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A NOVEL POTENTIAL INGREDIENT FOR FUNCTIONAL FOODS: PUNICALAGINS of POMEGRANATES



Artemis KARAALİ
Neşe ŞAHİN, Mine GÜLTEKİN

ITU
Istanbul Technical University
Department of Food Engineering

Outline of the Presentation



- Pomegranate
- Phenolics and Health
- Punicalagins of Pomegranate
- Other Phenolic Compounds in Pomegranates
- Experimental Study
- Methods
- Results and Discussion
- Conclusion

Pomegranate (*Punica granatum*)

- Pomegranate is one of the oldest known fruits.
- Considered a holy fruit in the Quran, and grown in Turkey, Iran, Mediterranean, Middle East and Arabic countries and USA.
- Mainly consumed as fresh fruit (seeds) or juice.
- Chemical composition differs depending on cultivar, growing region (soil and climate), harvest maturity, cultural practices, storage period and conditions (Tables 1 and 2).



Table 1. Food Value Per 100 g of Edible Portion
(USDA Nutritional Nutrient Database for Standard Reference, 2005).

| Proximates | Value per 100 g |
|---------------|-----------------|
| Calories | 68 kcal |
| Moisture | 80.97 g |
| Protein | 0.95 g |
| Fat | 0.30 g |
| Carbohydrates | 17.17 g |
| Sugars | 16.57 g |
| Fiber | 0.6 g |
| Ash | 0.61 g |

| Minerals | Value per 100 g |
|------------|-----------------|
| Calcium | 3 mg |
| Magnesium | 3 mg |
| Phosphorus | 8 mg |
| Iron | 0.3 mg |
| Sodium | 3 mg |
| Potassium | 259 mg |

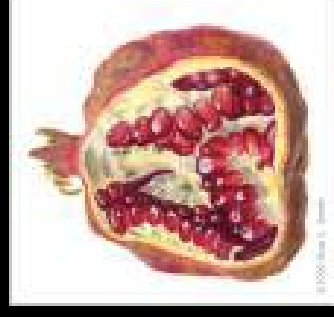
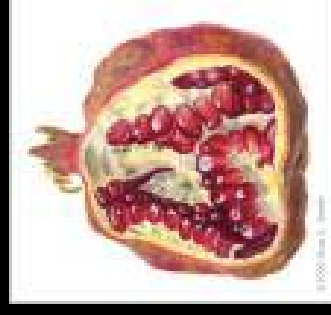


Table 2. Food Value Per 100 g of Edible Portion
 (USDA Nutritional Nutrient Database for Standard Reference, 2005).

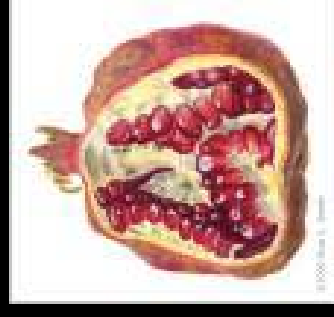
| | |
|------------------|----------|
| Vitamins | |
| Thiamine | 0.03 mg |
| Riboflavin | 0.03 mg |
| Niacin | 0.3 mg |
| Ascorbic Acid | 6.1 mg |
| Vit B6 | 0.105 mg |
| ProVit A | 108 IU |
| Pantothenic acid | 0.596 mg |
| Vit E | 0.6 mg |
| Vit K | 4.6 mcg |

| | |
|------------------------------------|---------|
| Lipids | |
| Fatty acids, total saturated | 0.038 g |
| Fatty acids, total polyunsaturated | 0.063 g |
| Others | |
| B-Carotene | 40 mcg |
| α -Carotene | 50 mcg |



Phenolics of Pomegranates

- ❑ Pomegranates are quite rich in hydrolysable tannins and anthocyanins.
- ❑ Major hydrolysable tannins in pomegranates are gallotannins, ellagic acid tannins and gallagyl tannins (these are termed “punicalagin”s).



Phenolics of Pomegranates



- Phenolic compounds have been shown to act as free radical scavengers and to have antioxidant activity.
- However, their bioavailabilities and the exact mechanism of their action in the human body is not yet totally elucidated.
- It a subject still under wide scientific investigation.

Health Claims for Polyphenols

Polyphenols were shown to have the ability to inhibit low-density lipoprotein (LDL) oxidation and macrophage foam cell formation. Therefore they are hypothesized to have:

- Anti-atherogenic effects, reducing the incidence of cardio-vascular and cerebro-vascular diseases (Kulkarni et al., 2004, Li et al., 2006).
- Antiproliferative activity against oral, colon and prostate cancers (Seeram, et al., 2005), reducing cancer mortality (Kulkarni et al., 2004, Li et al, 2006).



Health Claims for Polyphenols

- Apoptotic effects against human colon cancer cells (Seeram, et al., 2005).
- Therapeutic effect on colic, colitis, diarrhea, dysentery, leucorrhoea, menorrhagia, oxyuriasis, paralysis, rectocele and headache (Schubert et al., 1999).
- Therapeutic effect on infection of male or female sexual organs, mastitis, acne (Singh et al., 2002).
- Antiviral activity against genital herpes virus (Singh et al., 2002).



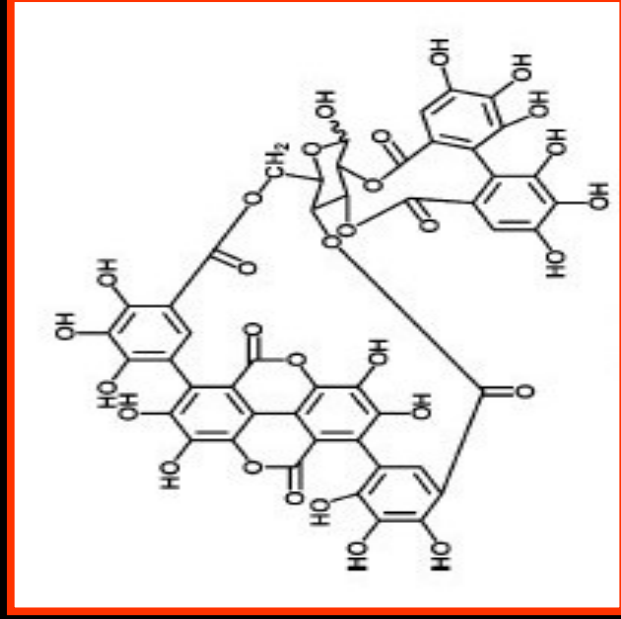
Health Claims for Polyphenols

- Further therapeutic actions such as vermifugal, taenicial, astringent, antispasmodic, antihysterical, diuretic, carminative, sudofiric, galactogogue, emmenagogue (Schubert et al., 1999).
- Folliculitis, pie, allergic dermatitis, tympanitis, diarrhea, dysentery (Singh et al., 2002).
- Neuroprotective effects through improving lipid profiles in diabetic patients (Schubert et al., 1999).



Punicalagins of Pomegranates

- The new term invented for pomegranate tannins is “punicalagins” .
- It is found to be quite abundant in the pomegranate juice (<2g/L).
- It is a superior antioxidant responsible for the free-radical scavenging ability of the pomegranate juice.



Other Phenolic Compounds in Pomegranates



- Ellagic acid derivatives
- Anthocyanins (Delphinidin, cyanidin, pelargonidin 3-glucoside, 3,5-diglucosides)

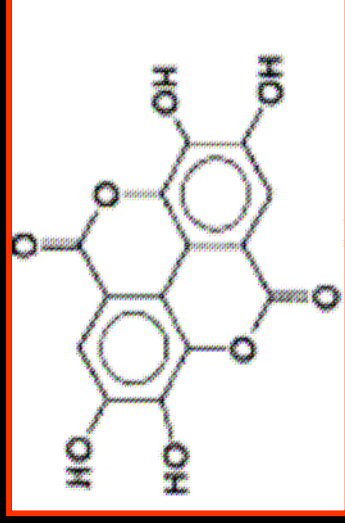


Figure 2. Chemical Structure of Ellagic Acid (Gil et al., 2000).

Experimental Study

MATERIALS:

Pomegranates: peel and seed extracts

Juices: Fresh juice prepared in the laboratory

Commercial juice sample



Methods



1. For Pomegranate Seed and Peel Samples (Singh et al., 2002).

Peeling pomegranates



Peel powder

Seed powder after
removal of fatty matter



Extraction of antioxidant fractions in methanol



Filtration and concentration under vacuum (40C)

2. For Pomegranate Juice Sample (Gil et al., 2000)



Peeling



Pressing and extracting the juice localised in sacs/commercial juice



Centrifuging for 2 min at 1400 rpm

3. “Phenolic profiling”: Analysis of Individual Phenolic Compounds by HPLC (Singh et al., 2002 and Gil et al., 2000)

Extracts dissolved in
MeOH:Water (6:4 v/v)

Centrifuged Juice in
MeOH:Water (6:4 v/v)



20 µl sample injected into HPLC equipped with PAD detector,
C18 supelcosil column (5 µm particle size)

Solvent A: 3% acetic acid + 97% water

Solvent B: 3% acetic acid + 25% acetonitrile + 72% water



4. Determination of Total Phenols (Folin-Ciocalteu Method, Singh et al., 2002).

Peel and seed extracts

Juice

Dissolving in MeOH:Water (6:4 v/v)

Sample (300 μ l)+10 fold-dilution with FC reagent (1.5 ml)+7.5% Na₂C₀3 (1.2 ml)

Standing for 1.5 h at room temperature

Absorbance measurement at 765 nm by UV-visible spectrophotometer



5. Determination of Antioxidant Activity (DPPH Method, Moreno et al., 1998)

100 μ l extracts/juice + 3.9 ml DPPH solution



Vigorous Mixing



Standing for 30 min at room temperature



Absorbance measurement at 515 nm by UV-visible spectrophotometer
(% of radical scavenged by test sample)



Results and Discussion

Total Phenolic Contents

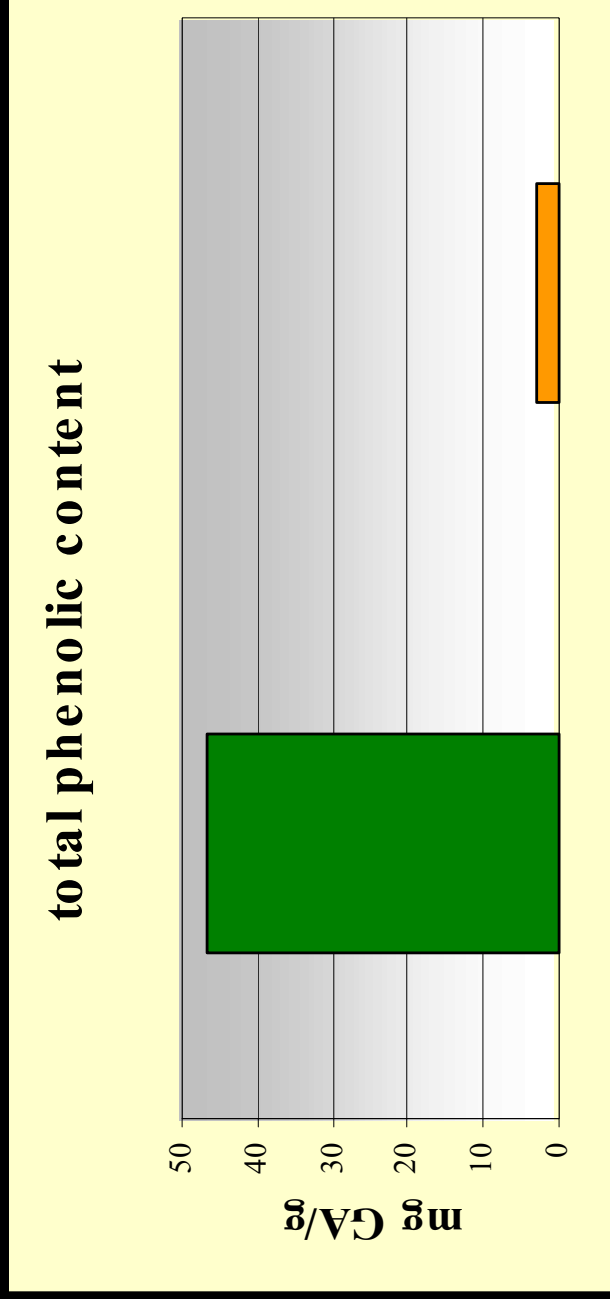
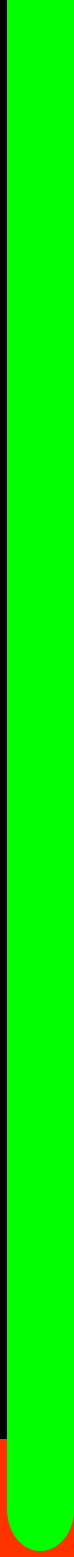


Table 2. Phenolic content of pomegranate peels and seed extracts

| Pomegranate | Phenolics (mg/g) | Phenolics (mg/g) |
|-------------|------------------------|------------------|
| | gallic acid equivalent | |
| Peel | 46.5 | 48.66 |
| Seed | 3.1 | 2.88 |



Total Phenolic Contents



peel seed

Figure 5. Total phenol content of pomegranate peel and seed extracts.

Total Phenolic Contents



Table 3. Phenolic content of pomegranate juices.

| Pomegranate | Phenolics (mg/L) | Phenolics (mg/L) (Alper et. Al., 2005, Gil et al., 2000). |
|------------------|------------------|---|
| Fresh juice | 1860 | gallic acid equivalent |
| Commercial juice | 2480 | |
| | | 2941.01 |

Total Phenolic Contents

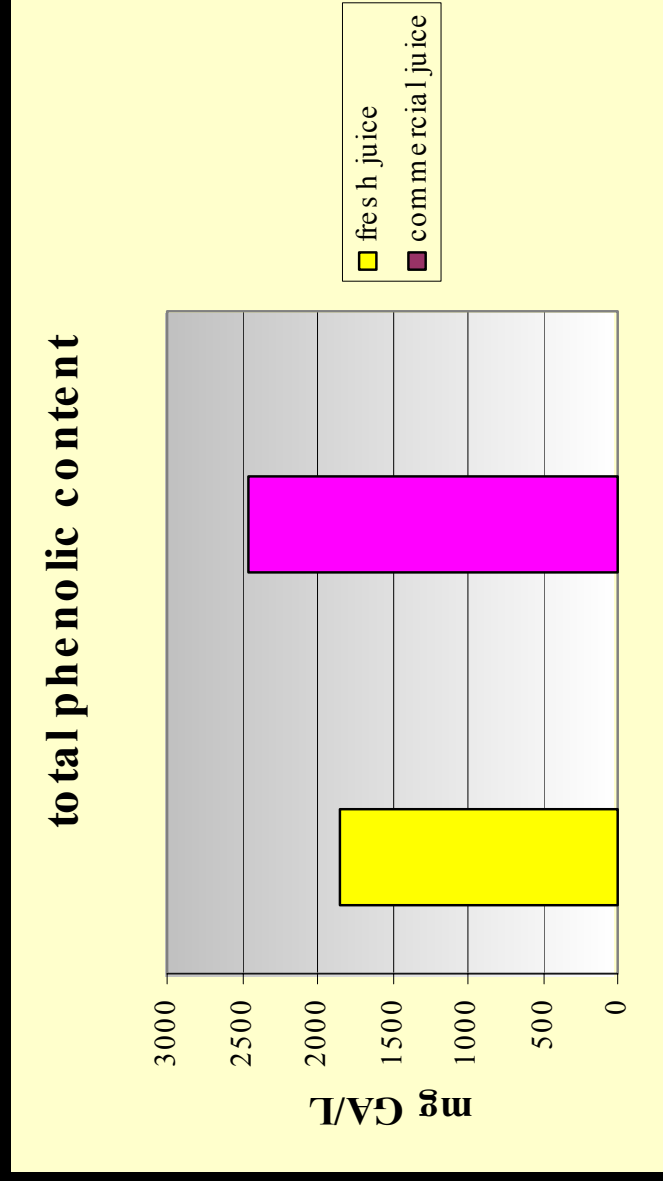


Figure 6. Total phenol content of pomegranate juices

*hydrostatic pressure used in the commercial products crushes the whole fruit, extracting also the ellagitannins in the peels

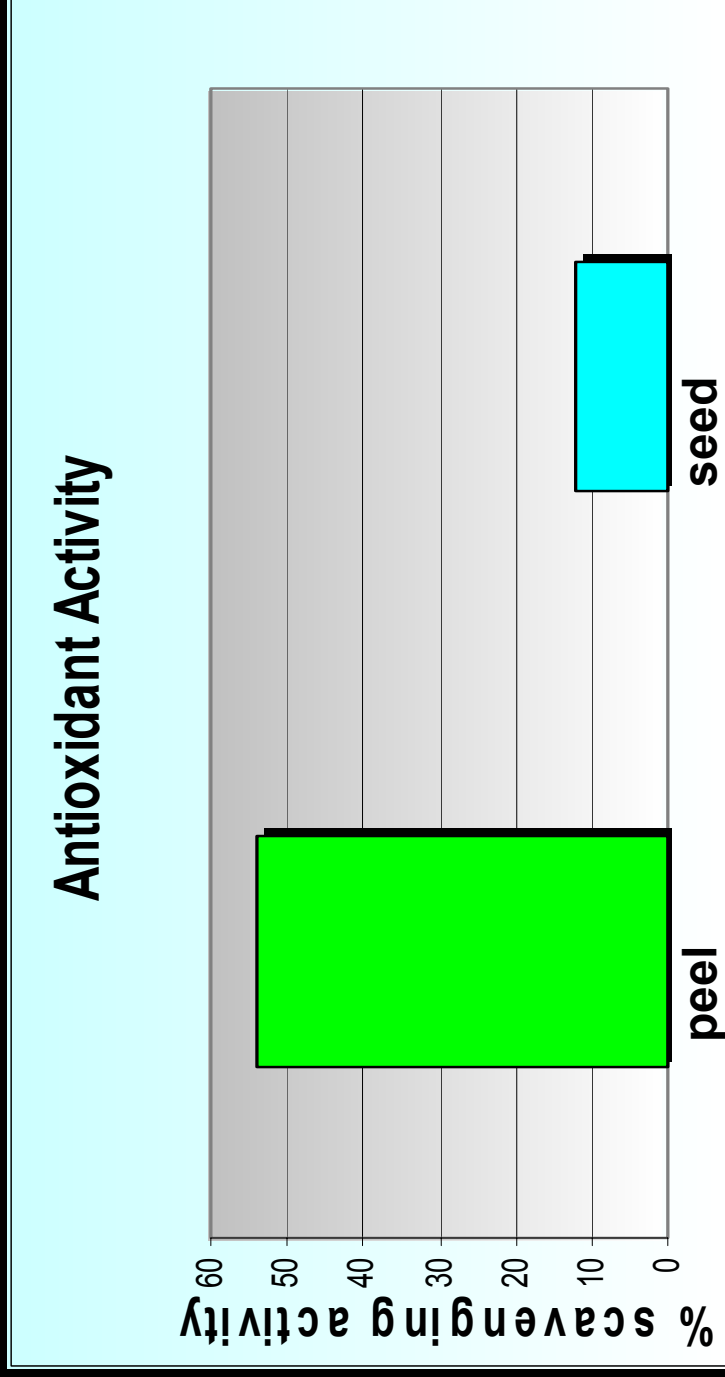


Radical Scavenging Activity

Table 4. Antioxidant activity of pomegranate seed and peel extracts

| Pomegranate | Antioxidant Activity (%) | Antioxidant Activity (%) (Singh et al., 2002) |
|-------------|--------------------------|--|
| Peel | 54.0 at 25 ppm | 81.0 at 50 ppm |
| Seed | 12.2 at 25 ppm | 23.2 at 100 ppm |

Radical Scavenging Activity



24 Figure 7. Antioxidant activity of pomegranate peel and seed extracts.



Radical Scavenging Activity

Table 5. Antioxidant activity of pomegranate juices.

| Pomegranate | Antioxidant Activity (%) | Antioxidant Activity (%) |
|------------------|--------------------------|--------------------------|
| Fresh juice | 32.5 at 5 mg/mL | - |
| Commercial juice | 54.8 at 5 mg/mL | 26.4 |



Radical Scavenging Activity:

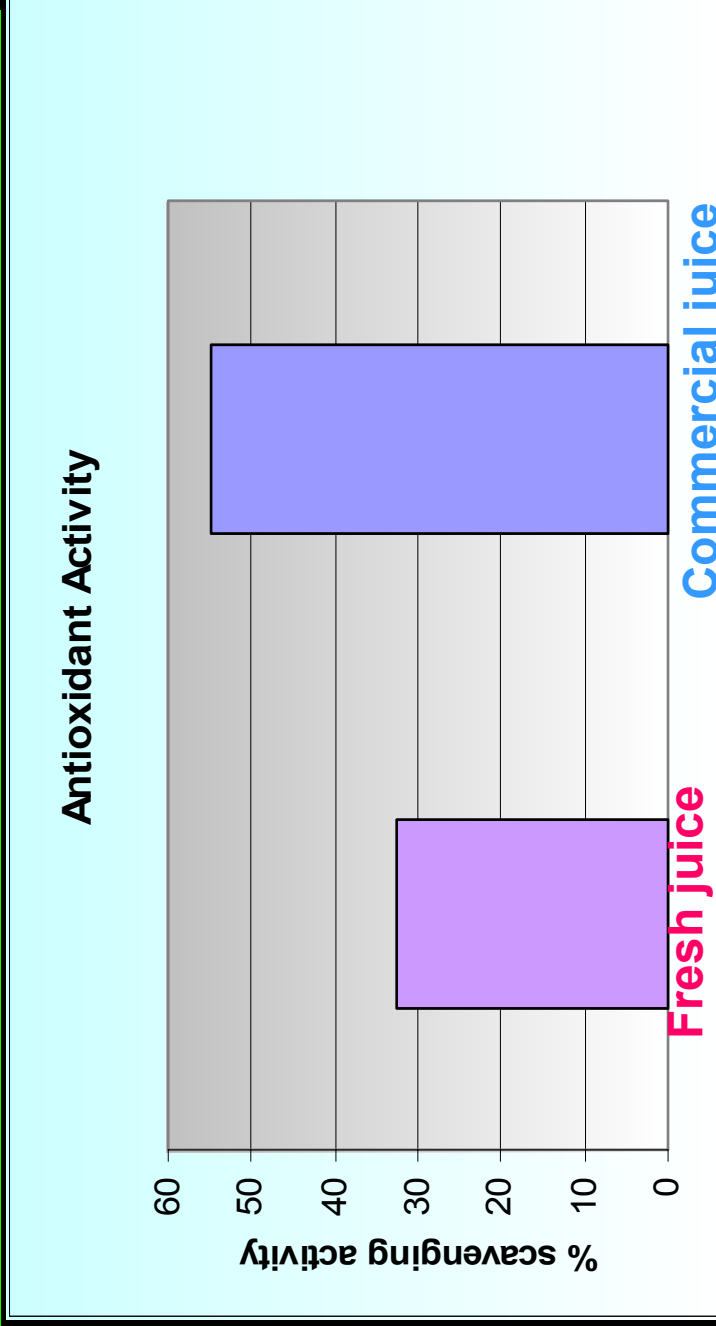


Figure 8. Antioxidant activity of pomegranate juices

*hydrostatic pressure used in the commercial products crushes the whole fruit, extracting also the ellagitannins in the peels



Phenolic Profiling

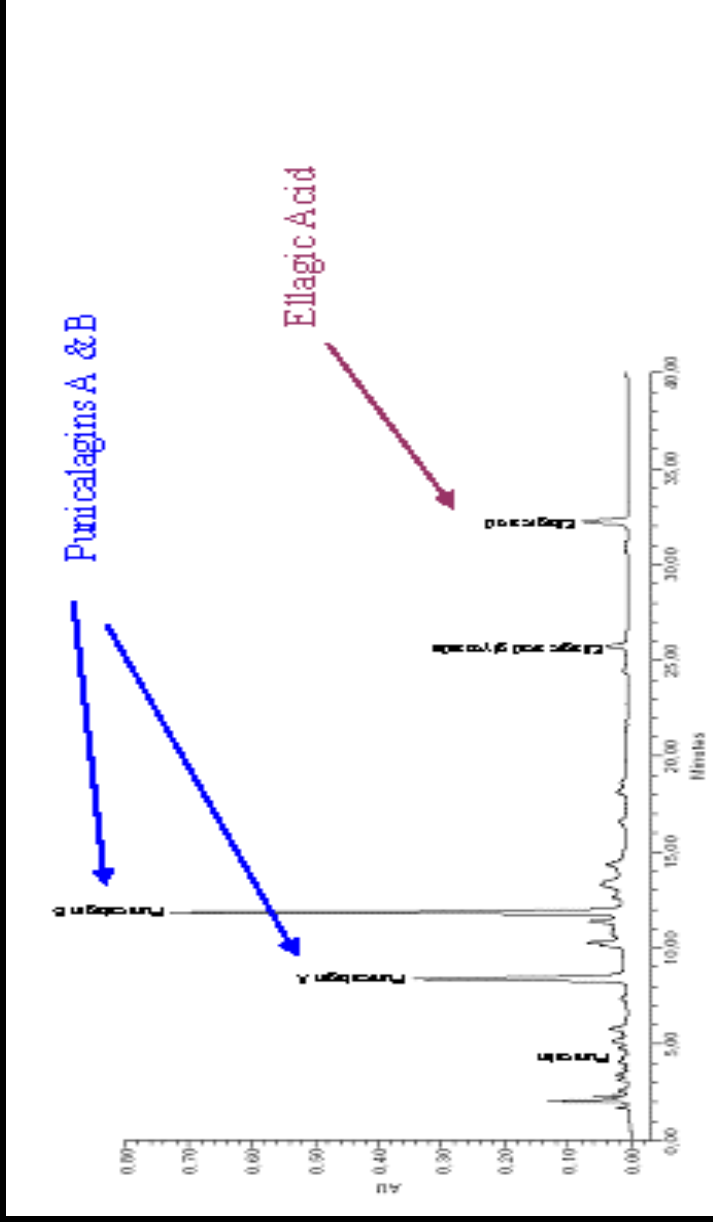


Figure 3. Phenolic composition of fresh pomegranate juice.



Phenolic Profiling

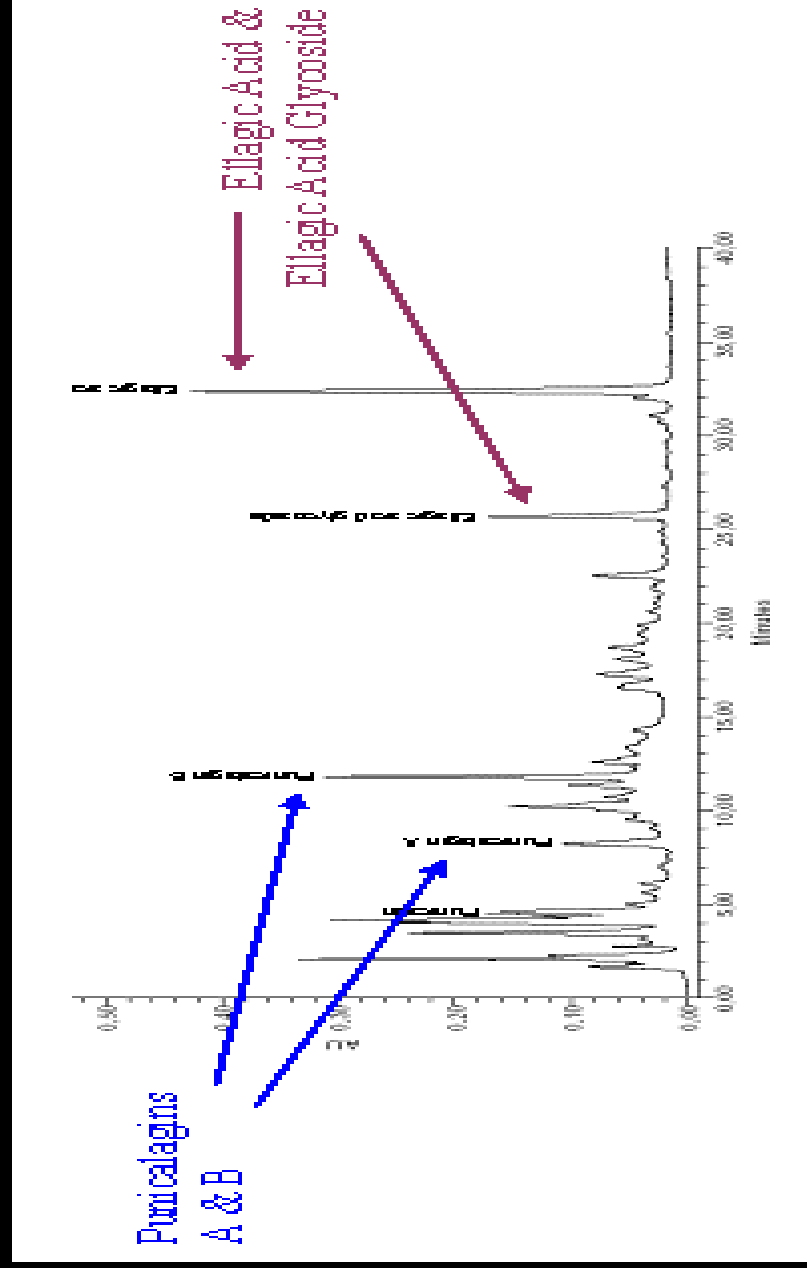


Figure 4. Phenolic composition of commercial pomegranate juice.



Conclusions

- ❖ Pomegranate, juice and other pomegranate products promise to have health-promoting effects.
- ❖ The factor responsible for the high antioxidant activity in the pomegranate is termed “punicalagin”, which is an ellagitannin.
- ❖ Pomegranate peels may be a rich source and raw material for natural antioxidants.



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THANK YOU