Phenols and antioxidant activity of apple, quince, pomegranate, bitter orange and almond-leaved pear methanolic extracts.

E. Tzanakis, Th. Kalogeropoulos, St. Tzimas, A. Chatzilazarou and E. Katsoyannos.

State Technological Institute (T.E.I.) of Athens
Food Technology and Oenology Department

Abstract

Fruits are valuable sources of natural phenolic antioxidants , which are known to have beneficial health promoting properties. Some rarely consumed fruits (almond-leaved pear) in the Greek province and peels processed to traditional homemade pastries (bitter orange, quince) as well as some wild types (wild pomegranate and wild pear) where investigated in relation to their phenol content and antioxidant activity potential of the methanolic extracts and compared to well known fruits (apple, pomegranate). Total phenols (TP) values of fruit flesh and peel where estimated by the Folin-Ciocalteu reagent after extraction with ethyl acetate and n-propanol in acid hydrolyzed and non hydrolyzed samples, while antioxidant activities values of methanolic extracts where estimated by the DPPH method. The obtained TP and antioxidant activity values where compared to the corresponding values of green apple, red apple and pomegranate.

Key words: antioxidant activity, phenols, quince, apple, pomegranate, bitter orange, almond-leaved pear

Corresponded author E. Katsoyannos ekatso@teiath.gr

Introduction

Phenolic compounds widely distributed in plants, attract significant scientific interest due to their bio-functional health-promoting properties (Robards, 1999; Rice-Evans, 2000; Antolovich et al. , 2000; Ryan et al, 1999; McDonald et al, 2001; Moure et al. 2001). Fruits are potential sources of natural phenolic antioxidants used as food additives for the prevention of lipid oxidation and thus prolongation of food self-life. Many fruits have been characterized as to their phenolic profile and antioxidant activity (Mayr et al., 1995; Pearson et al., 1999; Hamauzu et al., 2005; Scalbert et al., 2000; Li et al., 2006; Poyrazoglu et al., 2002; Miller and Rice-Evans, 2006; Challice et al., 1972; Vanamala et al., 2006). For example apple and orange extracts show remarkable antioxidants potential (Gorinstein et al., 1999; Scalzo et al.,2005; Gardner et al., 2000; Larrauri et al., 1996; Leong et al., 2002) as they show high phenolic content. The phenolic content in apple vary within the range 0,15 - 2,5 %, 839 mg/Kg (Salunkhe and Kadam, 1995; Escarpa et al., 2001) but fresh apple juice contains only 10% of fresh apple antioxidant activity and orange contains 217 mg / 100 g, 74 mg Gallic acid equivalent/100 g or 142 ± 22.6 L-ascorbic acid equivalent/100 g (Cieślik et al., 2006; Li et al., 2006; Leong et al., 2002). Scarce data on phenolic content of pomegranate exist, while for not usually consumed fruits like quince, wild pomegranate, bitter orange and almond-leaved pear there is a great leak of information regarding the composition and phenols content. Pomegranate contains 2,92 ± 0,19 mg / 100gr total phenols (Al-Maiman and Ahmad, 2002) and 0.2–1.0% (Aviram and Dornfeld, 2001) soluble phenols showing remarkable antioxidant activity and significant health properties.

Apples and apple juice decrease the possibility of incidences of prostate cancer, anti-influenza viral activity, are involved in LDL oxidation, decrease the risk of chronic diseases such as cardiovascular disease and cancer (Denis et al., 1999; Hamauzu et al., 2005; Pearson , 1999; Boyer et al., 2004). Quince phenolics showed the strongest anti-influenza viral activity, antiseptic and antidiarreatic abilities while quince sperms have emollient activity (Denis et al., 1999; Hamauzu et al., 2005; Nousis ,1984). Pomegranate peel show anti-mutagenic effects, and pomegranate juice - which is rich in tannins - possess anti-atherosclerotic properties, reduces blood pressure and may improve stress-induced myocardial ischemia in patients who have coronary heart disease (Aviram et al., 2001; Negi et al., 2003; Sumner, 2005). Sour orange juice is anti-septic, anti-bilious and hemostatic, while their leaves show sudorific, anti-spasmodic, stimulant, tonic and stomachic action. Flowers of bitter orange prepared as a sirup act as a sedative in nervous disorders, induce sleep and are used for weight-loss (Morton , 1987; Colker et al., 1999), almond-leaved pear has not any known medical uses. Quince and bitter orange peels are raw materials for tradition home-made pastries, while almond-leaved pear is consumed in some areas of Greek province.

The aim of this study was to measure the phenolic content and antioxidant activity of quince, wild pomegranate, bitter orange, almond-leaved pear methanolic extracts common in Greece and compare it with the phenolic content and antioxidant activity of apple and cultivated pomegranate methanolic extracts.

Materials and methods

Reagents and Equipment

Ethylacetate, n–propanol, methanol (used for the extraction of phenols), HCl 37 % (used for polyphenols hydrolysis) $N\alpha_2CO_3$ and Folin-Ciocalteu Reagent (used for the determination of total phenols) were obtained from MERCK. Gallic acid and caffeic acid (used as phenolic standards) were acquired from Sigma , while 2,2-Diphenyl-1-picrylhydrazyl Hydrat 95% (DPPH) used for the determination of antioxidant activity of phenols was purchased from MERCK . A Precisdig waterbath of SELECTA and a magnetic stirrer SM1 Stuart were used for the extractions and a UV-Vis Photometer SHIMADZU, UV mini 1240, spectrometer HITACHI U-3210 for the phenols determination and antioxidant activity determinations.

Samples

The following fruits were used:

Apples of varieties: Granny Smith and Starking Delicius (Malus pumila Mill. synonyms: M. communis, M. paradisiaca, M. sylevestris e.t.c.), Quince (Cydonia vulgaris

Pers. synonyms:Cydonia oblonga Miller and Pyrus cydonia L.) , a wild type as well as a cultivated pomegranate (Punica granatum). All cultivated varieties were acquired from the local market . A mature and a immature bitter orange (Citrus aurantium L.) was originated from a public garden in Athens , while the mature and the immature almond-leaved pear (Pyrus amygdaliformis L.) from Argos (Greece).

Procedures

Fruit samples handling and pre-treatment

The fruits were kept refrigerated (-18 ^{o}C) until used. Apples were peeled before use. Portions of 5 g of peel and 5 g of apple flesh were homogenized before the extraction procedure. Wild and cultivated pomegranates as well as mature and immature bitter-oranges were also peeled. Portions of 10 g of milled fruits were taken separately from each flesh and peel and used for the extractions. Milled mature and immature almond-leaved pears and quince were each applied in portions of 10 g without separating the peel from flesh. All these procedures have taken place at room temperature and in absence of light.

Extraction procedures

The weighted milled fruit samples were extracted three times with each time 50 ml of Ethyl-acetate at temperature of 55 ^{o}C for 20 min under magnet stirring. The residue was treated twice with 50 ml n-propanol under the same extraction conditions as with ethyl-acetate. The united extracts were evaporated at 40 ^{o}C under vacuum to dryness and the residue was dissolute with 5 ml of methanol and filtered and put at a refrigerator until photometric determination of total phenols and antioxidant activity. Total phenols were determined in methanolic extracts by means of Folin-Ciocalteu reagent according to Vasquez-Ronsero (1976) at 725 nm and expressed as ppm caffeic acid. Antioxidant activity were determined in methanolic extracts with 0,06 mM DPPH at 515 nm according the photometric method of Bandoniene et al (2002) and expressed as % inhibition. Antioxidant activity values were calculated by means of the formula :

$$\% \text{ Inhibition} = (\Delta A/Ao) \times 100 \quad \text{with} \quad \Delta A = Ao - A_{fin}$$

Whereby A is the absorbance at 515 nm. Ao is the initial absorbance of the control used (0,06 mM DPPH in methanol without antioxidant) at t=0. A_{fin} is the absorbance of the reaction solution at the end of the reaction.

Hydrolysis conditions

The residue of the above described extraction procedure of each fruit was then hydrolysed with 25 ml of HCl 2N at ambient temperature for 24 h. The hydrolyzed were extracted with ethyl-acetate and n-propanol as described above and total phenols and antioxidant activity were determined in methanolic extracts as previously.

Results and Discussion

The following tables and graphs present the results estimated concerning values of phenols and antioxidant activity

.

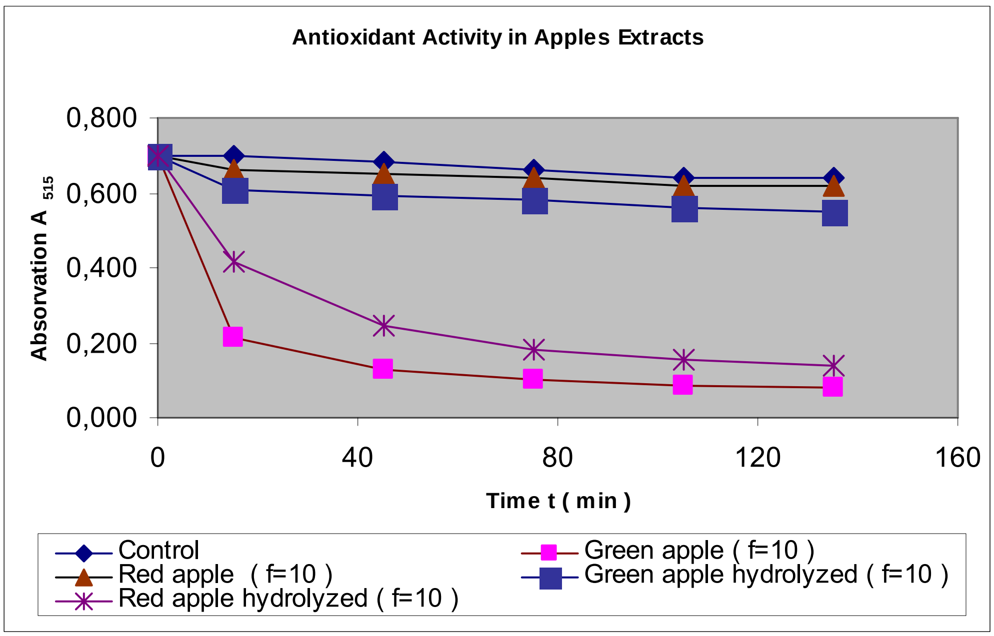
Table 1. Phenols content in various hydrolyzed and non hydrolyzed fruit samples.

Sample number	Sample origin	Phenols (mg / Kg)	% Phenols concentration	% total phenols (TP) concentration
1.	Green apple	258,0	0,026	0,106
2.	Green apple hydrolyzed	798,0	0,080	
3.	Red apple	309,0	0,031	0,077
4.	Red apple hydr.	462,0	0,046	
5.	Quince	119,0	0,012	0,037
6.	Quince hydr.	249,0	0,025	
7.	Pomegranate flesh	1099,6	0,11	1,170
8.	Pomegranate flesh hydrolyzed	10576,7	1,06	
9.	Pomegranate peel	27672,6	2,77	3,164
10.	Pomegranate peel hydr.	3937,2	0,394	
11.	Wild pomegranate	4892,9	0,49	0,743
12.	Wild pomegranate hydrolyzed	2527,5	0,253	
13.	Wild pomegranate peel	34192,9	3,419	3,530
14.	Wild pomegranate peel hydrolyzed	1107,8	0,111	
15.	Bitter orange immature peel	1407,8	0,141	0,284
16.	Bitter orange immature peel hydr.	1428,2	0,143	
17.	Bitter orange immature	911,4	0,091	0,114
18.	Bitter orange immature hydrolyzed	224,6	0,023	
19.	Bitter orange mature peel	800,1	0,080	0,212
20.	Bitter orange mature peel hydr.	1318,6	0,132	
21.	Bitter orange mature	1282,7	0,128	0,139
22.	Bitter orange mature hydrolyzed	113,2	0,011	
23.	Wild Pear immature (n-Propanol)	165,0	0,017	0,489
24.	Wild Pear immature hydr. (n-Prop.)	422,0	0,042	
25.	Wild Pear immature (Ethyl-Acetate)	407,0	0,041	
26.	Wild Pear immature hydr. (EtAc)	419,0	0,042	
27.	Wild Pear mature (n- Propanol)	525,0	0,053	0,222
28.	Wild Pear mature hydr. (n-Propanol)	383,0	0,038	
29.	Wild Pear mature (Ethyl-Acetate)	846,0	0,085	
30.	Wild Pear mature (EtAc) hydr.	464,0	0,046	

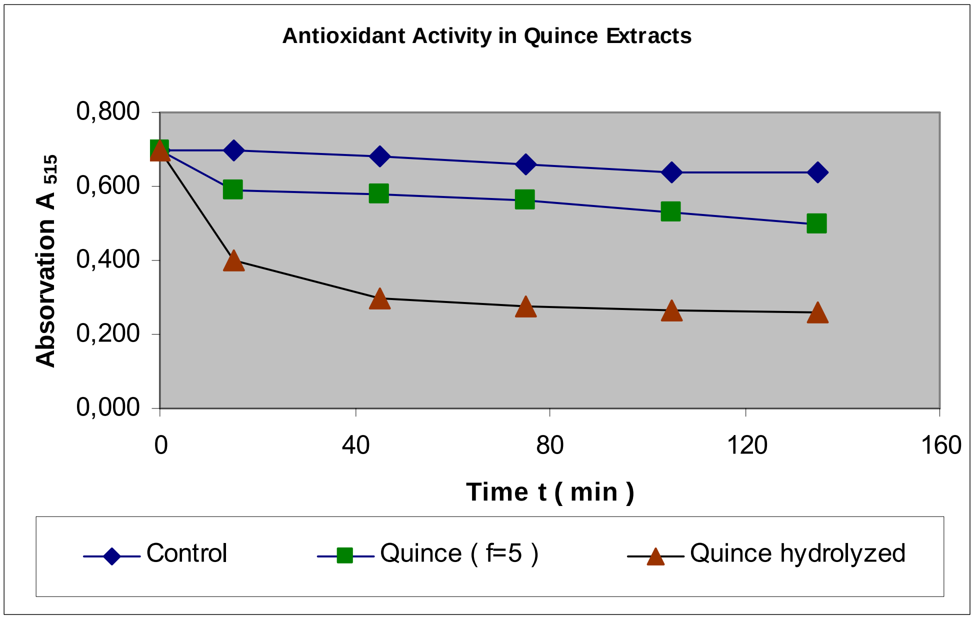
Notices: Phenols concentration values referred to fruit wet weight. EtAc: Ethyl-Acetate. Total phenols concentrations mean the sum of hydrolyzed and non hydrolyzed samples.

Antioxidants activity in fruits in methanolic extracts

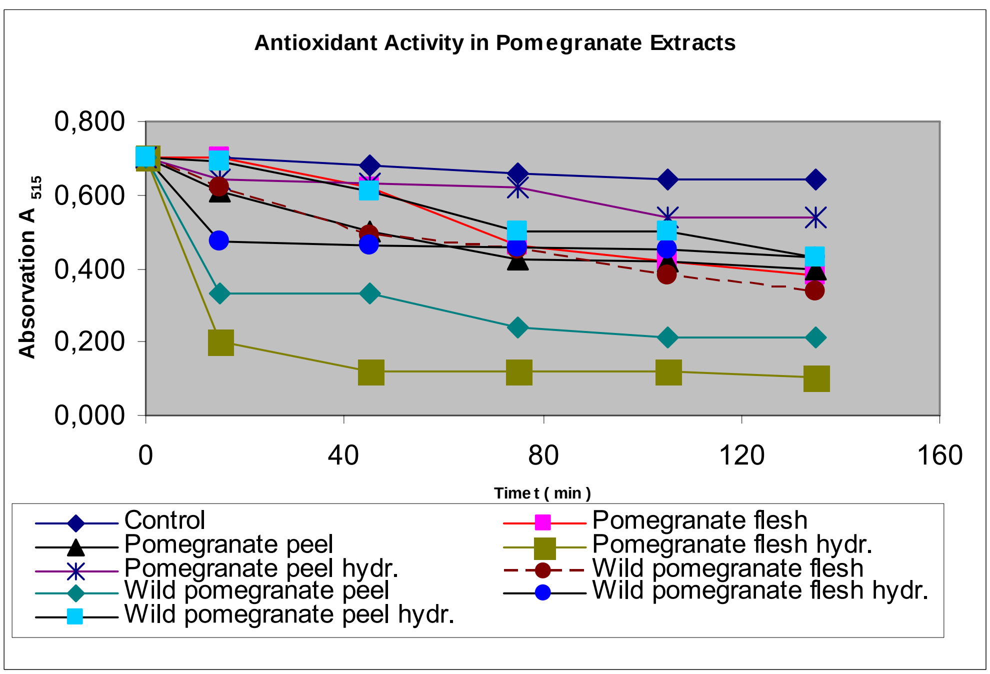
Graph 1. Antioxidants activity in apple methanolic extract.



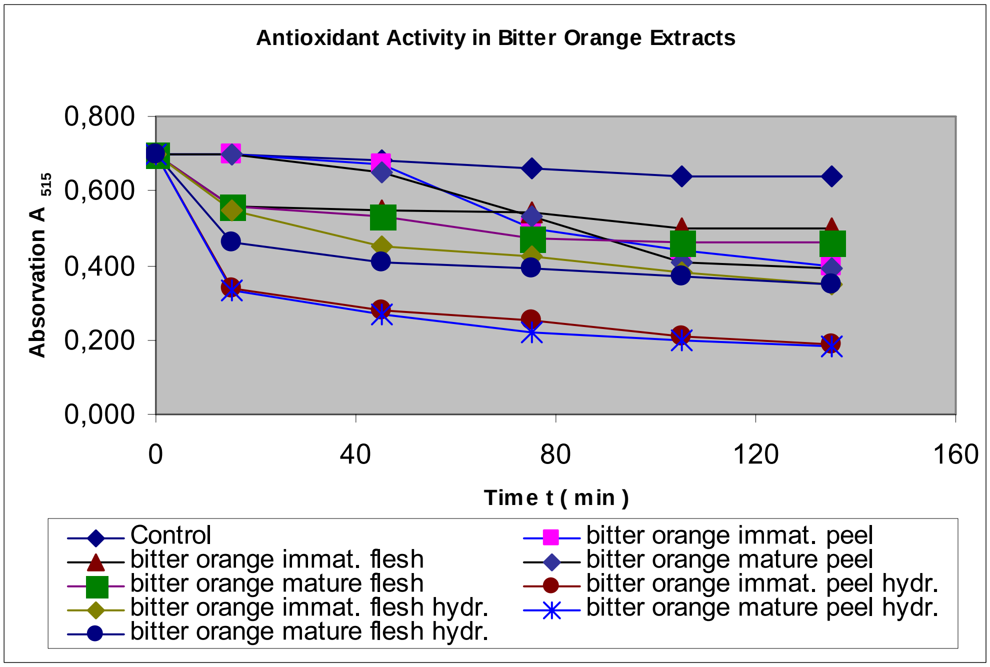
Graph 2. Antioxidants activity in quince methanolic extracts.



Graph 3. Antioxidants activity in pomegranate methanolic extracts.



Graph 4. Antioxidants activity in bitter orange methanolic extracts.



Graph 5. Antioxidants activity in almond-leaved pear (wild pear) methanolic extracts.

Sample number	sample origin	Phenols of non hydrolyzed samples	Phenols of hydrolyzed samples	Antioxidant activity of non hydrolyzed samples	Antioxidant activity of hydrolyzed samples
1.	Green apple	258,0	798,0	92,19	17,97
2.	Red apple	309,0	462,0	6,25	82,81
3.	Quince	119,0	249,0	-	65,63
4.	Pomegranate flesh	1099,6	10576,7	46,88	86,72
5.	Pomegranate peel	27672,6	3937,2	42,19	12,50
6.	Wild pomegranate flesh	4892,9	2527,5	50,00	28,13
7.	Wild pomegranate peel	34192,9	1107,8	73,44	32,81
8.	Bitter orange immat. flesh	911,4	224,6	27,34	46,88
9.	Bitter orange immat. peel	1407,8	1428,2	40,63	76,56
10.	Bitter orange mature flesh	1282,7	113,2	34,38	50,00
11.	Bitter orange mature peel	800,1	1318,6	43,75	79,69
12.	Wild Pear immat. (n - Prop.)	165,0	422,0	53,13	28,13
13.	Wild Pear immat. (EtAc)	407,0	419,0	35,94	43,75
14.	Wild Pear mature (n - Prop.)	525,0	383,0	54,69	62,50
15.	Wild Pear mature (EtAc)	846,0	464,0	45,31	43,75

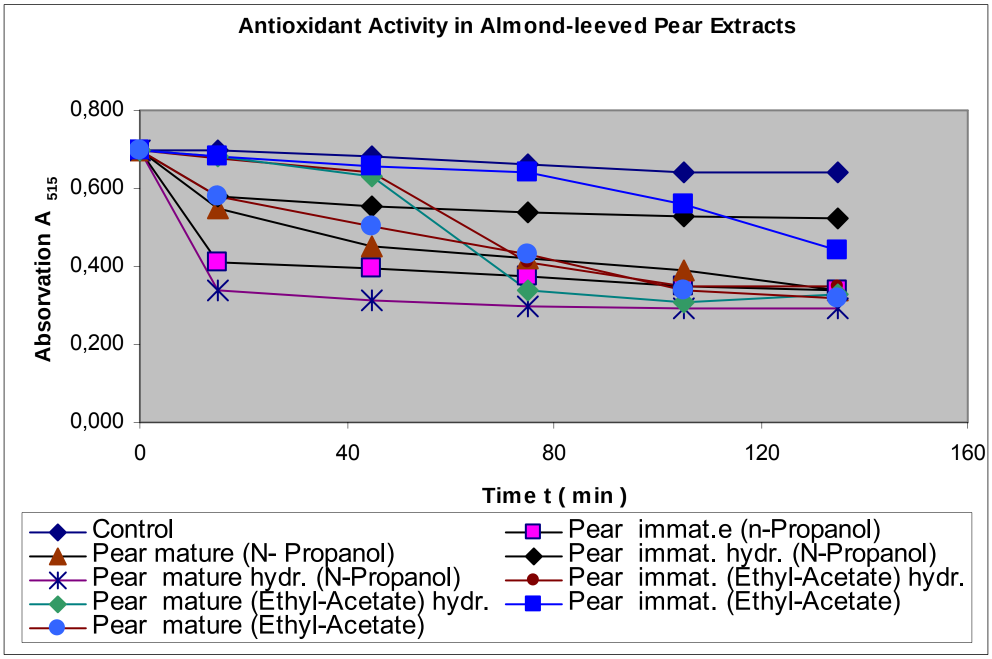


Table 2. Comparison of phenols concentration and antioxidant activity in fruit samples.

Notices: Phenols expressed as mg caffeic acid / kg wet fruit flesh or peel weight. Antioxidant activity as % Inhibition.

Conclusions

Fruits phenols content

Flesh

Among the tested fruits (Table 1) pomegranate shows in any case the highest values of total phenols (1,170 – 0,743 % TP wet weight) followed by wild pear (0,489 – 0,222 % TP w. w.), in relation to other analyzed fruits and especially to apple (0,106 – 0,077 % TP w. w.). Pomegranate contains more then seven to eleven higher phenols values then green apple. Wild type fruits show lower phenols values in the case of pomegranate. Immature wild pear contains double total phenols as mature wild pear, while in the case of bitter orange mature fruit show higher values of total phenolics. Among the fruits traditionally processed as homemade pastries bitter orange shows slightly higher total phenols values than apple, while quince clearly lower. Concerning the simple (free in flesh, non hydrolyzed) phenolics we consider that in any case the phenols values are much lower than the hydrolyzed ones, except in the case of wild pomegranate and bitter orange.

Peel

Pomegranate peel (3,164 % TP wet w.) and especially the wild type (3,53 % TP wet w.) shows exceptionally TP values, while bitter orange peel which is processed to traditional homemade pastry shows more than double TP values compared to apple (0,212 – 0,284 % TP w.w.). Thus pomegranate peel (by product of pomegranate juice processing) could be a valuable source of natural phenolic antioxidants.

Antioxidant activity of methanolic extracts

From the values in Table 2 is obvious that TP are non directed proportional to antioxidant activity measured by the DPPH method among the methanolic extracts of fruit tested. This reflects the high degree of differentiation of individual phenols content from fruit to fruit and fruit part to part (flesh to peel) as well as the differences concerning the antioxidant activity of individual phenols known from many other similar examples in the international literature related to phenolic antioxidants of plant materials. An elucidation of the relationships between antioxidant activity and TP content could be performed by means of advanced chromatography techniques such as HPLC , LC and MS. Among the hydrolyzed samples tested, pomegranate flesh shows the highest antioxidant activity value (86,72 %) comparable to red apple followed by bitter orange peel. Concerning the antioxidant activity values of non hydrolyzed samples all fruits tested where lower than green apple but higher than red apple. The differences in antioxidant activity values observed between hydrolyzed and non hydrolyzed samples vary from fruit to fruit. In the most of fruits (red apple, pomegranate flesh, bitter orange flesh and peel and pear) non hydrolyzed samples shows lower antioxidant activity values than hydrolyzed samples. From the differences between the values of TP and antioxidant activity in hydrolyzed and non hydrolyzed samples is obvious that the most significant antioxidant power of the fruits is concentrated in the higher (condensed, non hydrolyzed) phenols than in the simple phenolic compounds free in the fruit juice. Bitter orange peel and quince which are processed as traditional pastries show antioxidant values comparable to red apple and significant higher to green apple.

References

1. Aviram M., Dornfeld L, ″ Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure ″, Elsevier, Atherosclerosis 158 (2001) 195 –198.

2. Salunkhe D. K., Kadam S.S., ″ Handbook of fruit science and technology ″,Utah, U.S.A., 1995.

3. Salah A. Al-Maiman, Dilshad Ahmad, ″ Changes in physical and chemical properties during pomegranate (Punica granatum L.) fruit maturation ″, Elsevier, J. Food Chemistry 76 (2002) 437–441.

4. Vinson A. J., Xuehui Su, Zubik L., Bose P., ″ Phenol Antioxidant Quantity and Quality in Foods: Fruits ″, J. Agric. Food Chem, 49 (2001) 5315 - 5321.

5. Scalbert A. and Williamson G., ″ Dietary Intake and Bioavailability of Polyphenols ″, symposium “Chocolate: Modern Science Investigates an Ancient Medicine” , Annual Meeting and Science Innovation Exposition of the American Association for the Advancement of Science, Washington, D.C., 2000

6. Morton F. J., ″ Fruits of warm climates ″, Sour Orange. p. 130–133, Miami, Florida, U.S.A., 1987.

7. Poyrazoglu E., Gokmen V, Artik N., ″ Organic Acids and Phenolic Compounds in Pomegranates (Punica granatum L.) Grown in Turkey ″, Elsevier, Journal of food composition and analysis, 15 (2002) 567–575.

8. Negi P.S., Jayaprakasha G.K., Jena B.S., ″ Antioxidant and antimutagenic activities of pomegranate peel extracts ″, Elsevier, J. Food Chemistry 80 (2003) 393–397.

9. Vanamala J., Reddivari L.,. Yoo Kil Sun, Pike L. M., Patil Bh. S., ″ Variation in the content of bioactive flavonoids in different brands of orange and grapefruit juices ″, Elsevier, Journal of Food Composition and Analysis 19 (2006) 157–166.

10. B.B. Li, B. Smith, Md. M. Hossain, ″ Extraction of phenolics from citrus peels, Solvent extraction method ″, Elsevier, Separation and Purification Technology 48 (2006) 182–188.

11. L.P. Leong, G. Shui, ″ An investigation of antioxidant capacity of fruits in Singapore markets ″, Elsevier, Food Chemistry 76 (2002) 69–75.

12. Larrauri J. A., Rupbrez P., Bravo L., Saura-Calixto F. ″ High dietary fibre peels: associated powders from orange and lime polyphenols and antioxidant capacity ″, Elsevier, Food Research International, Vol. 29, No. 8, pp. 751-162, 1996

13. A. Escarpa, M.C. González, ″ Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromato-graphic and spectrophotometric methods ″, Elsevier, Analytica Chimica Acta 427 (2001) 119–127.

14. U. Mayr, D. Treutter, C. Santos-Buelga, H. Bauer and W. Feucht ″ Develomental changes in the phenol concentration of “ Golden Delicius ”, Apple fruits and leaves ″, Pergamon, Phytochemistry, Vol. 38, No. 5, pp. 1151--1155, 1995.

15. Cieślik E., Gręda A., Adamus W., ″ Contents of polyphenols in fruit and vegetables ″, Elsevier, Food Chemistry 94 (2006) 135–142.

16. Gorinstein Sh., Zemser M., Haruenkit R., Chuthakorn R., Grauer F., Martin-Belloso O., Trakhtenberg S. J. ″ Comparative content of total polyphenols and

dietary fiber in tropical fruits and persimmon ″, Elsevier, Nutr. Biochem. 10: 367–371, 1999

17. Miller N. J., Rice-Evans C. A. ″ The relative contribution of ascorbic acid and phenolic antioxidant to the total antioxidant activity of orange and apple juices and blackcurrant drink ″, Elsevier, Food Chemistry, Vol. 60, No. 3, pp. 135–142, 2006.
18. Denis L., Morton M. S., Griffiths K., ″ Diet and its preventive role in prostatic disease ″, Medline, European Urology, Volume 35, Issue 5-6 , 1999, Pages 377-387.

19. Hamauzu Y., Yasui H., Inno T., Kume Ch. , Omanyuda M., ″ Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (Pseudocydonia sinensis Schneid.), quince (Cydonia oblonga Mill.), and apple (Malus domestica Mill.) fruits ″, Medline, Journal Of Agricultural And Food Chemistry, Volume 53, Issue 4 , February 23, 2005, Pages 928-934.

20. Pearson D. A., Tan C. H. , German J. B., Davis P. A., Gershwin M. E., ″ Apple juice inhibits human low density lipoprotein oxidation ″, Medline, Life Sciences, Volume 64, Issue 21 , 1999, Pages 1913-1920.

21. Boyer J., Liu R. H.,″ Apple phytochemicals and their health benefits ″, Medline, Nutr J,Volume 3 , May 12, 2004, Page 5.

22. Li Y., Guo Ch., Yang J., Wei J., Xu J., Cheng Sh. ″ Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract ″, Elsevier, Food Chemistry 96 (2006) 254–260.

23. Sumner M. D., Elliott-Eller M., Weidner G., Daubenmier J. J., Chew M. H., Marlin R., Raisin C. J., and Ornish D., ″ Effects of Pomegranate Juice Consumption on Myocardial Perfusion in Patients With Coronary Heart Disease ″, Elsevier, 2005.

24. Colker C. M., Kalman D. S., Torina G. C., Perlis T., Street Ch., ″ Effects of Citrus aurantium Extract, Caffeine, and St. John's Wort on Body Fat Loss, Lipid Levels, and Mood States in Overweight Healthy Adults ″, Current Therapeutic Research, Vol. 60, No. 3, March 1999.

25. Challice J. S., M N Westwood, ″ Phenolic compounds of the Genus Pyrus ″, Pergamon, Phytochemtstry, 1972, Vol 11. pp 37 to 44.

26. Scalzo J., Politi Al., Pellegrini N., Mezzetti Br., Battino M., ″ Plant genotype affect total antioxidant capacity and phenolic contents in fruit ″, Elsevier, Nutrition 21, (2005), 207–213.

27. Gardner P.T., White T.A.C., McPhal D.B., DuthieG.C., ″ The relative contri-

butions of vitamine C, carotenoids and phenolics to the antioxidants potential of fruit juices ″, Elsevier, Food Chemistry 68 (2000) 471–474.

28. Nousis K. J. , ″ The new Arboriculture ″, volume B′, 2nd edition, Athens, 1984.

29. Rice-Evans A. C. Measurement of total antioxidant action as a marker of antioxidant status in vivo. Proceedings and limitations. Free Radical Research 33 (2000) 59−68.

30. Antolovich M., Prenzler P., Robards K., Ryan D. Sample preparation in the determination of phenolic compounds in fruits. Analyst 125(2000) 989-1009.

31. Ryan D., Robards K., Lavaee S. Determination of phenolic compounds in olives by reverse-phase chromatography and mass spectrometry J. Chromatography A, 832 (1999) 87-96.

32. McDonald S., Prenzler P.D., Antolovich M., Robards K. Phenolic content and antioxidant activity of olive extracts Food Chemistry 73(2001) 73-84

33. Vazquez Roncero A., Del Valle L.J., Del Valle C.J. Componentes fenolicos de la aceituna III. Polifenoles del aceite. Grasas y aceites. 27 (1976) 185-191.

34. Vazquez Roncero A., Graciani C., Maestro Duran R. Componentes fenolicos de la aceituna I. Polifenoles de la pulpa. Grasas y aceites , 25(5), (1974) 269-279.

35. Robards K., Prenzler P.D., Tucker G., Swatsitang P., Glover W. Phenolic compounds and their role in oxidative processes in fruits. Food Chem. (1999), 66: 401–36.

36. Bandoniene D., Murcovic M., Pfannhauser W., Venskutonis P.R. and Gruzdiene D., Eur. Food Res.Technol., 214(2002) 143-147.

37. Moure A. ,Cruz J.M., Franco D., Dominguez J.M.,Sineiro J., Dominguez H., Nunez M.J., Parajo J.C., Natural antioxidants from residual sources. Food Chemistry (72), (2001), 145-171.