Overview

• Introduction
• Botany
• Origin and Production: global scenario
• Phenolic composition
• Pre and post harvest factors
• Health benefits
Introduction

- Pomegranate: ‘Pome’ means apple and ‘granatum’ means seedy
- *Punica granataum* L. belongs to family *punicaceae*
- *Punicaceae* contains only one genus and two species
- *Punica protopunica* Baf.f. : another species found only in Socotra Island of Yemen

(Ed and Eric, 2007/ *HORTSCIENCE*, 42, 1087-1092)
Botany

- **Plant**: Attractive deciduous tall shrub
- **For high quality fruits**: Hot summer & mild cold winters
- **Cannot tolerate temp. below**: -12°C

Bears bright red flowers & attractive lush green foliage
Planted as ornamental shrub

(Ed and Eric, 2007/ HORTSCIENCE, 42, 1087-1092)
Origin and Distribution

- **Origin:** Pomegranate- Iran  
  (Chadha and Patel, 2007/Acta Hort. 752, 49-54)

- **Distribution:**
  - By 3000 BC- was in cultivation in Iran and Israel  
    (Goor, 1967 Economic Botany, 21, 215-230)
  - In 1500s and 1600s the Spanish brought it to central America
  - In 1700s it was in Spanish Florida and English Georgia and by 1770 in California  
    (Heber and Bowerman, 2009/ Nutrition Today: 44(4)180-184)
Cultivar ‘Wonderful’ : Most popular in United States

29,000 acres area under pomegranate cultivation in San Joaquin Valley of California

(Holland. et al., 2008/ Horticultural Reviews 35, 127-191)

(http://www.agmrc.org/commodities__products/fruits/pomegranates_profile.cfm)
Parts of Fruit

- Peel
- Seed
- Membrane
- Aril

Products
- Juice
- Cosmetics
- Nutraceuticals
Questions.....?

• What are the major phytochemicals present in pomegranate?
• Name any other fruit which have edible arils?
Bioactives reported in pomegranate

- **Anthocyanins**: 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin
- **Phenolic acids**: punicalagin, punicalin, gallic acid, ellagic acid, citric acid and ascorbic acid
- **Tannins**: ellagitannins and gallotannins
Chemical structures

Anthocyanin Biosynthesis

CO₂ → Photosynthesis → Acetate pathway (C₂ Unit)

Shikimic acid pathway → Protein production, etc.

- Phenylalanine → Phenylalanine-ammonia lyase (PAL)
- Cinnamic acid → Cinnamic acid 4-hydroxylase

3 Malonyl CoA (3-carbon unit) → p-Coumaric acid

Coenzyme A (CoA) → p-Coumaric acid-CoA ligase

- CO₂ → CoA → Chalcone synthase (CHS)
- Chalcone isomerase (CHI)

- Oxidases, etc. → Anthocyanin

Jack Sullivan, March 14, 1998
Anthocyanins

**Figure 2:** Structures of anthocyanidins and anthocyanins that were studied.

- **Aglycone**
- **Glucosides**

<table>
<thead>
<tr>
<th>Anthocyanidins &amp; Anthocyanins</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelargonidin</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Peonidin</td>
<td>OMe</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Malvidin</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
</tr>
</tbody>
</table>

| Pelargonidin-3-galactoside    | H  | H  | galactose |
| Cyanidin-3-galactoside        | OH | H  | galactose |
| Cyanidin-3-rutinoside         | OH | OH | rutinoside |
| Cyanidin-3-glucosylrutinoside | OH | OH | glucose-rutinoside |
| Delphinidin-3-galactoside     | OH | OH | galactose |

Ellagic acid

- **Free form:** ellagitannins (EA)- Punicalagin
- **Bound form:** EA glycosides
- **Punicalagin:** accounts for >50% of the juice’s antioxidant activity

» PJ > PT > punicalagin > EA
Factors influencing phenolic content

- **Pre harvest factors**
  - Cultivars
  - Environmental
  - Maturity
- **Post harvest factors**
  - Processing
  - Packing
Cultivars

- A. Rosh Hapered
- B. PG 127-28
- C. PG 116-17
- D. PG. 118-19
- E. Wonderful
- F. Shani Yonay
- G. Kamel
- H. PG. 128-29
- I. Emek

- Different stages of fruit color development in three cultivars in Israel

(Holland. et al., 2008/ Horticultural Reviews 35, 127-191)
## Maturity

### Chemical composition of pomegranate (*Punica granatum* L.) fruit seeds

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Stages</th>
<th>Unripe fruits(^a)</th>
<th>Half-ripe fruits(^a)</th>
<th>Full-ripe fruits(^a)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture %</td>
<td></td>
<td>79.45 ±0.96(^a)</td>
<td>80.66 ±3.39(^a)</td>
<td>77.72 ±3.36(^a)</td>
<td>79.28 ±2.89(^a)</td>
</tr>
<tr>
<td>2</td>
<td>Protein (%)</td>
<td></td>
<td>3.99 ±0.4(^a)</td>
<td>3.74 ±0.89(^a)</td>
<td>4.45 ±0.68(^a)</td>
<td>4.06 ±0.26(^a)</td>
</tr>
<tr>
<td>3</td>
<td>Fat (%)</td>
<td></td>
<td>0.2 ±0.0(^a)</td>
<td>0.01 ±0.0(^a)</td>
<td>0.25 ±0.3(^a)</td>
<td>0.15 ±0.10(^a)</td>
</tr>
<tr>
<td>4</td>
<td>Saturated</td>
<td></td>
<td>18.6 ±3(^a)</td>
<td>17.9 ±3.6(^a)</td>
<td>16.4 ±2(^a)</td>
<td>17.5 ±1.02(^a)</td>
</tr>
<tr>
<td>5</td>
<td>Unsaturated</td>
<td></td>
<td>81.6 ±4.26(^a)</td>
<td>82.1 ±2.1(^a)</td>
<td>84.6 ±2.5(^a)</td>
<td>82.8 ±17.96(^a)</td>
</tr>
<tr>
<td>6</td>
<td>Ascorbic acid (mg/100 g)</td>
<td></td>
<td>0.26 ±0.07(^a)</td>
<td>0.25 ±0.14(^a)</td>
<td>0.18 ±0.32(^a)</td>
<td>0.23 ±0.11(^a)</td>
</tr>
<tr>
<td>7</td>
<td>Phenolic compounds (mg/100 g)</td>
<td></td>
<td>3.65 ±0.17(^a)</td>
<td>3.22 ±0.72(^a)</td>
<td>1.90 ±0.57(^a)</td>
<td>2.92 ±0.19(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Means of 20 fruits in each row followed by different letters are significantly different (*P* < 0.05); S.D., ± standard deviation.
Maturity: phenolics & anthocyanins

Fig. 6. Total phenolics of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leq 0.05$) compared to previous sample.

Fig. 5. Total anthocyanin pigment content of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leq 0.05$) compared to previous sample.

**Figure 1.** Evolution of total anthocyanin concentration in juice of the “Assaria” pomegranate fruits, during storage at 5°C, quantified by comparison with an external standard of cyanidin 3-rutinoside (Apin Chemicals, UK). Total amount of anthocyanins in the samples was calculated as the sum of the mean of individual pigments.
Heat treatment

Figure 1. Total antioxidant activity (a) and total phenolics content (b) in arils during cold storage + 3 days at 20 °C (SL) of control and heat-treated pomegranates.

J. Agric. Food Chem. 2006, 54, 8495-8500
Antioxidant Potential

- Ability to scavenge free radicals
- Different methods for determining:
  - ORAC, TRAP, IOU
  - TEAC, FRAP, DPPH
  - TOSC
- What do these values really mean?
- Can we compare values from different assays?
Antioxidant activity

- Higher antioxidant activity than wine, tea and water extract of husk
- 1 & 2 experimental pomegranate juice (lab)
- 3 & 4 commercial juice

Figure 1. Antioxidant capacity of pomegranate juice.

Bioavailability

• **Case Study:**
  - 800 mg pomegranate extract (300 mg punicalagins, 22 mg ellagic acid)
  - 11 healthy volunteers
  - Samples drawn 0.5, 2, 24 hours after consumption

• **Results:**
  - Ellagic acid, urolithins, glucuronides found in plasma
  - AOX capacity increased by 32% 0.5 hours postprandial

• Similar conclusions were drawn from 2 subsequent studies
• Individual variability plays a role in absorption

Mertens-Talcott, S., et al.; 2006

![Graph showing plasma concentrations of EA](image1)

![Graph showing antioxidant capacity](image2)
Bioavailability

Figure 2. Chromatograms of anthocyanins before and after pancreatin bile salt digestion. Numbers are as in Figure 1.

1, delphinidin 3,5-diglucoside; 2, cyanidin 3,5-diglucoside; 3, pelargonidin 3,5-diglucoside; 4, delphinidin 3-glucoside; 5, cyanidin 3-glucoside; 6, pelargonidin 3-glucoside.
Cardiovascular Disease

- Leading cause of death in U.S.A
- Atherosclerosis: build up of waxy plaque in blood vessels
- Pomegranate juice showed potential for reducing plaque formation
  - Mice; Humans
  - attributed to AOX activity


Cardiovascular Disease

Case Studies:

(1) 50 ml Pom juice; Hypertensive patients
   Results: 36% reduction in ACE activity
   5% reduction in systolic blood pressure

(2) 240 mL Pom juice;
   Daily, 3 months ; 45 patients with CVD
   Results: Improved stress-induces myocardial ischemia.

Pomegranate and Cancer

- The four leading types of cancer in the US are lung, prostate, breast, and colon cancer.
- Chemoprevention: use of non-toxic dietary botanical substances to prevent the onset of cancer
- Studies suggest potential benefit
- Need in vivo studies to relate in vitro findings
Pomegranate and Breast Cancer

- *In vitro* studies
  - Cell lines: MDA-231 and SUM 149: both aggressive

Pomegranate and Prostate Cancer

- Clinical Trial
  - 8 oz. pom juice, 570 total phenolics GAE
  - 46 men, reoccurring prostate cancer

Pomegranate and Lung Cancer

(Khan, Afaq et al. 2007)
Pomegranate and Colon Cancer

- HT-29 colon cancer cells

Fig. 3. Antiproliferative activities of punicalagin (●), EA (○) TPT (△) and PJ (▽) against human colon tumor cell lines: (A) SW 460; (B) SW 620; (C) HT 29; (D) HCT 116. Cells were exposed to punicalagin, EA or TPT (at 100–12.5 μg/ml concentrations) and PJ (normalized to punicalagin content) for 48 h. Data are expressed as percentage of untreated cells, mean±S.E. (n=3). Asterisk indicates a significant difference compared to untreated controls, P≤.05, two-tailed t test.

# Pomegranate Juice and Cancer

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of cancer</th>
<th>Cell lines/clinical</th>
<th>Percent Inhibition : Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Seeram, Adams et al. 2005)</td>
<td>Colon</td>
<td>SW480, SW620, HT29 and HCT116</td>
<td>30% to 100%: 12.5 and 100 μg/ml of fresh juice</td>
</tr>
<tr>
<td>(Saruwatari, Okamura et al. 2008)</td>
<td>Colon</td>
<td>Caco-2</td>
<td>(IC50) value calculated at 2.7% (vol/vol) of fresh juice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Punicalagin: IC50 of 45 microM,</td>
</tr>
<tr>
<td>(Adams, Seeram et al. 2006)</td>
<td>Colon</td>
<td>HT-29</td>
<td>79%: 50 mg/L of fresh juice</td>
</tr>
<tr>
<td>(Khan, Gorin et al. 2009)</td>
<td>Breast</td>
<td>MDA-231 and SUM 149</td>
<td>67% when treated with 200 mg/mL Pomegranate fruit extracts</td>
</tr>
<tr>
<td>(Jeune, Kumi-Diaka et al. 2005)</td>
<td>Breast</td>
<td>MCF-7</td>
<td>50% of the cells at concentrations of 40 μg/ml of fresh PJE</td>
</tr>
<tr>
<td>(Malik, Afaq et al. 2005)</td>
<td>Prostrate</td>
<td>PC3 and CWR22Rv1</td>
<td>Approximately 80% when treated with 100 μg/ml of PFE</td>
</tr>
<tr>
<td>(Pantuck, Leppert et al. 2006)</td>
<td>Prostate</td>
<td>clinical trial</td>
<td>2% decrease in cell proliferation and a 17% increase in apoptosis 8 ounces of PJ daily</td>
</tr>
<tr>
<td>(Khan, Afaq et al. 2007)</td>
<td>Lung</td>
<td>in vivo</td>
<td>Tumor reduction was 53.9% and 61.6% by PFE group at 84 and 140 days after inducing cancer</td>
</tr>
<tr>
<td>(Khan, Hadi et al. 2007)</td>
<td>Lung</td>
<td>A549</td>
<td>PFE treatment of A549 cells resulted in 33, 40 and 47% decrease in cell viability at the doses of 50–150 μg/ml of PFE.</td>
</tr>
</tbody>
</table>
Pomegranate: Supplements

• POM Wonderful: largest US pomegranate producer.3
• First pomegranate supplement submitted for FDA for review.

* Scale represents antioxidant potency index.
University of Michigan Ann Arbor tested the best-known supplements using the three leading antioxidant potency tests: DPPH, ORAC and FRAP on a per-gram basis. To get an accurate reflection of free radical-fighting ability, results were combined into a single index. POMx was indexed at 100 since it had the highest score. For a complete list of the supplements tested, along with their raw scores and index values, see pompilli.com/potency.
Consumer awareness

• Comment on the study involving POMx

Comment:
“Although pomegranate consumption has not been reported to possess deleterious health effects, apparent innocuousness cannot be simply extended to enriched pomegranate extracts. Heber et al. (1) reported no difference in adverse effects between pomegranate extract-treated and placebo groups, as well as no apparent treatment-related changes of clinical significance or laboratory results in the chemistry, hematology, or urinalysis testing.” J. Agric. Food Chem. 2008, 56, 12143–12144

“continuous consumption of pomegranate juice decreases CYP 1A2 and 3A hepatic expression in mice”.

(1) Heber et al.
Potential Implications

Case Study
• A 48-year-old man was on cholesterol lowering drugs, ezetimibe and rosuvastatin for 17 months
• Pomegranate juice - 200 ml twice a week
• Three weeks later; thigh pain and an elevated serum creatinine kinase level (138,030 U/L, normal < 200 U/L)
• The condition may lead to the breakdown of muscle fibers and kidney failure.

(Sorokin et al, 2006/ American Journal of Cardiology Volume 98, Issue 5, Pages 705-706)
What We Can Conclude...

- Pomegranate juice has an effect *in vitro* on cancer cell death and growth.
- More research needs to be done *in vivo*.