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Tannin content in acorns (*Quercus* spp.) from Poland

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Abstract: Oak acorns used to be an important human food, up until recent times. The major factor inhibiting their use in modern nutrition is their high tannin content. Polish oak trees were screened in order to find out whether there are any major geographical or interspecific differences in tannin and total phenolic contents in acorns, which might help us to establish further directions in the search for low-tannin individuals. We studied the level of phenolic compounds using the standard Polish and European Pharmacopoeia method, with Folin-Ciocalteu reagent and hide powder. Altogether 49 acorn samples of *Quercus robur*, 13 of *Q. petraea*, 1 of *Q. pubescens* (all native) and 12 of *Q. rubra* (introduced) were collected in different regions of Poland. The amount of investigated phenolics in *Q. robur* and *Q. petraea* acorns was similar, and tannins constituted the main component. The *Q. pubescens* sample was distinguished by the lowest tannin and total phenolic content and a relatively high amount of non-tannin phenolics. *Q. rubra* had a slightly lower tannin content than *Q. robur* and *Q. petraea* acorns, but the level of other phenolics was much higher. The results for *Q. robur* suggest geographical variability of phenolic content as well as a relationship between the phytochemical and biometric parameters of oak acorns.

Additional key words: polyphenols, oak acorns, phytochemistry, food security

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Introduction

Oaks (*Quercus* spp.) are one of the key species in temperate ecosystems in the Northern hemisphere. They not only harbor a large fauna of invertebrates, which feed on their leaves and acorns (Southwood 1961; Kennedy and Southwood 1984), but they have constituted one of the most sought after sources of calories for vertebrates (e.g. boar, rodents, jays etc.)

and for humans in foraging economies (Maurizio 1927; Bainbridge 1986; Johns and Duquette 1991; Mason 1995, 2000; Massei et al. 1996; Moerman 1998; Mason and Nesbitt 2009; Turner et al. 2011). Oak acorns were also used as emergency food in war times in societies, which had previously abandoned their use. Oak kernels (*Quercus semen*), especially roasted ones (*Quercus semen tostum*), were also applied in traditional medicine throughout Europe

(e.g. Paluch 1984; Rakić et al. 2007). Their past importance in human and animal nutrition, as well as in phytotherapy, has caused some interest in their chemical composition (Ofcarcik and Burns 1971; Cantos et al. 2003; Rakić et al. 2004, 2005, 2006, 2007; Kobs 2008).

The limiting factor in the use of acorns in human and animal nutrition is their high tannin content (Smallwood and Peters 1986; Rakić et al. 2007; Pour et al. 2010). Plant raw materials with high levels of tannins are widely used in phytotherapy, and they show antibacterial, astringent, antihemorrhagic and antidiarrhoeal properties. Internally, they are used to treat catarrh in the intestines and stomach (e.g. oak bark), as well as diarrhea (Kozłowski et al. 2009), but in large quantities, tannins are toxic to humans and disturb the ingestion of nutrients from the digestive tract (Chung et al. 1998). At high concentrations, tannins can also damage the gut epithelium and liver and kidney tissues (Singleton and Kratzer 1973; Chung-MacCoubrey et al. 1997). Finally, tannins at high levels can incur metabolic costs because of increased detoxification requirements (Chung-MacCoubrey et al. 1997). High levels of tannins also give food a bitter taste, making it unattractive for consumption. Some indigenous methods of removing tannins from acorns, or at least decreasing tannin content, have been described (Moerman 1998). These are soaking in running water, leaching, and cooking with clay as an adsorbent. In Poland and other Eastern European countries, acorns were commonly used to make a coffee substitute until the mid-20th century, and this tradition is reviving now (e.g. Łuczaj 2008; Dénes et al. 2012; Kalle and Sõukand 2012). They were also commonly used as an admixture to flour to make bread, but only within the context of food scarcity (Maurizio 1927; Łuczaj and Szymański 2007; Łuczaj 2008). Within Europe, acorns had much more importance in the Mediterranean than in northern Europe. In some parts of the Mediterranean, such as Spain and Sardinia, the acorns of *Q. ilex* L. are still eaten even nowadays (Johns and Duquette 1991; Tardío et al. 2006; Pignone and Laghetti 2010), and the use of roasted *Q. virgiliana* (Ten.) Ten. acorns was also recorded in southern Italy (Pieroni et al. 2005), whereas in northern Europe, where *Q. robur* is the commonest species, they have been forgotten even as famine food (Łuczaj 2011; Dénes et al. 2012; Kalle and Sõukand 2012; Łuczaj et al. 2012). It is not unlikely that their larger importance in the south stems from the fact that *Q. ilex*, *Q. virgiliana* and *Q. pubescens*, the commonest oaks in the Mediterranean, are perceived as sweeter and less bitter than *Q. robur*, the dominant species in northern Europe. Additionally, Mazuelos Vela et al. (1967) found a very low tannin content in *Q. ilex* acorns (0.4–0.5%). The acorns of *Quercus* have also been used as food in Asia (Pember-

ton and Lee 1996; Hu 2005; Kang et al. 2012, 2013). As oaks are often components of climax vegetation, growing varieties of native oaks for food could enable food production in semi-natural woodlands. However, oaks are slow-growing plants, thus it is difficult to produce new cultivars. Intraspecific variability in the content of secondary plant metabolites is widespread in nature (e.g. Sidjimova et al. 2011; Adamczak et al. 2012a; Mirgos et al. 2012; O'Reilly-Wapstra 2013), and perceived by animals as they make their feeding choices (Smallwood and Peters 1986; Kimball et al. 2012). Phytochemical differentiation, even in closely related species, is also very widespread (e.g. Nowak 2005; Adamczak 2012b; Çaliskan et al. 2012; Edwards et al. 2012). There is quite a lot of data on tannin content in oak acorns in North America (Trimble 1896; Ofcarcik and Burns 1971; Fleck and Layne 1990; see also Mason 1992 for an overview of some of these papers), although very few such studies were performed in Europe (Rakić et al. 2004, 2005, 2006, 2007), and the tannin content of many common species (e.g. *Q. petraea*) has never been studied before. No screening of oak populations has been performed on a larger scale to study the natural variation in their levels of tannins. All previous studies of oak acorns were limited to samples from one or few localities. Our aim was to fill this gap and study the diversity of tannin and total phenolic content in acorns originating from Poland.

Only two native species of oaks and one exotic taxon are common in Poland. The English oak (*Q. robur* L.) can be found in all parts of Poland, except for higher elevations, and it is very common in a variety of habitats, from dry to moist. It is both an important forestry tree and a component of parks and gardens. The sessile oak (*Q. petraea* (Matt.) Liebl., syn. *Q. sessilis* Ehrh.) occurs mainly in the lowlands, except for some parts of NE Poland. It is not as common as the English oak, and only occasionally becomes sub-dominant in forests. This species is mainly restricted to drier habitats, where it usually grows together with Scots pine (*Pinus sylvestris*) or English oak. The third taxon of oak, the pubescent oak (*Q. pubescens* Willd.), can be found only in one small location in NW Poland, in the Odra valley (Chybicki et al. 2012). It is a sub-Mediterranean species widely distributed in southern and south-central Europe, mainly in calcareous soils. All three native oaks belong to the *Quercus* subgenus (= *Lepidobalanus* Endl. *pro parte*) and *Quercus* section (Boratyński et al. 2006). After the English oak, the second most common oak species in Poland is the introduced red oak (*Q. rubra* L.), extensively planted in forests and parks and often naturalized.

As oaks of the subgenus of *Erythrobalanus* (Spach) Oerst. (e.g. *Q. rubra*) are regarded as more bitter than other oaks (e.g. Ofcarcik and Burns 1971), our hypothesis was that the introduced *Q. rubra* would have

the most bitter acorns. In North America the mean content of tannins found in the *Erythrobalanus* oaks ("red oaks") was 9.8% (ranging from 2.9 to 20.3%), and in *Lepidobalanus* oaks ("white oaks") it was 7.0% (ranging from 3.3 to 10.9%) (Mason 1992). However we also assumed that some differences would be found between *Q. robur* and *Q. petraea*, or between geographical races/forms of *Q. robur*.

Material and Methods

Plant material

Oak acorns were collected, in October and November 2011 and 2012, from under individual trees. For each sample 20–30 acorns were usually taken, air-dried at room temperature (23–25°C), then shelled out and powdered. For phytochemical analysis, oak kernels – seeds (*Quercus semen*) were used. A variety of measurements were also made (weight before and after air-drying, and after shelling, as well as length and width) for acorns collected in 2012. Altogether, 49 acorn samples of *Quercus robur*, 13 of *Q. petraea*, 1 of *Q. pubescens* and 12 of *Q. rubra* were collected in various parts of Poland (Fig. 1, Appx 1). As most of our *Q. robur* samples came either from western or south-eastern Poland, we compared these two groups of localities in some of our analyses (Fig. 1). The single sample of *Q. pubescens* (which is extremely rare in Poland) was collected from the Adam Mickiewicz University Botanical Garden in Poznań, from a tree originating from the only native population of this species in Poland (from the Bielinek reserve).

Phytochemical analysis

Phytochemical analysis was conducted according to the Polish and European Pharmacopoeia (2006, 2008, 2010). Binding by raw hide was used as a way of determining tannin content. Use of collagen-rich raw hide powder is an easy way to estimate the level of tannins, due to their protein-binding properties (Seigler et al. 1986). The amounts of total polyphenols (phenolics) and polyphenols unadsorbed on hide powder (non-tannin phenolics) were determined spectrophotometrically with Folin-Ciocalteu reagent, for 1.0 g of air-dried, powdered oak seeds after 30 min of hot water extraction in a water bath. The absorbance was measured at $\lambda=760$ nm. The tannin content (expressed as pyrogallol equivalent) was calculated as the difference between total phenolics and non-tannin phenolics (Rakić et al. 2006; Adamczak et al. 2012a). The obtained results were calculated for dry matter (DM). The moisture content [%] in the raw material was measured after drying it at 105°C to constant mass. Folin-Ciocalteu reagent and sodium carbonate decahydrate were purchased

from POCh. Pyrogallol (1,2,3-trihydroxybenzene) and hide powder were obtained from Sigma-Aldrich.

All analytical procedures were performed with protection from light. Acorn samples were extracted with 150 ml of water for 30 min in a boiling water bath, then the flask with extract was cooled and made up with water to 250 ml. After the sedimentation of plant material, the water extract was filtered, and the first 50 ml of filtered liquid was rejected. Total polyphenols were determined from 5 ml of filtrate diluted with water to 25 ml. To 2 ml of this solution 1 ml of Folin-Ciocalteu reagent was added, then 10 ml of water, and the flask was filled up to 25 ml with sodium carbonate solution (290 g/l). The absorbance was measured after 30 min of incubation in darkness, using water for compensation. The content of non-tannin phenolics was determined from 10 ml of filtrate with addition of 0.1 g hide powder, after shaking (60 min) and filtering of the solution. From this solution, 5 ml was made up with water to 25 ml and the further procedure was repeated as above. The reference standard solution was prepared from 50 mg pyrogallol in water diluted up to 100 ml. Afterwards, 5 ml of this solution was diluted with water to 100 ml, and 2 ml was used for further analysis with Folin-Ciocalteu reagent, as described above. The tannin content (%) was calculated with the following formula:

$$X = 62.5 \times (A_1 - A_2) \times m_2 / A_3 \times m_1$$

where: X – tannin content; A_1 – absorbance of total phenolics; A_2 – absorbance of phenolics unadsorbed on hide powder; A_3 – absorbance of reference solution of pyrogallol; m_1 – mass of investigated sample [g]; m_2 – mass of pyrogallol [g].

Statistical analysis

The Shapiro-Wilk test was applied to check the normality of variable distribution, and F-test and Levene's test were used to analyse the homogeneity of variances. For the skewed distribution of variables, square root, logarithmic and inverse proportion transformations of data were performed. In the analysis of intra- and interspecific differences, Student, Mann-Whitney and Kruskal-Wallis tests were used. Pearson's correlation and Spearman's rank correlation were applied to evaluate the relationships between variables. An open access statistical program PAST (Hammer et al. 2001; PAST 2012) and Statistica 7.1 software (Statistica 2005) were used for analyses.

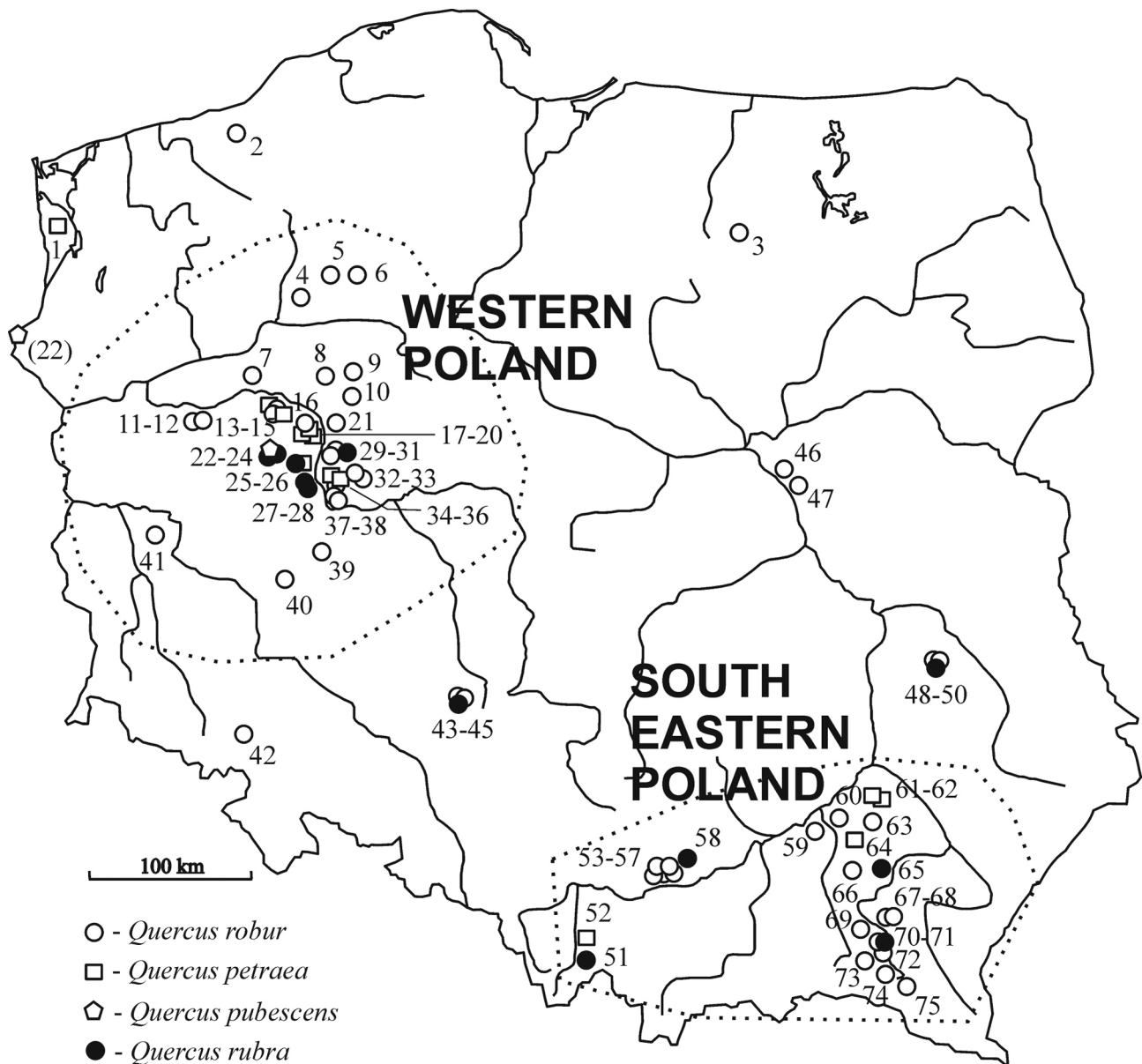


Fig. 1. Distribution of acorn samples

1 – Police near Szczecin (*Q. petraea*), 2 – Strzekęcino near Koszalin (*Q. robur*), 3 – Tylkowo near Olsztyn (*Q. robur*), 4 – Skórka near Piła (*Q. robur*), 5 – Śmiardowo Złotowskie near Złotów (*Q. robur*), 6 – Dorotowo near Złotów (*Q. robur*), 7 – Klempicz near Czarnków (*Q. robur*), 8 – Gościejewo near Rogoźno (*Q. robur*), 9 – Wągrowiec (*Q. robur*), 10 – Roszkowo near Wągrowiec (*Q. robur*), 11–12 – Międzychód (*Q. robur*), 13 – Obrzycko near Szamotuły (*Q. petraea*), 14–15 – Obrzycko near Szamotuły (*Q. robur*), 16 – Jaryszewo near Szamotuły (*Q. petraea*), 17 – Biedrusko near Poznań (*Q. robur*), 18–20 – Biedrusko near Poznań (*Q. petraea*), 21 – Zielonka near Poznań (*Q. robur*), 22 – Adam Mickiewicz University Botanical Garden in Poznań (*Q. pubescens*), (22) – the Bielinek reserve (the original locality of *Q. pubescens*), 23–24 – Adam Mickiewicz University Botanical Garden in Poznań (*Q. rubra*), 25 – Dendrological Garden of University of Life Sciences in Poznań (*Q. rubra*), 26 – Dendrological Garden of University of Life Sciences in Poznań (*Q. petraea*), 27–28 – Poznań Stare Miasto (*Q. rubra*), 29–30 – Poznań Nowe Miasto (*Q. robur*), 31 – Poznań Nowe Miasto (*Q. rubra*), 32–33 – Koninko near Poznań (*Q. robur*), 34–35 – Daszewice near Poznań (*Q. petraea*), 36 – Daszewice near Poznań (*Q. robur*), 37–38 – Rogalin near Mosina (*Q. robur*), 39 – Dusina near Gostyń (*Q. robur*), 40 – Bojanowo near Leszno (*Q. robur*), 41 – Zielona Góra (*Q. robur*), 42 – Modlęcín near Świebodzice (*Q. robur*), 43–44 – Bąków near Kluczbork (*Q. robur*), 45 – Bąków near Kluczbork (*Q. rubra*), 46 – Warszawa Rembertów (*Q. robur*), 47 – Pilawa near Warszawa (*Q. robur*), 48 – Lublin Śródmieście (*Q. robur*), 49 – Lublin Konstantynów (*Q. robur*), 50 – Lublin Konstantynów (*Q. rubra*), 51 – Porąbka near Bielsko-Biała (*Q. rubra*), 52 – Kęty near Bielsko-Biała (*Q. petraea*), 53–54 – Kraków Bronowice (*Q. robur*), 55 – Kraków Czyżyny (*Q. robur*), 56 – Kraków Grzegórzki (*Q. robur*), 57 – Kraków Stare Miasto (*Q. robur*), 58 – Raciborowice near Kraków (*Q. rubra*), 59 – Suchy Grunt near Mielec (*Q. robur*), 60 – Mielec (*Q. robur*), 61–62 – Nowa Dęba (*Q. petraea*), 63 – Werynia near Kolbuszowa (*Q. robur*), 64 – Leszcze near Kolbuszowa (*Q. petraea*), 65 – Rzeszów (*Q. rubra*), 66 – Gnojnica near Ropczyce (*Q. robur*), 67–68 – Rzępnik near Krosno (*Q. robur*), 69 – Ustrobnia near Krosno (*Q. robur*), 70 – Krosno (*Q. robur*), 71 – Krosno (*Q. rubra*), 72 – Haczów near Krosno (*Q. robur*), 73 – Chorkówka near Krosno (*Q. robur*), 74 – Wróblík Królewski near Krosno (*Q. robur*), 75 – Mymoń near Krosno (*Q. robur*).
Geographical coordinates of acorn samples – Appx 1.

Table 1. Size and weight variability of oak acorns collected in 2012 (mean as well as minimum and maximum given in brackets)

Variables\Species	<i>Q. robur</i> (n=36)	<i>Q. petraea</i> (n=11)	<i>Q. rubra</i> (n=9)
acorn length [cm]***	2.71 (2.26–3.66)	2.63 (1.96–3.06)	2.14 (1.98–2.28)
acorn width [cm]***	1.48 (1.16–1.73)	1.56 (1.32–1.85)	1.87 (1.75–2.02)
length/width of acorns***	1.83 (1.48–2.23)	1.69 (1.48–1.81)	1.14 (1.02–1.21)
air-dry weight of acorns [g]*	3.15 (1.16–5.32)	3.12 (1.36–4.39)	3.86 (3.09–4.57)
air-dry weight of seeds [g] ^{N.S.}	2.63 (0.85–4.55)	2.52 (1.05–3.33)	2.39 (1.98–2.82)
seed share in the acorn weight [%]***	83 (73–87)	81 (75–85)	62 (55–68)

The length and width of acorns were determined on the basis of 20–30 measurements for each sample, while the weight of acorns and seeds – on the basis of one measurement for the whole sample. Kruskal-Wallis test for interspecific differentiation of the size and weight of acorns: N.S. – not significant ($p > 0.05$); * – $p < 0.05$; *** – $p < 0.001$; $n = 56$.

Results

Our research showed that oak acorns lose about 30% of their weight after air-drying, then acorn shells contain an average of 6.9% water, and acorn kernels (seeds) – 6.4%. Acorns of *Q. robur* (English oak) and *Q. petraea* (sessile oak) were characterized by a high variability of size and weight, but they did not differ from each other. Acorns of *Q. rubra* (red oak) were significantly different from the oak species native to Poland, and they showed a low variation in size and weight. It is interesting that the percentage share of seeds at the air-dry weight of *Q. rubra* acorns was significantly lower than in the case of *Q. robur* and *Q. petraea* acorns. Although *Q. rubra* acorns were on average heavier than those of the native oaks, their mean seed mass was slightly lower than that of the native species (Table 1). The investigated samples of oak acorns indicated a wide range of phytochemical variability. *Q. pubescens* acorns were distinguished by the lowest content of total phenolics (2.61% DM) and tannins (0.97%), and by a relatively high amount of non-tannin phenolics (1.64%). The level of total phenolics and tannins in the case of other species did not differ significantly. Tannins, especially for *Q. robur* and *Q. petraea* acorns, constituted the main component of polyphenols. Attention should be paid to the relatively low tannin content of *Q. rubra* acorns in relation to the level of non-tannin phenolics (Table 2). The total amounts of phenolics and tannins in *Q. robur* acorns collected in 2012 were significantly higher in SE Poland compared to W Poland. The geographical origin of samples did not affect the content

of non-tannins or the ratio of tannins to non-tannins. Acorns from western Poland were characterized by a slightly larger average size and weight, but these differences were not statistically significant (Table 3).

For *Q. robur* we found negative correlations between the size and weight of acorns and the level of the investigated groups of active compounds, but these correlations were not strong. The highest correlation was detected between the total level of phenolics and the air-dry weight of acorn seeds. Tannin content was most strongly associated with seed weight. In the case of the level of non-tannins, the highest correlation was found with acorn width. On the other hand, the ratio of tannins to non-tannins was not connected with the size and weight of *Q. robur* acorns. Similarly, the shape index of acorns (length/width) was not correlated with the amount of chemical compounds (Table 4).

Discussion

Our biometric studies of acorns indicate a high variability of *Q. robur* and *Q. petraea*, and lower differentiation of *Q. rubra* (Table 1). According to the literature, both native species are characterized by a large morphological variation. The length of *Q. robur* acorns ranges from 1.5 to 4.0 cm, width: 1.0–2.0 cm and weight: 2.00–6.90 g. In the case of *Q. petraea* acorns it is: 1.6–3.5 cm, 0.8–1.6 cm and 1.54–7.58 g, respectively (Boratyńska et al. 2006). The smaller variability of *Q. rubra* from Poland may be due to the fact that it is an alien species (the so-called “founder

Table 2. Mean content of total phenolics, tannins and non-tannin phenolics in the seeds of oak acorns collected in the years 2011–2012 (the minimum and maximum given in brackets)

Variables\Species	<i>Q. robur</i> (n=49)	<i>Q. petraea</i> (n=13)	<i>Q. rubra</i> (n=12)	<i>Q. pubescens</i> (n=1)
total phenolics [% DM] ^{N.S.}	4.33 (3.05–6.58)	4.29 (3.36–6.66)	4.58 (3.48–5.80)	2.61
tannins [% DM] ^{N.S.}	3.48 (2.43–5.17)	3.39 (2.57–4.75)	2.97 (2.10–4.26)	0.97
non-tannins [% DM]***	0.86 (0.57–1.89)	0.90 (0.58–2.06)	1.60 (1.29–2.00)	1.64
tannins/non-tannins***	4.25 (1.84–6.26)	4.39 (1.25–5.98)	1.90 (1.19–2.77)	0.59

Total phenolics, tannins and non-tannins – expressed as pyrogallol equivalent; tannins/non-tannins – the ratio of tannins to non-tannins; DM – dry matter of raw material. Kruskal-Wallis test for interspecific differentiation of the content of phenolic compounds (without *Q. pubescens*): N.S. – not significant ($p > 0.05$); *** – $p < 0.001$; $n = 74$.

Table 3. Differentiation of the polyphenol content and the biometric parameters of *Quercus robur* acorns collected in 2012 from sides in two regions of Poland (mean \pm SD)

Variables	Western Poland	South-Eastern Poland	<i>p</i> -value
total phenolics [% DM] ^a	3.89 \pm 0.45	4.35 \pm 0.45	0.0127
tannins [% DM] ^a	3.15 \pm 0.39	3.56 \pm 0.45	0.0146
non-tannins [% DM] ^a	0.74 \pm 0.12	0.78 \pm 0.12	0.3677
tannins/non-tannins ^b	4.32 \pm 0.67	4.67 \pm 0.93	0.3627
acorn length [cm] ^a	2.75 \pm 0.27	2.67 \pm 0.26	0.4360
acorn width [cm] ^a	1.52 \pm 0.12	1.47 \pm 0.11	0.3021
length/width of acorns ^a	1.81 \pm 0.13	1.82 \pm 0.20	0.8823
air-dry weight of acorns [g] ^a	3.28 \pm 0.73	3.15 \pm 0.56	0.5901
air-dry weight of seeds [g] ^a	2.76 \pm 0.61	2.62 \pm 0.52	0.5126
seed share in the acorn weight [%] ^b	84 \pm 1.8	83 \pm 2.4	0.2737

Total phenolics, tannins and non-tannins – expressed as pyrogallol equivalent; DM – dry matter of raw material; SD – standard deviation. Statistical tests: a – Student's test; b – Mann-Whitney test; n=28.

effect”, or “bottle-neck effect”). The high similarity of acorn size and weight, as well as phenolic content, in *Q. robur* and *Q. petraea* (Table 1–2) confirms that both species belong to one section and subsection of *Quercus* and are closely related (Boratyński et al. 2006). As expected, the acorns of *Q. pubescens* (*Galliferae* subsection), were low in tannin content, however not as low as *Q. ilex* (Mazuelos Vela et al. 1967). On the other hand it was surprising that *Q. rubra* acorns were not characterized by a higher level of tannins than *Q. robur* and *Q. petraea* (Table 2). The oaks of the *Erythrobalanus* subgenus, to which the red oak (*Q. rubra*) belongs, are generally regarded as more bitter and tannin-rich than other oaks (Ofcarcik and Burns 1971; Weckerly et al. 1989; Mason 1992).

The obtained results of phytochemical investigations are difficult to compare with the literature data, due to different methods of drying, extraction and analysis of acorn samples. Tannins are a very versatile group of usually polymeric compounds, and different analytical methods may exclude or include certain subgroups of this category of compounds. We used the hide powder method, as it is widely used in many countries (Seigler 1986; Rakić et al. 2006; Kobs 2008), although detailed procedures varied between the studies.

According to Rakić and coauthors (2005, 2006, 2007), the tannin content in *Q. robur* acorns varies, depending on the method of drying and extraction of samples, from 7.76 to 20.4%. It is interesting that the ratio of tannins to non-tannins was similar to our results (Table 2) and ranged from 2.78 to 10.73 (Rakić et al. 2006, 2007). On the other hand, Smallwood and Peters (1986) indicate that the tannin level in the white oak group *Lepidobalanus* is much lower and ranges from 0.5 to 2.5%. Pour et al. (2010) also show a low level of tannins in oak kernels (1.76%). Older American studies of acorn composition usually report larger tannin contents, which may be the result of different procedures used in analyses.

Comparative analysis of *Q. robur* samples collected in one year from western and south-eastern Poland showed significant differences in the total content of phenolics and tannins between these regions (Table 3). This may indicate the geographical variability of the level of investigated chemical compounds in oak acorns. It was interesting that these samples were different, although only slightly, in terms of size and weight, too (Table 3). Therefore, the relations between phytochemical and biometric parameters of *Q. robur* acorns were studied (Table 4). The results obtained suggest that the amount of polyphenols is connected with the size and weight of acorns. This may be a clue towards the gathering of lower tannin fruits. However, it is important to find out whether this phenomenon is caused by the fact that oaks growing in better habitat (climatic) conditions produce larger and lower-tannin acorns or whether genetic factors are responsible for this relationship.

Further research on the tannin content of south European oak acorns should be carried out in order to find out whether the larger popularity of acorns as food in southern Europe rather than in the north stems from differences in tannin and polyphenol contents. This hypothesis is supported by the low tannin content of *Q. pubescens* acorns, originating from the only native Polish population of this species (Table 2), and in the case of some samples of *Q. ilex* (Mazuelos Vela et al. 1967 cited by Mason 1992). We pro-

Table 4. Correlation between the polyphenol content and the biometric parameters of *Quercus robur* acorns

Variables	total phenolics	tannins	non-tannins
acorn length ^a	-0.37*	-0.33*	
acorn width ^a	-0.44**	-0.38*	-0.43**
air-dry weight of acorns ^a	-0.45**	-0.42*	-0.38*
air-dry weight of seeds ^a	-0.48**	-0.44**	-0.39*
seed share in the acorn weight ^b			-0.39*

a – Pearson's correlation; b – Spearman's rank correlation; *p*-value: *** – *p*<0.001, ** – *p*<0.01, * – *p*<0.05, n=36.

pose further research into the chemistry and nutritional value of acorns, as oaks have been key species during food crises in temperate and Mediterranean climates and have huge potential as food plants, both as emergency food and an alternative major source of starch. This is proven by the fact that the Dary Nature company (from Koryciny, NE Poland) sells large amounts of tannin-deprived *Q. robur* flour in health food shops in Poland. A tannin removal technique (the firm's secret) was developed by Mr Mirosław Angielczyk, the owner of the company (<http://www.ziolowyzakatek.sklep.pl/maka.z.zoledzi.eko.1kg.dary.natury.html>). As oaks harbor a rich insect flora, and acorns are very susceptible to insect and pathogen invasions, another branch of oak research should be the study of tannin-removal techniques. Efficient tannin removal from acorns may be a more economically viable option than growing low-tannin cultivars.

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References

- Adamczak A., Buchwald W., Gryszczyńska A. 2012a. Phytochemical variability of coltsfoot (*Tussilago farfara* L.) in Poland. *Herba Polonica* 58: 7–14.
- Adamczak A., Buchwald W., Zieliński J., Mielcarek S. 2012b. Flavonoid and organic acid content in rose hips (*Rosa* L., section *Caninae* DC. em. Christ.). *Acta Biologica Cracoviensia* 54: 105–112.
- Bainbridge D.A. 1986. The use of acorns for food in California: past, present, future. In: *Proceedings of the Symposium on Multiple-use Management of California's Hardwood Resources*. San Luis Obispo, pp. 453–458.
- Boratyńska K., Filipiak M., Boratyński A. 2006. Budowa morfologiczna i zmienność. In: *Dęby. Nasze Drzewa Leśne*. Tom 11. Bugała W. (ed.). Instytut Dendrologii PAN, Poznań-Kórnik, pp. 63–85.
- Boratyński A., Boratyńska K., Filipiak M. 2006. Systematyka i rozmieszczenie. In: *Dęby. Nasze Drzewa Leśne*. Tom 11. Bugała W. (ed.). Instytut Dendrologii PAN, Poznań-Kórnik, pp. 85–114.
- Çalışkan O., Gündüz K., Serçe S., Toplu C., Kamiloglu Ö., Sengül M., Ercisli S. 2012. Phytochemical characterization of several hawthorn (*Crataegus* spp.) species sampled from the Eastern Mediterranean region of Turkey. *Pharmacognosy Magazine* 8: 16–21. <http://dx.doi.org/10.4103/0973-1296.93305>
- Cantos E., Espin J.C., Lopez-Bote C., de la Hoz L., Ordonez J.A., Tomas-Barberan F.A. 2003. Phenolic compounds and fatty acids from acorns (*Quercus* spp.), the main dietary constituent of free-ranged Iberian pigs. *Journal of Agricultural and Food Chemistry* 51: 6248–6255. <http://dx.doi.org/10.1021/jf030216v>
- Chung K.T., Wong T.Y., Wei C.I., Huang Y.W., Lin Y. 1998. Tannins and human health: a review. *Critical Reviews in Food Science and Nutrition* 38: 421–464. <http://dx.doi.org/10.1080/10408699891274273>
- Chung-MacCoubrey A.L., Hagerman A.E., Kirkpatrick R.L. 1997. Effects of tannins on digestion and detoxification activity in gray squirrels (*Sciurus carolinensis*). *Physiological Zoology* 70: 270–277.
- Chybicki I.J., Oleksa A., Kowalkowska K., Burczyk J. 2012. Genetic evidence of reproductive isolation in a remote enclave of *Quercus pubescens* in the presence of cross-fertile species. *Plant Systematics and Evolution* 298: 1045–1056. <http://dx.doi.org/10.1007/s00606-012-0614-8>
- Dénes A., Papp N., Babai D., Czucz B., Molnár Z. 2012. Wild plants used for food by Hungarian ethnic groups living in the Carpathian Basin. *Acta Societatis Botanicorum Poloniae* 81: 381–396. <http://dx.doi.org/10.5586/asbp.2012.040>
- Edwards J.E., Brown P.N., Talent N., Dickinson T.A., Shipley P.R. 2012. A review of the chemistry of the genus *Crataegus*. *Phytochemistry* 79: 5–26. <http://dx.doi.org/10.1016/j.phytochem.2012.04.006>
- European Pharmacopoeia. 2010. 7th ed. CE, Strasbourg, pp. 243–244.
- Farmakopea Polska. 2006. 7th ed., vol. I. Wyd. PTFarm, Warszawa, pp. 329–330.
- Farmakopea Polska. 2008. 8th ed., vol. I. Wyd. PTFarm, Warszawa, p. 232.

- Fleck D.C., Layne J.N. 1990. Variation in tannin activity of acorns of seven species of central Florida oaks. *Journal of Chemical Ecology* 16: 2925–2934. <http://dx.doi.org/10.1007/BF00979484>
- Hammer O., Harper D.A.T., Ryan P.D. 2001. PAST: paleontological statistics software package for education and data analysis. *Paleontologia Electronica* 4: 9.
- Hu S.Y. 2005. Food plants of China. The Chinese University Press, Hong Kong.
- Johns T., Duquette M. 1991. Traditional detoxification of acorn bread with clay. *Ecology of Food and Nutrition* 25: 221–228. <http://dx.doi.org/10.1080/03670244.1991.9991170>
- Kalle R., Sõukand R. 2012. Historical ethnobotanical review of wild edible plants of Estonia (1770s–1960s). *Acta Societatis Botanicorum Poloniae* 81: 271–281. <http://dx.doi.org/10.5586/asbp.2012.033>
- Kang Y., Łuczaj Ł., Ye S., Zhang S., Kang J. 2012. Wild food plants and wild edible fungi of Heihe valley (Qinling Mountains, Shaanxi, central China): herbophilia and indifference to fruits and mushrooms. *Acta Societatis Botanicorum Poloniae* 81: 405–413. <http://dx.doi.org/10.5586/asbp.2012.044>
- Kang Y., Łuczaj Ł., Kang J., Zhang S. 2013. Wild food plants and wild edible fungi in two valleys of the Qinling Mountains (Shaanxi, central China). *Journal of Ethnobiology and Ethnomedicine* 9: 26. <http://dx.doi.org/10.1186/1746-4269-9-26>
- Kennedy C.E.J., Southwood T.R.E. 1984. The number of species of insects associated with British trees: a re-analysis. *Journal of Animal Ecology* 53: 455–478. <http://dx.doi.org/10.2307/4528>
- Kimball B.A., Russell J.H., Ott P.K. 2012. Phytochemical variation within a single plant species influences foraging behavior of deer. *Oikos* 121: 743–751. <http://dx.doi.org/10.1111/j.1600-0706.2011.19515.x>
- Kobs L. 2008. Dietary polyphenolic intake from acorns and acorn meal. Masters thesis, The University of Georgia, Athens.
- Kozłowski J., Buchwald W., Forycka A., Szczygielska D. 2009. Rośliny i surowce lecznicze. IWNiRZ, Poznań, pp. 13–28.
- Łuczaj Ł. 2008. Archival data on wild food plants used in Poland in 1948. *Journal of Ethnobiology and Ethnomedicine* 4: 4. <http://dx.doi.org/10.1186/1746-4269-4-4>
- Łuczaj Ł. 2011. Dziko rosnące rośliny jadalne użytkowane w Polsce od połowy XIX w. do czasów współczesnych. *Etnobiologia Polska* 1: 57–125.
- Łuczaj Ł., Pieroni A., Tardío J., Pardo-de-Santayana M., Sõukand R., Svanberg I., Kalle R. 2012. Wild food plant use in 21st century Europe: the disappearance of old traditions and search for new cuisines involving wild edibles. *Acta Societatis Botanicorum Poloniae* 81: 359–370. <http://dx.doi.org/10.5586/asbp.2012.031>
- Łuczaj Ł., Szymański W.M. 2007. Wild vascular plants gathered for consumption in the Polish countryside: a review. *Journal of Ethnobiology and Ethnomedicine* 3: 17. <http://dx.doi.org/10.1186/1746-4269-3-17>
- Mason S.L.R. 1992. Acorns in human subsistence. PhD thesis, Institute of Archaeology, University College, London.
- Mason S. 1995. Acornutopia? Determining the role of acorns in past human subsistence. In: *Food in Antiquity*. Wilkins J., Harvey D., Dobson M. (eds). University of Exeter Press, Exeter, pp. 12–24.
- Mason S. 2000. Fire and Mesolithic subsistence-managing oaks for acorns in northwest Europe? *Palaeogeography, Palaeoclimatology, Palaeoecology* 164: 139–150. [http://dx.doi.org/10.1016/S0031-0182\(00\)00181-4](http://dx.doi.org/10.1016/S0031-0182(00)00181-4)
- Mason S., Nesbitt M. 2009. Acorns as food in southeast Turkey: implications for prehistoric subsistence in Southwest Asia. In: *From foragers to farmers: papers in honour of Gordon C. Hillman*. Fairbairn A., Weiss E. (eds). Oxbow Books, Oxford, pp. 71–85.
- Massei G., Genov P.V., Staines B.W. 1996. Diet, food availability and reproduction of wild boar in a Mediterranean coastal area. *Acta Theriologica* 41: 307–320. <http://dx.doi.org/10.4098/AT.arch.96-29>
- Maurizio A. 1927. Die Geschichte unserer Pflanzennahrung von den Urzeiten bis zur Gegenwart. Verlag Paul Parey, Berlin.
- Mazuelos Vela F., Ramos Ayerbe F., Ros de Ursino, J.A.F. 1967. Le fruit du Chêne (*Quercus ilex*). *Oléagineux* 22: 169–171.
- Mirgos M., Przybyszewska E., Capecka E., Angielczyk M., Przybył J.L., Bączek K., Węglarz Z. 2012. Intraspecific variability of great burnet (*Sanguisorba officinalis* L.) in respect of sterols content. *Herba Polonica* 58: 16–23.
- Moerman D.E. 1998. Native American Ethnobotany. Timber Press, Portland.
- Nowak R. 2005. Fatty acids composition in fruits of wild rose species. *Acta Societatis Botanicorum Poloniae* 74: 229–235. <http://dx.doi.org/10.5586/asbp.2005.029>
- O'Reilly-Wapstra J.M., Miller A.M., Hamilton M.G., Williams D., Glancy-Dean N., Potts B.M. 2013. Chemical variation in a dominant tree species: population divergence, selection and genetic stability across environments. *PLoS ONE* 8: e58416. <http://dx.doi.org/10.1371/journal.pone.0058416>
- Ofcarcik R.P., Burns E.E. 1971. Chemical and physical properties of selected acorns. *Journal of*

- Food Science 36: 576–578. <http://dx.doi.org/10.1111/j.1365-2621.1971.tb15133.x>
- Paluch A. 1984. Świat roślin w tradycyjnych praktykach leczniczych wsi polskiej. Uniwersytet Wrocławski, Wrocław.
- PAST version 2.17b: <http://folk.uio.no/ohammer/past>, accessed 15 Nov 2012.
- Pemberton R.W., Lee N.S. 1996. Wild food plants in South Korea; market presence, new crops, and exports to the United States. *Economic Botany* 50: 57–70. <http://dx.doi.org/10.1007/BF02862113>
- Pieroni A., Nebel S., Santoro R.F., Heinrich M. 2005. Food for two seasons: culinary uses of non-cultivated local vegetables and mushrooms in a south Italian village. *International Journal of Food Sciences and Nutrition* 56: 245–272. <http://dx.doi.org/10.1080/09637480500146564>
- Pignone D., Laghetti G. 2010. On sweet acorn cake (*Quercus* spp.) tradition in Italian cultural and ethnic islands. *Genetic Resources and Crop Evolution* 57: 1261–1266. <http://dx.doi.org/10.1007/s10722-010-9625-x>
- Pour M.B., Bahmaninia E., Ebrahimi R., Fayazi J. 2010. Evaluate effects of different inclusion of oak kernel with determine food potential oak kernel substitute with corn seed on broiler chicken's ration. *Research Journal of Biological Sciences* 5: 17–19. <http://dx.doi.org/10.3923/rjbsci.2010.17.19>
- Rakić S., Maletić R., Perunović M., Svrzić G. 2004. Influence of thermal treatment on tannin content and antioxidation effect of oak acorn *Quercus ceris* extract. *Journal of Agricultural Sciences* 49: 97–107.
- Rakić S., Petrović S., Kukić J., Jadranin M., Tešević V., Povrenović D., Šiler-Marinković S. 2007. Influence of thermal treatment on phenolic compounds and antioxidant properties of oak acorns from Serbia. *Food Chemistry* 104: 830–834. <http://dx.doi.org/10.1016/j.foodchem.2007.01.025>
- Rakić S., Povrenović D., Maletić R., Živković M. 2005. Drying of the aqueous extract of acorn *Quercus robur* in a spouted-fluid bed. *Journal of Agricultural Sciences* 50: 173–182.
- Rakić S., Povrenović D., Tešević V., Simić M., Maletić R. 2006. Oak acorn, polyphenols and antioxidant activity in functional food. *Journal of Food Engineering* 74: 416–423. <http://dx.doi.org/10.1016/j.jfoodeng.2005.03.057>
- Seigler D.S., Seilheimer S., Keesy J., Huang H.F. 1986. Tannins from four common *Acacia* species of Texas and Northeastern Mexico. *Economic Botany* 40: 220–232. <http://dx.doi.org/10.1007/BF02859146>
- Sidjimova B., Evstatieva L., Ivanova A., Mechkarova P., Lazarova I., Tchorbantov B. 2011. Intraspecific variability of main phytochemical compounds in *Tribulus terrestris* L. from North Bulgaria. *Biotechnology and Biotechnological Equipment* 25: 2348–2351. <http://dx.doi.org/10.5504/BBEQ.2011.0036>
- Singleton V.L., Kratzer F.H. 1973. Plant Phenolics. In: *Toxicants Occurring Naturally in Foods*. National Research Council, National Academy of Sciences, Washington, pp. 309–345.
- Smallwood P.D., Peters W.D. 1986. Grey squirrel food preferences: the effects of tannin and fat concentration. *Ecology* 67: 168–174. <http://dx.doi.org/10.2307/1938515>
- Southwood T.R.E. 1961. The number of species of insect associated with various trees. *Journal of Animal Ecology* 30: 1–8. <http://dx.doi.org/10.2307/2109>
- StatSoft Inc. STATISTICA (data analysis software system), version 7.1. 2005, <http://statsoft.com>.
- Tardío J., Pardo-de-Santayana M., Morales R. 2006. Ethnobotanical review of wild edible plants in Spain. *Botanical Journal of the Linnean Society* 152: 27–71. <http://dx.doi.org/10.1111/j.1095-8339.2006.00549.x>
- Trimble H. 1896. The tannin of some acorns. *American Journal of Pharmacology* 68: 601–604.
- Turner N.J., Łuczaj Ł.J., Migliorini P., Pieroni A., Dreton A.L., Sacchetti L.E., Paoletti M.G. 2011. Edible and tended wild plants, traditional ecological knowledge and Agroecology. *Critical Reviews in Plant Sciences* 30: 198–225. <http://dx.doi.org/10.1080/07352689.2011.554492>
- Weckerly F.W., Sugg D.W., Semlitsch R.D. 1989. Germination success of acorns (*Quercus*): insect predation and tannins. *Canadian Journal of Forest Research* 19: 811–815. <http://dx.doi.org/10.1139/x89-124>
- Ziołowy Zakątek. Mąka z żołądki. <http://www.ziolowyzakatek.sklep.pl/maka.z.zoledzi.eko.1kg.dary.natury.html>