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Nutritional composition and flavonoid content of edible wild greens and green pies: a potential rich source of antioxidant nutrients in the Mediterranean diet

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Abstract

The traditional Greek diet is dominated by the high consumption of olive oil, fruit and vegetables. Antioxidants represent a common element in these foods and may be important mediators of the beneficial effect of this diet. Wild edible greens are frequently consumed throughout Greece. Seven edible wild greens and traditional Cretan green pies were analyzed for their nutritional composition and flavonoid content, in particular flavonols and flavones. A high nutritional value and a low energy value characterize the wild greens. These wild greens have a very high flavonol content when compared with regular fresh vegetables, fruits and beverages commonly consumed in Europe. *Rumex obtusifolius* was found to contain twice the amount of quercetin contained in onions. Two pieces of Cretan green pie (100 g) contain approximately 12 times more quercetin than one glass of red wine (100 ml) and three times more quercetin than a cup of black tea (200 ml). Wild greens potentially are a very rich source of antioxidant flavonols and flavones in the Greek diet. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Wild greens; Flavonoids; Nutritional composition; Mediterranean diet

1. Introduction

The Mediterranean diet and, in particular the traditional Greek diet, is currently attracting considerable interest because of its likely health benefits (Willett, 1994). Olive oil, fruit and vegetables are central to this diet and many components associated with these foods could contribute to the apparent health effects of the Greek diet. In Greece, the consumption of vegetables includes the various wild greens, which are traditionally collected throughout the country and consumed in various manners. Several authors have hypothesized that antioxidants may be important mediators of this beneficial dietary effect (Trichopoulou, Lagiou & Papas, 1998; Visioli & Galli, 1998). Lionis and his colleagues have recently demonstrated that popular herb extracts on the island of Crete have a high antioxidant capacity towards lipid peroxidation (Lionis, Fatesjo, Skoula, Kapsokefalou & Faresjo, 1998). To our knowledge, however, no attempt has been made to measure specific antioxidants of wild greens commonly consumed as part of the traditional Greek diet. Flavonoids are generally considered as an important category of antioxidants in the human diet (Cotelle, Bernier, Herichart, Catteau, Gaydon & Wallet, 1992; Limasset, Le Doucen, Dore, Ojasoo, Damon & de Paulet, 1993; Middleton & Kandaswami, 1992).

We report here on the nutritional composition and the content of flavonols and flavones, two subclasses of flavonoids, in wild edible greens frequently eaten in rural Greece in the form of salads, mixed dishes and

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pies. Indicatively we present data for one of the many traditional green dishes, the Cretan green pies.

2. Materials and methods

A traditional village of Crete was visited by E.V. in mid-April 1997 and with the valuable guidance and help of the women of the village, 13 different types of edible wild greens were collected. These greens were used for the preparation of the traditional Cretan green pies, which were studied. Cretan green pies are small half-moon shaped pastry filled with a mixture of wild greens and fried in virgin olive oil. According to the local recipe, the finely chopped wild greens are not boiled but cooked with plenty of virgin olive oil and only a little water for approximately an hour at a medium temperature. They are then left to cool and drain before filling the pastry.

The nutritional composition and flavonoid contents were determined in the pie and in 7 of the greens. The English, Greek, and botanical names of the greens are presented in Tables 1 and 3. Each type of green consisted of about 50 individual sub-samples taken at various locations in the village. These sub-samples were mixed in equal portions to give the composite sample that was analyzed. The greens were thoroughly washed with plenty of water in order to remove any soil or dirt and chopped very finely. Then the raw greens were homogenized by a common household vegetable blender, pre-frozen at −30°C and freeze dried. Thus, these freeze-dried samples represented the raw edible parts of the greens, the leaves and the tender stems including variation in location. Using the 13 types of greens collected about 15 pies were prepared. Representative samples of about equal weight of each pie were mixed to give a composite sample. This composite pie sample was homogenized by an Ultra Turrax T25 mixer, pre-frozen at −30°C and freeze dried. Freeze-dried samples of the greens and the fried pies were sent to RIKILT DLO, Wageningen, The Netherlands for analysis of their favonoid content, in particular flavonols and flavones, as well as to the Greek State Laboratory and ETAT S.A. for the determination of the nutritional composition and the inorganic constituents.

The analytical methods performed were the following: Flavonoid *determination*. The quantative determination of the following six major flavonols and flavones, quercertin, kaempferol, myrecetin, isorhamnetin, luteolin and apigenin after acid hydrolysis of their glycosides was performed by a Reversed-Phase HPLC method with UV detection. (Hertog, Hollman & Venema, 1992). In brief, flavonoid glycosides were extracted and hydrolysed to their aglycones with HCl in 50% aqueous methanol. Completeness of hydrolysis was checked using 3 different hydrolysis conditions as described. (Hertog, Hollman & Venema). Subsequently, the resulting aglycones were separated on a NOVA-PAK C18 column using methanol/phosphate buffer (45/55, v/v, pH 2.4) as mobile phase, and UV detection (370 nm). Peak identity and purity was checked using a photodiode array detector to record UV-spectra of the flavonoids in samples on-line. Flavonoids were quantified using a calibration curve of pure aglycone standards (FLUKA: myricetin No.70050, quercetin dihydrate No.83370, kaempferol No.60010, and apigenin No.10790; ROTH: luteolin No.5801; EXTRASYNTHESE: isorhamnetin No.1120S). Samples were analysed in duplicate; RSDduplicates $<4\%$. With each series of samples 3 different quality control samples (mixture onions and leek; celery; a mixture of lettuce and endive) were included. RSD of reproducibility of these quality control samples was within 8%. (Fig, 1)

Moisture determination. The moisture content was evaluated during the freeze drying procedure by difference. (Flink & Knudsen, 1983) *Protein determination* — the detemination of nitrogen was carried out using the Kjeldahl method (Association of Analytical Chemists [AOAC, 1980]). *Lipids determination* — the total lipid content was determined by extraction, according to the Soxhlet method, after acid hydrolysis of the sample (Egan, Kirk & Sawyer, 1987) *Carbohydrate*

Table 1
Flavonoid content of composite samples of Greek edible wild greens and green pies[a]

Food			Myricetin mg/100 g	Quercetin mg/100 g	Kaempferol mg/100g	Isorhamnetin mg/100 g	Luteolin mg/100g	Apigenin mg/100 g
Fennel	Maratho	(*Foeniculum vulgare* Mill)	19.8	46.8	6.5	9.3	0.1	<0.07
Chive	Agriopraso	(*Allium schoenoprasm*)	<0.03	10.4	12.5	8.5	0.3	<0.07
Annual Saw-thistle	Zochos	(*Sonchos oleraceus* L.)	3.6	16.0	3.8	0.7	6.5	3.8
Hartwort	Kaukalithra	(*Tordylium apulum*)	1.6	29.3	2.9	5.1	0.6	<0.08
Corn poppy	Koutsounada	(*Papaver rhoeas* L.)	1.1	26.3	2.3	1.1	0.1	0.1
Broad leaf Dock	Lapatho	(*Rumex obtusifolius* L.)	5.7	86.2	10.3	<0.03	<0.02	<0.05
Queen Anne's Lace	Stafilinakas	(*Daucus carota*)	0.4	1.1	0.2	<0.06	34.1	12.6
Cretan green pie			1.4	12.4	4.3	1.8	6.6	<0,3

[a] Composite samples of edible wild greens consisted of about 50 individual sub-samples taken at various locations in a village. Composite sample of the pies consisted of 15 pies individually prepared. Each composite sample was analysed in duplicate. Contents are expressed as mg/100g of food.

Table 2
Published data on the flavonoid content of selected vegetables, fruits and beverages commonly consumed in Europe[a,b,c]

Food		Myricetin mg/100 g	Quercetin mg/100 g	Kaempferol mg/100 g	Luteolin mg/100 g	Apigenin mg/100 g
Lettuce	(*Lactuca Sativa* L. cv. *capitata* L.)	< 0.1	1.4–7.9	< 0.2	< 0.1	< 0.2
Onion	(*Allium cepa* L.)	< 0.1	34.0–34.7	< 0.2	< 0.1	< 0.2
Endive	(*Chicorium endiva* L.)	< 0.1	< 0.1	4.6	< 0.1	< 0.2
Broad beans	(*Vicia Faba* L.)	2.6	2.0	< 0.2	< 0.1	< 0.2
Celery	(*Apium graveolens* var. *dulce Pers*)	< 0.1	< 0.1	< 0.2	0.5–2.2	1.6–10.8
Apple	(*Malus pumila* Mill.)	< 0.1	2.0–3.6	< 0.2	< 0.1	< 0.2
Red wine		0.9	1.1	< 0.1	< 0.05	< 0.1
Black tea		0.3	1.4–1.7	1.4–1.6	< 0.05	< 0.1
Apple juice		< 0.05	0.3	< 0.1	< 0.05	< 0.1

[a] Source: Crozier et al., 1997; Hertog et al., 1993; Hertog, Hollman & Katan, 1992; Justesen et al., 1998..
[b] Average values reported in these papers are given. Whenever average values of two or more papers were available, the lowest and highest averages are given.

Table 3
Nutritional composition of composite samples of Greek edible wild greens and green pies[a]

Food 100 g	Water g	Protein g	Lipids[b] g	Carbohydrate g	Dietary fiber	Energy value kcal	K mg	Na mg	Ca mg	Mg mg	Fe mg	P mg	Zn mg
Fennal, Maratho *Foeniculum vulgare* Mill	86.7	3.8	N/A	4.9	3.5	48	618.9	81.1	341.3	54.4	1.3	47.2	0.3
Chive, Agriopraso, *Allium schoenoprasm*	87.1	1.8	N/A	7.0	3.6	46	286.3	14.3	162.8	17.4	0.6	26.2	0.2
Annual Saw-thistle, Zochos, *Sonchus oleraceus* L.	91.9	1.8	N/A	3.1	2.6	29	319.3	73.8	126.6	24.9	0.6	41.5	0.5
Hartwort, Kaukalithra, *Tordylium apulum*	85.2	2.7	N/A	6.5	4.5	53	535.7	120.4	366.4	36.7	2.1	32.7	0.6
Corn Poppy, Koutsounada, *Papaver rhoes* L.	91.0	2.9	N/A	3.1	2.5	36	188.1	42.6	181.8	19.6	0.9	26.5	0.2
Broad leaf Dock, Lapatho, *Rumex obtusifolius* L.	89.9	2.9	N/A	3.9	2.7	38	420.1	50.4	60.2	51.5	1.1	61.2	0.4
Queen Anne's lace, Stafilinakas, *Daucus carota*	82.7	2.1	N/A	9.5	4.4	57	687.3	123.3	450.2	45.8	3.5	27.4	0.6
Cretan green pies	35.5	5.8	23.2	31.2	3.8	370	195.6	99.5	135.3	22.5	0.9	32.6	0.3

[a] Composite samples of edible wild greens consisted of about 50 individual sub-samples taken at various locations in a village. Composite sample of the pies consisted of 15 pies individually prepared. Each composite sample was analysed in duplicate.
[b] N/A = not available.

determination — the carbohydrate content of the food samples was estimated through the equation: Carbohydrate % = 100 — moisture%–lipids%–proteins%–metals%–dietary fiber% (McCance & Widdowson, 1991) *Dietary fiber determination* — the dietary fiber content was determined by the Englyst method. (Englyst, Quigley & Hudson, 1994) *Energy determination* — Energy determination was carried out using bomb calorimetry. (Box & Cameron, 1982) *Mineral determination* — the potassium (K) and sodium (Na) content of the samples was determined through the flame photometric method (Association of Official Analytical Chemists [AOAC], 1990a). The calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) content was determined by atomic absorbtion spectrophotometry (AOAC, 1990a). The total phosphorus (P) content was determined by assessing the orthophosphoric ions in an aqueous solution, by the ascorbic acid method (In house method, AOAC, 1990b; Pearson, 1981).

Quality control was conducted by the food analysis performance Assessment Scheme (FAPAS) which belongs to the Ministry of Agriculture and Fisheries of the United Kingdom for protein (Series X, Round 13: December 1997, z-score -1.5), dietary fiber (Series XVIII, Round 4: August 1998, z-score –1), Ca, Fe and Zn determination (Series XVIII, Round 3: April 1998, RSZ 1.2) and for the determination of lipids (Series I, Round 24: September 1999, z-score –1).

3. Results

The specific flavonoid groups that were analysed are the flavonols (-myricetin, quercetin, kaempferol, and isorhamnetin-) and the flavones (-luteolin and apigenin-) (Table 1). It is clear that these wild greens have a very high flavonol content when compared with regular fresh vegetables, fruits and beverages commonly consumed in Europe (Crozier, Lean, McDonald & Black, 1997; Hertog, Hollman & Katan, 1992; Hertog, Hollman & van de Putte, 1993; Justesen, Knuthsen & Leth, 1998) (Table 2). The prominent flavonol in the examined wild greens was quercetin, followed by kaempferol. Lapatho

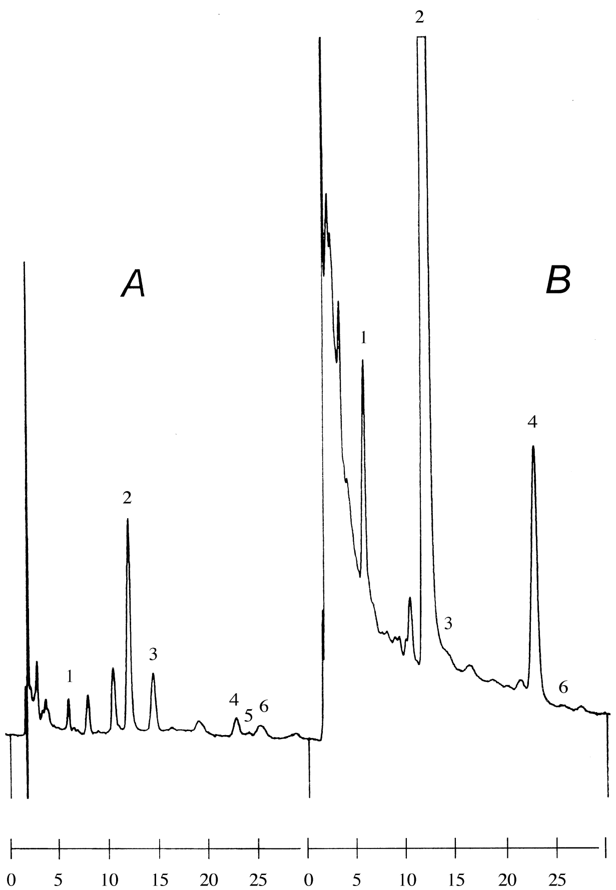

Fig. 1. Typical chromatograms of a pie extract (A) and a Lapatho extract (B) monitored in methanol/phosphate buffer (45/55, v/v, pH 2.4). Detection at 370 nm. 1. Myricetin; 2. Quercetin; 3. Luteolin; 4. Kaempferol; 5. Apigenin; 6. Isorhamnetin.

proved to contain extremely high quantities of quercetin. It is worth pointing out that it contains twice the amount of quercetin contained in onions. Onions are a principal source of this flavonoid in The Netherlands (Hertog, Hollman & Katan, 1992). Maratho, kaukalithra, and koutsounada also have a high level of quercetin, whereas in addition, agriopraso and lapatho are rich in kaempferol. Stafilinakas shows a low flavonol content, but in contrast to the other greens, shows a high flavone content (-luteolin and apigenin-). Whilst in each food item a certain flavonoid subgroup prevails, the green pies contain significant quantities of both subgroups (flavonols and flavones) due to the mixture of wild greens used for their preparation.

A high nutritional value and a low energy value characterizes the wild greens (Table 3). Their contribution to the protein and dietary fiber content of the green pies is apparent. Several greens contain significant quantities of iron. Specifically stafilinaka's iron content tends to approach the iron content of spinach. The high mineral content of the Cretan greens could possibly be partly due to the composition of the Cretan soil.

Extra virgin olive oil is the only lipid used for the preparation of the Cretan green pies as the traditional recipe does not include the addition of other types of lipids like vegetable oils, cooking fats, butter, cheese or milk. Consequently the total lipid content and over 50% of the energy value of the pies derives exclusively from extra virgin olive oil.

4. Discussion

The consumption of vegetables and olive oil prevails in the Mediterranean Diet. (Trichopoulou & Lagiou, 1997, 1998). In Greece, the consumption of vegetables includes the various wild greens, which are traditionally collected throughout the country and consumed in various ways, usually prepared with virgin olive oil. The daily availability of edible wild greens is 20 g /person, which is equal to 10% of the total daily availability of vegetables (the data has been retrieved from the Dafne Databank). The present paper reports data on 7 wild greens, which represent only a fraction of the total number of edible wild greens consumed in Greece, which surpasses 150.

These wild greens, raw and cooked (fried pies), proved to contain high quantities of quercetin. In fact they contain significantly higher quantities than both red wine and black tea, which are considered main sources of quercetin for North European countries (Hertog, 1994). Lapatho contains twice the amount of quercetin as onions, which are also considered a principal source of quercetin. Two pieces of Cretan green pie (100 g) contain about 12 times more quercetin than one glass of red wine (100 ml), and three times more quercetin than a cup of black tea (200 ml). (Tables 1 and 2)

Thus, wild greens potentially are a very rich source of antioxidant flavonols and flavones in the Greek diet.

Wild greens are usually consumed with virgin olive oil, which is the only edible lipid that contains polyphenolic compounds. Subsequently the wild green dishes contain a rich mixture of polyphenolic antioxidants. This combination of wild greens with olive oil might possibly contribute to the apparent health promoting effects of the Greek diet (Trichopoulou, Lagiou & Papas, 1998).

Until recently the evaluation of the Mediterranean diet focused on the low content of saturated fatty acids and the high content of composite carbohydrates and dietary fiber. Recent research implies that other compounds of the Mediterranean diet, the antioxidants, which exist in abundance in vegetables, fruit, beverages and also virgin olive oil, may contribute to the prevention of heart diseases and possibly some forms of cancer and other diseases (Duarte et al., 1993; Frankel et al., 1993; Negre-Salvagyre & Salvagyre, 1992). Fruits, vegetables and beverages contain a significant amount of flavonoids (flavonols, flavones, flavanones, flavans and anthocyanins) whereas virgin olive oil, which is the basis of the Mediterranean diet, contains high amounts of polyphenolic antioxidants of various subclasses, such as hydroxytyrosol, phenolic acids, and oleuropein.

While there is no direct evidence that these antioxidants are central to the benefits of the Mediterranean Diet, indirect evidence from epidemiological data and the increasing understanding of their mechanisms of action suggest that antioxidants may play a major role. Ongoing research will help elucidate the role of antioxidants in the significant benefits of the traditional Mediterranean diet.

Wild greens are central to the Greek traditional diet and they have generally been overlooked as minor or trivial constituents. Their high content in flavonoids and the potential importance of the latter in the prevention of several chronic diseases requires reconsideration of the role of these greens in the traditional as well as the contemporary diet.

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References

Association of Official Analytical Chemists. (1980) *Official methods of analysis*. 7.015 Kjeldahl method, p. 126.

Association of Official Analytical Chemists. (1990a) *Official methods of analysis* (15th ed.) (K, Na) method 956.01, p.47, (Ca, Mg, Fe, Zn) method 975.03, p.42.

Association of Official Analytical Chemists. (1990b). In house method. *Official methods of analysis* (15th ed.). (p.56).

Box, B. A., & Cameron, A. G. (1982). *Food Science a chemical approach* (4th ed.). UK: Hodder and Stoughton Ltd.

Cotelle, N., Bernier, J. L., Henichart, J. P., Catteau, J. P., Gaydou, E., & Wallet, J. C. (1992). Scavenger and antioxidant properties of ten synthetic flavones. *Free Rad Biol Med., 13*, 211–219.

Crozier, A., Lean, M. E. J., McDonald, M. S., & Black, C. (1997). Quantative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal of Agriculure and Food Chemistry, 45*, 590–595.

Duarte, J., Perez-Vizcaino, F., Zarzuelo, A., Jimenez, J., & Tamargo, J. (1993). Vasodilator effects of quercetin in isolated rat vascular smooth muscle. *European Journal of Pharmacology, 239*, 1–7.

Egan, H., Kirk, R. S., & Sawyer, R. (1987). *Pearson's chemical analysis of foods*, (8th ed.). Longman Scientific & Technical (p. 22).

Englyst, H. N., Quigley, M. E., & Hudson, G. J. (1994). Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst, 119*, 1497–1509.

Flink, M. J., & Knudsen, H. (1994). *An introduction to freeze drying*. Denmark: Strandberg Bogtryk/Offset a·s.

Frankel, E. N., Kanner, J., German, J. B., Parks, E. and Kinsella, J. E. (1993). Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet, 43* 454–457

Hertog, M. G. L. (1994) Flavanols and flavones in foods and their relation with cancer and coronary heart disease risk. PhD thesis, Agricultural University Wageningen. Grafisch Service Centrum Van Gils BV, Wageningen, the Netherlands.

Hertog, M. G. L., Hollman, P. C. H., & Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *Journal of Agricultural and Food Chemistry, 40*, 2379–2383.

Hertog, M. G. L., Hollman, P. C. H., & van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of Agricultural and Food Chemistry, 41*, 1242–1246.

Hertog, M. G. L., Hollman, P. C. H., & Venema, D. P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultral and Food Chemistry, 40*, 1591–1598.

Justesen, U., Knuthsen, P., & Leth, T. (1998). Quantative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photodiode array and mass spectrometric detection. *Journal of Chromatography A, 799*, 101–110.

Limasset, B., Le Doucen, C., Dore, J.-C., Ojasoo, T., Damon, M., & de Paulet, A. C. (1993). Effects of flavonoids on the release of reactive oxygen species by stimulating human neutrophils: multivariate analysis of structure-activity relationships. *Biochemical Pharmacology, 46*, 1257–1271.

Lionis, C., Fatesjo, A., Skoula, M., Kapsokefalou, M., & Faresjo, T. (1998). Antioxidant effects of herbs in Crete. *Lancet, 352*, 1987–1988.

McCance & Widdowson. (1991). *The composition of foods* (5th ed.). Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food. Bungay, Suffolk: Richard Clay.

Middleton, E., & Kandaswami, C. (1992). Effects of flavonoids on immune and inflammatory cell functions. *Biochemical Pharmacology, 43*, 1167–1179.

Negre-Salvagyre, A., & Salvagyre, R. (1992). Quercetin prevents the cytotoxity of oxidized low-density lipoproteins by macrophages. *Free. Rad. Biol. Med., 12*, 101–106.

Pearson's Chemical analysis of foods. (1981). Churchill Livingstone (p. 30).

Trichopoulou, A., Lagiou, P., & Papas, A. (1998). Mediterranean diet: are antioxidants central to its benefits? In A. Papas, *Antioxidant status, diet, nutrition and health*. (pp 107–116). CRC Press.

Trichopoulou, A., & Lagiou, P. (1998). *Methodology for the exploitation of HBS food data and results on food availability in six European countries*. EC publication ISBN 92-828-4294-0.

Trichopoulou, A., & Lagiou, P. (1997). Methodology for the exploitation of HBS food data and results on food availability in 5 European countries. EC publication ISBN 92-828-1570-6

Visioli, F., & Galli, C. (1998). The effect of minor constituents of olive oil on cardiovascular disease: new findings. *Nutrition Reviews, 56*, 142–147.

Willett, W. C. (1994). Diet and health: What we should eat? *Science, 264*, 532–753.