicinal properties. The importance of ellagic acid in inhibiting protein glycation was fully documented by Muthenna *et al.* (2012). In this present study, the quite potent anti-glycative activity of the pomegranate was found to be highest in the ellagitannin-enriched membrane fraction. To slow the glycation process, current therapy relies on the lowering of plasma glucose

EXTENDED ABSTRACT

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concentrations by a variety of pharmacological agents. The inhibitory effect of phenolic compounds, such as ellagitannins, offers an alternative strategy to possibly delay the progression of diabetic complications.

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