The European Journal of Plant Science and Biotechnology ©2010 Global Science Books

Buckwheat as a Source for the Herbal Drug *Fagopyri herba*: Rutin Content and Activity of Flavonoid-Degrading Enzymes during Plant Development

Andreas Baumgertel • Andreas Loebers • Wolfgang Kreis*

Department Biologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Staudtstraße 5, D-91058 Erlangen, Germany

Corresponding author: * wkreis@biologie.uni-erlangen.de

ABSTRACT

Rutin content, flavonol 3-*O*-β-heterodisaccharidase (FHG) and polyphenol oxidase (PPO) were analysed in common buckwheat cv. 'Rutina' during various stages of plant development. Leaves, roots and inflorescences (if present) were removed from the stems immediately after harvest and samples of all plant parts were analyzed utilizing HPLC (rutin and quercetin content) or suitable enzyme assays (FHG and PPO activity). Highest levels of rutin were seen in inflorescences and leaves of 58-day-old plants. Inflorescences possessed about 100 times higher PPO activities than any other parts of the plant. Diurnal changes of rutin, FHG and PPO were also monitored and a slightly higher concentration of rutin and PPO was seen in the evening. Since rutin content is high and the activities of the rutin-degrading enzymes FHG and PPO are low in the morning during early flowering we propose that for the production of buckwheat herb (*Fagopyri herba*) the starting material (overground parts of *Fagopyrum esculentum*) should be harvested at that time in order to achieve optimal product quality.

Keywords: disaccharidase, *Fagopyri herba*, Polygonaceae, polyphenol oxidase, rutin-degrading enzyme(s)
Abbreviations: FHG, flavonol 3-*O*-β-heterodisaccharidase; **PPO**, polyphenol oxidase; **POX**, peroxidase

INTRODUCTION

Rutin is regarded as the active principle of *Fagopyri herba*, an herbal drug derived from common buckwheat (*Fagopyrum esculentum* Moench) used in the treatment of vascular diseases (Griffith and Barrow 1972; Ihme *et al.* 1996). The functionalities and properties of buckwheat compounds have been extensively investigated and buckwheat can now be regarded a "functional food" (Li and Zhang 2001). Rutin (quercetin 3-*O*-rutinoside) is the dominant flavonol glycoside in common buckwheat (Couch *et al.* 1946) and was reported to exhibit antioxidative, antihemorrhagic and blood vessel protecting properties (Gabor 1972; Wojcicki *et al.* 1995; Rice-Evans *et al.* 1997). The optimal moment for harvest, resulting in highest possible rutin levels in the herb has been determined (Hagels *et al.* 1995). Harvesting, drying and processing cause the destruction of cell compartments which results in the degradation or modification of important drug constituents due to active enzymes still present in the drug. Endogenous plant enzymes may hence influence the quality and stability of the final product. It has been demonstrated that the rutin content decreases during harvesting and drying of the plant and processing of the drug, and that an endogenous flavonol 3-*O*-β-heterodisaccharidase (FHG) (sometimes termed as "rhamnodiastase" or "rutinosidase", e.g., Bourbouze *et al.* 1975) contributes to rutin degradation in the dried herb during processing (Baumgertel *et al.* 2003). Polyphenol oxidases (PPO) are involved in oxidative processes occurring after plant harvest and are largely responsible for the browning reactions observed in fruits, mushrooms and also herbal drugs and drug preparations (Nüßlein *et al.* 2000; Mayer 2006). PPO might also contribute to the degradation of rutin and quercetin in common buckwheat.

The present study was performed with the commercial *Fagopyrum esculentum* cultivar "Rutina". Rutin content, FHG activity and PPO activity were assessed during plant development to establish the optimal time for harvest with optimal drug quality as the determining factor.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of *Fagopyrum esculentum* cv. 'Rutina', were provided by GlaxoSmithKline Consumer Healthcare, Herrenberg, Germany. Seeds were sown in April 1997 and germinated in standard soil (Plantaflor® Aussaaterde) containing 20% sand in a greenhouse at 20°C. Plants were collected at different stages of development precisely at 10 a.m. After 20 days the seedlings were pricked out in single pots and after 45 days (end of May) the plants were transferred to the experimental field station and planted in loamy soil rich in humus (June climate data 1997, Nuremberg area: 7.3 h sunshine/d (average); 16.3°C (average); rain 72.9 mm). On day 78 after sowing (end of June) the field-grown plants were collected at different times of day. Leaves, roots and inflorescences (if present) were removed from the stems immediately after harvest and samples of all plant parts were frozen in liquid nitrogen and stored at -20°C until further use.

Rutin and quercetin were purchased from Carl Roth GmbH & Co KG (Karlsruhe, Germany).

Dried buckwheat herb was provided by GlaxoSmithKline Consumer Healthcare, Herrenberg, Germany. *F. esculentum* plants were collected during flowering and dried at 60-80°C (temperature gradient). The drug was stored in the dark at 17°C and 30% humidity. The residual moisture of the drug was determined by drying at 105°C for 3 h (triplicate).

Extraction of rutin and quercetin from dried herb

75 mg of dried plant material were extracted with 10 mL methanol (80%) under ultrasonication for 45 min. After centrifugation for 10 min at 13,000 rpm, the supernatant was analyzed by HPLC.

Received: 4 April, 2009. Accepted: 27 February, 2010.

Original Research Paper

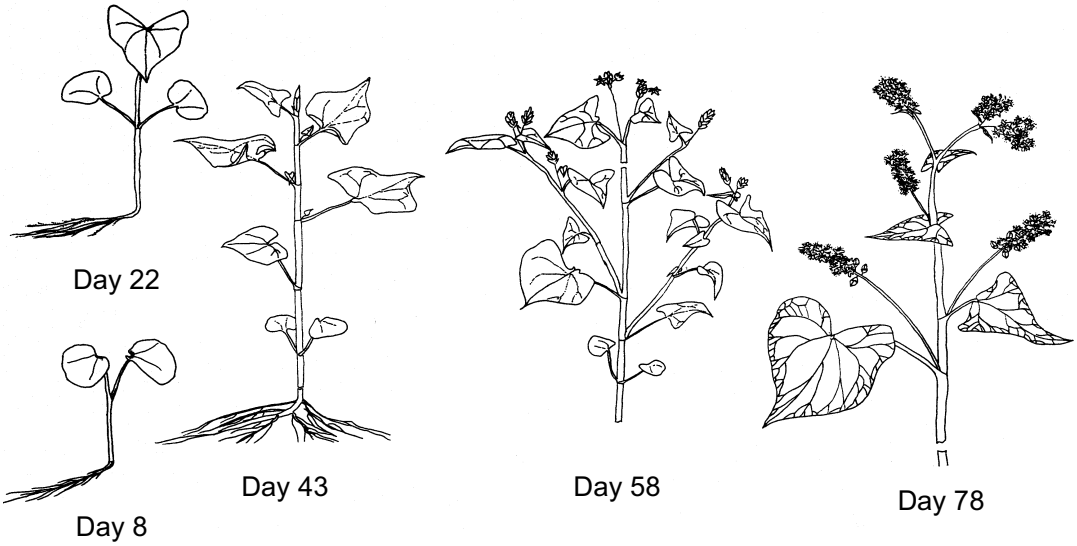

Fig. 1 Schematic presentation of the stages of buckwheat development. Figures represent the days after sowing. Drawings are not to scale.

Extraction of protein

The following procedures were carried out at 4°C in a cold room. 1 g of frozen plant material was homogenized with 10 mL of McIlvaine-buffer, pH 6.0, using an Ultra-Turrax. After centrifugation (10,000 × *g*, 20 min) the supernatant was removed. Protein present in the supernatant was precipitated by adding 2 volumes of cold (-18°C) acetone. The precipitate was spun down (3000 × *g*, 10 min) and subsequently dissolved in 1 mL of McIlvaine-buffer, pH 4.8. This protein solution was used to determine FHG and PPO activities.

HPLC-based determination of FHG

The procedure described by Baumgertel et al. (2003) was followed. 25 µL of a rutin solution were added to 500 µL of the protein solution. The concentration of the substrate was 1 mM per assay. After incubation for 20 min at 30°C and pH 4.8 the assay was stopped by adding 1575 µL methanol. The solution was centrifuged at 3,000 × g for 10 min to remove any precipitates and the supernatant analyzed by HPLC. FHG activity was calculated on the basis of quercetin released from rutin. Heat-inactivated protein solution served as control.

Photometric determination of PPO

The method outlined by Nüßlein *et al.* (2000) was employed. The standard enzyme assay contained: 500 µL buffer (0.5 mM SDS in 50 mM Na-P_i, pH 6.0), 100 µL of a 0.5 M L-proline solution, 275 µL Na-P_i pH 6.0, and 0.3 mL of the protein solution. The reaction was started adding 100 µL 4-methylcatechol solution (25 mM). Absorption at 525 nm was used to calculate the formation of a prolin-catechol adduct after 30 min. Heat-inactivated protein solution served as control.

High performance liquid chromatography (HPLC)

Flavonoids were extracted with MeOH-H_2O (4:1) as described by Hagels *et al.* (1995) and the extracts then analyzed by HPLC. The flavonoids were separated on an RP 18 column (Lichrosphere 100, 5 µm, 125 × 4, Merck) equilibrated with solution A (aq. Phosphoric acid 0.85% (v/v) + 4% (v/v) acetonitrile). The flavonoids were eluted with solution B (acetonitrile containing 12.5% (v/v) phosphoric acid 0.85% (v/v)) using the following gradient: 0 min 6% B, 5 min 15% B, 11 min 15% B, 16.5 min 24% B, 22 min 41% B, 25 min 41% B, 33 min 6% B and detected by their UV-absorption at 340 nm. Flavonoids were identified on the basis of their retention times in HPLC by direct chromatographic comparison with authentic samples.

Statistics

Statistical analyses was conducted by one-way analysis of variance (ANOVA) and correlation analysis (Pearson's correlation) using STATISTICA for Windows (release 5.1) and Microsoft Excel ($p < 0.05$). Group means were compared by Fisher's least significant difference (LSD) with $p < 0.05$ and Bonferroni correction.

RESULTS

Rutin content, FHG activity and PPO activity during plant development

PPO and FHG, as well as rutin contents in the commercial *F. esculentum* cv. 'Rutina' were determined during various stages of development in different plant parts. Plants were harvested at different stages of their development (**Fig. 1**). The rutin content, as determined by HPLC, increased until the onset of flowering (d 58), reaching as much as 6% in young leaves and 8% in the flower buds. Fully developed flowers contained about 4% rutin. Independently of the moment of harvest older leaves seem to contain less rutin than younger ones (**Table 1**). Primary leaves contained rutin only whereas the typical spectrum of rutin, hyperoside, quercitrin could be found after the onset of flowering (data not shown).

FHG activity was not detectable in older roots (> 20 d after sowing) and very low activity (< 0.5 nkat/mg) in stalks. FHG activity as high as 2.8 nkat/mg protein was detected in young leaves (day 43) as well as in mature leaves when flowers were fully developed (day 78). During flowering a significant decrease of FHG activity in flowers and other

Table 1 Rutin content (% of dry mass) of different parts of buckwheat plants and its dependence on plant development (n = 3 or more, mean values are shown, SD was always less than 20 %).

Plant part	Days after sowing					
	8	20	22	43	58	78
Root	0	0.01	0.08	0	0.14	0.08
Stalk				0.40	0.70	0.50
Primary leaf 1			3.06	0.40	2.94	2.47
Primary leaf 2				0.41	2.70	2.48
Primary leaf 3				0.77	2.94	1.96
Primary leaf 4				1.65	3.21	2.16
Primary leaf 5				4.04	2.77	2.06
Twigs					7.47	3.40
Inflorescence					8.40	4.20
Whole plant (mean)	1.22	0.59	1.14	0.98	3.5	2.04

Table 2 FHG 1 activity (nkat/mg protein) in different plant parts during plant development (n = 3 or more, mean values are shown). Where significant differences ($p < 0.05$) were found LSD values are given.

Plant part	Days after sowing							
	8	20	22	43	58	78	Mean	LSD
Root	0.039[c]	0.048[c]	0	0	0	0		
Stalk				0.019	0.04	0.27	0.11	0.07
Primary leaf 1			1.83	0.86	0.43	0.15	0.82	0.40
Primary leaf 2				1.82	0.73	2.45	1.67	0.58
Primary leaf 3				2.51	0.97	2.10	1.53	
Primary leaf 4				2.68	0.66	1.50	1.61	0.66
Primary leaf 5				2.79	0.79	0.6	1.39	0.37
Twigs					2.04[a]	0.81[b]	0.88	S*
Inflorescence					0.69[a]	0.12[b]	0.27	S*
Whole plant (mean)	0.02	0.07	0.60	0.65	0.78	0.74		
LSD			0.35	0.40	0.16	0.38		

* S – Significant according to Student's *t*-test using a and b values; c – single determinations only.

Table 3 PPO activity (nkat/mg protein) in different plant parts during plant development (n = 3 or more, mean values are shown). Where significant differences ($p = 0.05$) were found LSD values are given.

Plant part	Days after sowing							
	8	20	22	43	58	78	Mean	LSD
Root	2.96[c]	8.84[c]	1.07[c]	5.69	11.4	1.97	6.35	3.30
Stalk				1.85	1.49	0.87	1.40	0.46
Primary leaf 1			1.89	1.1	0.26	4.98	2.06	1.08
Primary leaf 2				1.55	0.21	2.18	1.31	0.53
Primary leaf 3				1.23	0.09	1.37	0.90	0.36
Primary leaf 4				3.81	0.11	0.85	1.59	0.44
Primary leaf 5				4.22	0.35	0.45	1.71	1.40
Twigs					2.15	2.04	2.10	
Inflorescence					228.00[a]	94.10[b]	161.05	S*
Whole plant (mean)	2.51	1.21	1.05	2.61	18.05	8.15		
LSD			0.26	1.47	16.70	2.92		

* S – Significant according to Student's *t*-test using a and b values; c – single determinations only.

plant parts was observed which continued until the end of the flowering period (**Table 2**).

PPO activity increased in young leaves (day 43), roots and mature leaves (day 78) during plant development, but did not reach more than about 5 to 12 nkat/mg protein. PPO is tremendously active in the inflorescences where its specific activity reaches more than 200 nkat/mg protein during the onset of flowering (**Table 3**). Thus with regard to PPO activity inflorescences significantly differ from other plant parts.

Diurnal changes of rutin content, FHG activity and PPO activity

Samples of buckwheat plants were collected during the flowering period every 4 hours for one day starting at 6 a. m. Lowest concentrations in diurnal changes in rutin contents were observed in the morning (1.65%), the highest in the evening (2.42%). Highest absolute variations were observed in the flowers and the lateral twigs (**Table 4**). FHG activity was also monitored and proved to be significantly higher in the evening as in the morning (**Table 5**). Small diurnal changes were detected for PPO activity on a whole plant basis with levels being slightly higher in the evening. This fact is mainly due to the usually elevated PPO concentration in the inflorescences and its 40–50% increase in the late evening (**Table 6**).

Rutin content, FHG activity and PPO activity in dried buckwheat herb

The dried buckwheat herb used in the present study contained about 5.5% rutin and 0.05% quercetin as determined by HPLC. The residual moisture of the drug at 25°C was 5.6 and 14.5% at 20 and 79% relative humidity, respectively. In this range rutin was found to be stable. Only in very humid conditions (93% relative humidity) and with a residual moisture of 21% rutin hydroysis became evident. After 48 h of storage under these conditions the quercetin content increased by a factor of 3. Interestingly, FHG activities as high as 11 nkat/mg protein could be detected in crude protein preparations of the dried herb kept under standard storage conditions (about 5% residual humidity) which is about 10 times higher than that detected in fresh plant material. Interestingly, PPO activity could not be demonstrated in 1 year old samples of the drug.

Table 4 Diurnal changes of rutin content (% of dry weight) in different plant parts on day 78 after sowing (n = 3 or more, mean values are shown, SD was always less than 20 %).

Plant part	Time of day (h)				
	6	10	14	18	22
Root	0.02	0.08	0.05	0.03	0.04
Stalk	0.21	0.33	0.24	0.37	0.38
Primary leaf 1	1.09	2.47	1.98	1.74	1.69
Primary leaf 2	2.21	2.48	1.61	1.97	1.97
Primary leaf 3	1.48	1.96	1.95	2.00	2.10
Primary leaf 4	1.50	2.16	1.30	1.76	2.50
Primary leaf 5	1.25	2.06	1.46	2.41	2.07
Twigs	2.42	3.88	2.77	3.88	4.49
Inflorescence	3.55	4.20	4.39	5.00	5.64
Whole plant (mean)	1.65	2.04	1.83	2.15	2.42

In order to find out whether PPO in inflorescences contributes to the degradation of rutin and quercetin during drying and processing of the *F. esculentum* herb, samples (flowers, stalks and leaves) were collected and dried at 40 and 60°C for 6 h. PPO activity was still present in the freshly dried drug, reaching levels of 60 nkat/mg protein and 18 nkat/mg protein when dried at 40 and 60°C, respectively.

DISCUSSION

In order to investigate the influence of rutin-degrading enzymes, namely FHG and PPO, *F. esculentum* plants were cultivated in the greenhouse and in the field. Samples were

Table 5 Diurnal changes of FHG 1 activity (nkat/mg protein) in different plant parts on day 78 after sowing (n = 3, mean values are shown). Where significant differences ($p < 0.05$) were found LSD values are given.

Plant part	Time of d (h)						
	6	10	14	18	22	Mean	LSD
Root	0.02	0	0	0	0.33	0.07	0.03
Stalk	0.06	0.27	0.19	0.15	0.26	0.19	0.12
Primary leaf 1	2.45	0.15	3.16	0.68	0.80	0.66	0.12
Primary leaf 2	1.08	2.45	4.63	5.80	1.98	3.19	1.66
Primary leaf 3	0.77	2.10	2.66	2.57	4.44	2.51	1.14
Primary leaf 4	0.26	1.50	0.61	2.30	2.33	1.40	0.45
Primary leaf 5	0.26	0.60	0.35	1.28	1.27	0.75	0.31
Twigs	1.14	0.84	3.08	1.74	1.90	1.74	1.10
Inflorescence	0.20	0.12	0.12	0.39	0.41	0.25	0.14
Whole plant (mean)	0.73	0.80	1.28	1.50	1.35		
LSD	0.20	0.40	0.89	0.90	0.71		

Table 6 Diurnal changes of PPO activity (nkat/mg protein) in different plant parts on day 78 after sowing (n = 3, mean values are shown). Where significant differences ($p = 0.05$) were found LSD values are given.

Plant part	Time of d (h)						
	6	10	14	18	22	Mean	LSD
Root	3.95	1.97	4.8	7.0	14.0	6.34	1.43
Stalk	3.27	0.87	0.84	2.04	0.64	1.53	0.53
Primary leaf 1	2.29	4.98	1.89	4.06	14.69	5.58	2.89
Primary leaf 2	1.1	2.18	0.92	3.37	10.57	3.75	2.25
Primary leaf 3	0.98	1.37	0.62	1.8	13.85	3.72	3.29
Primary leaf 4	0.22	0.85	0.33	0.9	5.83	1.63	1.43
Primary leaf 5	0.23	0.45	0.29	1.58	4.48	1.41	1.51
Twigs	9.73	0.47	0.62	1.1	0.65 5	2.51	1.18
Inflorescence	114.5	94.1	108.9	117.3	152.42	117.44	22.89
Whole plant (mean)	10.30	8.19	9.12	10.87	16.17		
LSD	2.92	6.96	4.64	10.54	7.45		

collected during plant development and analyzed for rutin content, FHG activity and PPO activity. Highest rutin concentrations were always detected in the youngest parts of the plants and in their inflorescences where they reached up to 8% during the early flowering stages. These observations are fully consistent with previous results (Hagels *et al.* 1995). Besides rutin other flavonoids were found in considerably lower amounts only. This is also consistent with the findings of Hagels *et al.* (1995) who reported quercitrin concentrations of up to 0.35% and hyperoside concentrations of 0.005-0.04% in the inflorescences. Only favourably low concentrations of quercetin were present (< 0.007%).

The focus of this study is measuring enzyme activities involved in rutin degradation (FHG and PPO) and discussing how these enzymes influence the quality of the herbal drug *Fagopyri herba*. Cell lysis, during drying, transport and/or storage under conditions causing high residual humidity, i.e. high local temperatures, might activate FHG and PPO.

Rutin-cleaving enzymes, termed flavonol 3-*O*-β-heterodisaccharidases (FHG), were isolated from dried buckwheat leaves and one of them, FHG 1, was purified to apparent homogeneity and partially sequenced (Baumgertel *et al.* 2003). Endogenous enzyme(s) may become active during the isolation of rutin from the dried herb and could therefore be responsible for the degradation of some of the rutin during processing. Enzymes, termed "rhamnodiastases" or "rutinosidases", releasing the disaccharide rutinose from rutin, have previously been isolated from plants (Bourboze *et al.* 1975) and fungi (Narikawa *et al.* 2000).

Leaves are accountable for over 90% of the total FHG activity of the whole plant and are the main contributor of the dried herb. Schanajewa (1998) observed a similar correlation between leaf development and the occurrence of rutin-degrading enzymes. We here demonstrated that in younger leaves FHG activity paralleled rutin contents whereas in older leaves during flowering they were negatively correlated. More than 50% of leaf rutin seems to be located in the upper epidermis which is compatible with its putative role as a UV-protective screen. This is consistent with a low activity of rutin-degrading enzyme(s) in ther epidermis (Schanajewa 1998). Inflorescences with high levels of rutin possessed low FHG activity only (**Tables 1, 2**).

Rutin content and FHG activity exhibited diurnal fluctuations. The influence of light on flavonoid accumulation and the activity of enzymes involved in their biosynthesis is well documented (Barz *et al.* 1971; Barz and Hösel 1971). Amrhein and Zenk (1970) reported that in buckwheat seedlings grown in darkness, illumination caused a drastic increase in the activity of L-phenylalanine ammonium lyase (PAL), a key enzyme in flavonoid biosynthesis.

Peroxidases (POX) or polyphenol oxidases (PPO) are involved in oxidative processes occurred after plant harvest. Most probably PPO is the enzyme responsible for the oxidative degradation of polyphenols (Nüßlein *et al.* 2000), but the contribution of endogenous peroxidase(s) has not been investigated here. It has been shown however, that horseradish peroxidase is able to degrade both quercetin and rutin (Takahama 1986). Yao *et al.* (2008) showed that PPO activity in common buckwheat remained at low levels under low UV-B radiation conditions but significantly increased under higher UV-B radiation. They also found changes among different cultivars and reported that rutin and ferulic acid concentrations also increased in high UV-B conditions. In the present study PPO could not be detected in the dried herb using the standard PPO assay, indicating that it may not have an adverse effect on the stability of quercetin derivatives. That does not mean that there is no PPO present. Many plant PPO show latency, i.e., these enzymes can be reactivated when treating protein preparations with proteases or SDS (Sellés-Marchart *et al.* 2006).

Like other secondary metabolites, flavonoids have a considerable turn-over, i.e., synthesis, storage, re-metabolization and degradation determine the level of their accumulation. However, compartmentalization, distribution or co-localization of rutin and its degrading enzymes at the cellular level was not investigated here. Hence physiological roles of rutin-degrading enzymes and their integration in rutin metabolism still remain obscure.

In summary, we deduce from our findings that optimal quality of common buckwheat herb (*Fagopyri herba*) can be expected, when plants are harvested in the morning hours of a sunny day during early flowering because: 1) the

rutin content is high, which is desirable, being the active ingredient; 2) high UV-B might be beneficial for rutin production; 3) FHG and PPO are relatively low, which will minimize the initial degradation of rutin during drying; 4) PPO in the drug will still be high enough to degrade unwanted quercetin released from rutin after harvesting or during processing.

ACKNOWLEDGEMENTS

This study was partially sponsored by SmithKline Beecham Fink GmbH, Herrenberg, Germany. We thank Barbara White for linguistic advice.

REFERENCES

Amrhein N, Zenk MH (1970) Aktivität der Phenylalanin-Ammonium-Lyase (PAL) und Akkumulation von phenylpropanoiden Verbindungen während der Keimung von Buchweizen (*Fagopyrum esculentum* Moench) im Dunkeln. *Zeitschrift für Pflanzenphysiologie* **63**, 384-388

Barz W, Hösel W (1971) Über den Umsatz von Flavonolen und Isoflavonolen in *Cicer arietinum. Phytochemistry* **10**, 335-341

Barz W, Hösel W, Adamek C (1971) Über den Einfluß von Licht auf den Umsatz von Flavonolen und Isoflavonolen in *Cicer arietinum. Phytochemistry* **10**, 343-349

Baumgertel A, Grimm R, Eisenbeiss W, Kreis W (2003) Purification and characterization of flavonol 3-*O*-β-heterodisaccharidase from the dried herb of *Fagopyrum esculentum* Moench. *Phytochemistry* **64**, 411-418

Bourbouze R, Pratviel-Sosa F, Percheron F (1975) Rhamnodiastase et α-L-rhamnosidase de *Fagopyrum esculentum. Phytochemistry* **14**, 1279-1282

Couch JF, Naghski J, Krewson CF (1946) Buckwheat as a source of rutin. *Science* **103**, 197-198

Gabor M (1972) Pharmagologic effects of flavonoids on blood vessels. *Angiologica* **9**, 355-374

Griffith LA, Barrow A (1972) The fate of orally and parenterally administered flavonoids in the mammal. *Angiologica* **9**, 162-174

Hagels H, Wagenbreth D, Schilcher H (1995) Phenolic compounds of buckwheat herb and influence of plant and agricultural factors (*Fagopyrum esculentum* Moench and *Fagopyrum tataricum* (L.) Gaertn.). In: Matano T, Ujihara A (Eds) *Current Advances in Buckwheat Research*, Shinshu University Press, Matsumoto, Japan, pp 801-809

Ihme N, Kiesewetter H, Jung F, Hoffmann KH, Birk A, Müller A, Grützner KI (1996) Leg oedema protection from a buckwheat herb tea in patients with chronic venous insufficiency: a single centre, randomised, double-blind, placebo-controlled clinical trial. *European Journal of Clinical Pharmacology* **50**, 443-447

Li SQ, Zhang QH (2001) Advances in the development of functional foods from buckwheat. *Critical Reviews in Food Science and Nutrition* **41**, 451-464

Mayer AM (2006) Polyphenol oxidases in plants and fungi: Going places? A review. *Phytochemistry* **67**, 2318-2331

Narikawa T, Shinoyama H, Fuji T (2000) A β-rutinosidase from *Penicillium rugulosum* IFO 7242 that is a peculiar flavonoid glycosidase. *Bioscience, Biotechnology and Biochemistry* **64**, 1317-1319

Nüßlein B, Kurzmann M, Bauer R, Kreis W (2000) Enzymatic degradation of cichoric acid in *Echinacea purpurea* preparations. *Journal of Natural Products* **63**, 1615-1618

Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. *Trends in Plant Science Reviews* **2**, 152-159

Selles-Marchart S, Casado-Vela J, Bru-Martinez R (2006) Isolation of a latent polyphenol oxidase from loquat fruit (*Eriobotrya japonica* Lindl.): Kinetic characterization and comparison with the active form. *Archives of Biochemistry and Biophysics* **446**, 175-185

Schanajewa TA (1998) Rutin and rutin-decomposing enzymes in various tissues of buckwheat leaves. *Fiziologija Rastenij* **45**, 74-78

Takahama U (1986) Spectrophotometric study on the oxidation of rutin by horseradish peroxidase and characteristics of the oxidized products. *Biochimica et Biophysica Acta* **882**, 445-451

Wojcicki J, Barcew-Wisniewska B, Samochowiec L, Rozewicka L (1995) Extractum Fagopyri reduces atherosclerosis in high-fat diet fed rabbits. *Pharmazie* **50**, 560-562

YaoY, Yang Y, Li Y, Lutts S (2008) Intraspecific responses of *Fagopyrum esculentum* to enhanced ultraviolet B radiation. *Journal of Plant Growth Regulation* **56**, 297-306