Variations of the orthodiphenol content of *Cynara scolymus* L. during the plant growing seasons

V. Lattanzio and Irene Morone

Istituto di Agronomia e Coltivazioni Erbacee, via Amendola, 165/A, I – 70126 Bari (Italy), 23 October 1978

Summary. The variations undergone by chlorogenic acid, cynarine, cynazoside and scolymoside during the biological cycle of the artichoke plant were studied. Furthermore, the differences in the composition of the orthodiphenolic fraction between different organs of the plant were studied.

The plants polyphenol content, which is considered to be important in the physiology of the growth and development of the plant itself, undergoes marked variations during the biological cycle, and thus its determination can be an index for evaluating the plant's age[1]. Some phenomena of accumulation and reduction suggest that polyphenol compounds are not the final products of a metabolic process but constitue reserves of phenolic units formed during some growth period and used later[2]. The biosynthesis of these components has been well studied[2-4], but little informations are available about their metabolism[5-7,9,15]. A new analytical method, which utilized gel-chromatography on Sephadex LH-20[10-16], has therefore been applied to study the composition of the orthodiphenol fraction of artichoke and its variations during the plant's biological cyle.

Results and discussion. In the plant, considered as a whole, the orthodiphenol content falls gradually and continuously as the plant grows (from 6% of dry wt) and this course is determined by the variations of the chlorogenic acid content which represents the most abundant component, percentage-wise.

It is interesting to observe that the chlorogenic acid content falls from 4.4% to 1.6% d.m. as the plant gradually develops to the stage of differentiation of the capitulum. From this moment onwards it remains stable at an almost constant level. A similar tendency can be detected for cynaroside (0.4%–0.16% dry matter) and scolymoside (0.7%–0.15% dry matter), while the behaviour of cynarine is just the opposite, increasing gradually as the plant grows (0.01%–0.05% dry matter). This is explained by considering that the capitula continually increase their weight with regard to the total weight of the plant's green mass after differentiation; moreover, they represent the organ richest in cynarine (0.08%–0.13% dry matter). On studying the accumulation of the various orthodiphenolic components (mg/plant in time), it is seen that chlorogenic acid and scolymoside gradually increase as the plant grows, at the moment of differentiation. That is, the rate at which these components are synthesized exceeds that of their transformation. Their level remains constant from this moment until the maturation of the secondary capitula and this means that we are faced by systems of comparable rates of accumulation and transformation. Transformation processes predominate in the last stage of the biological cycle. Cynaroside accumulates in a similar way, with the 'plateau' remaining even during the senescence stag. During the senescence stage cynarine returns to accumulating and this is related to the fact that the plant presents only a stem without leaves and capitula which are flowering.

It has already been mentioned that chlorogenic acid represents the most abundant component, 83% of the orthodiphenol fraction up to the moment of differentiation, when it abruptly falls to 75% and then remains always at this level. The same phenomenon (abrupt fall of the chlorogenic acid content) has also been observed by other authors with regard to *Nicotiana*[14].

Observing the individual organs in detail, the presence of chlorogenic acid and scolymoside is seen in the young apical leaves (table); while the relative abundance of other components and scolymoside increase during growth of the leaf, chlorogenic acid regularly falls. The presence of chlorogenic acid and scolymoside only, in the ratio of 93:7, has been found also in stems; while in the seeds only chlorogenic acid out of all the orthodiphenol components is present. Finally, the capitulum is the plant organ richest in cynarine, and the direct correlation between physiological stage and relative abundance of chlorogenic acid, which falls with increase of age, is seen here, as has been observed already in the leaves but not in the stems.

Different ways of accumulation of the polyphenol compounds are found in the various organs exmined, and this is explained by the fact that polyphenols are metabolized on the spot, without migration between the various parts of the plant[1]. It is to add that, in good agreement with what has been observed up to now, diminution of that total polyphenol components and of chlorogenic acid is observed within the plant passing from its highest to its lowest part, in good agreement with the age of the individual organs, while scolymoside and cynaroside reach their maximum level in the adult organs in full vegetative vigour and cynarine increases continuously with increase in age of the organ.

The analyses performed on the various parts of the plant during the entire biological cycle show another phenomenon: the sequence with which the various orthodiphenol components appear in the plant:

Chlorogenic acid → Chlorogenic acid + scolymoside → Chlorogenic acid + scolymoside + cynaroside → Chlorogenic acid + scolymoside + cinaroside + cynarine

Conclusions. The literature contains little data regarding the composition of the orthodiphenol fraction of the artichoke and in any case such data do not take into account the variability of said fraction in function of the physiological state of the plant or the organ examined.

Orthodiphenol fraction composition in leaves at different physiological stage

	Relative composition	% of dry matter
Apical leaves		
Chlorogenic acid	94%	6.65–5.97%
Scolymoside	6%	0.49–0.44%
Growing leaves		
Chlorogenic acid	90–82%	6.14–3.57%
Scolymoside	7–11%	0.62–0.38%
Cynaroside	2– 6%	0.26–0.11%
Cynarine	0.15–0.68%	0.04–0.01%
Full-grown leaves		
Chlorogenic acid	85–63%	1.33–4.83%
Scolymoside	10–24%	0.96–0.33%
Cynaroside	4–13%	0.48–0.18%
Cynarine	0.2– 1%	0.04–0.01%
Senescent leaves		
Chlorogenic acid	75–52%	2.71–0.85%
Scolymoside	15–31%	0.73–0.22%
Cynaroside	8–14%	0.29–0.09%
Cynarine	1.3–3.2%	0.06–0.04%
Dry leaves		
Chlorogenic acid	50–31%	0.28–0.04%
Scolymoside	33–38%	0.19–0.05%
Cynaroside	15–24%	0.10–0.03%
Cynarine	4– 7%	0.02–0.01%

This research has pointed the relations between chlorogenic acid content and age of the plant or organ, changes in composition of the orthodiphenol fraction in function of the physiological stage, changes in composition of the orthodiphenolic fraction between the various organs and metabolism of the various components.

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Differential developmental pattern of acid and alkaline phytase and phosphatase activities in rat intestine

C.V. Ramakrishnan and S.D. Bhandari

Department of Biochemistry, Faculty of Science, M.S. University of Baroda, Baroda 390 002 (India), 9 October 1978

Summary. Rat intestine was found to show a distinct acid phytase activity (pH optimum 4.7) in addition to that of an alkaline phytase (pH optimum 8.0). The phytase and phosphatase activities were found to differ in their developmental pattern and responded differentially to some inhibitors. Thus the two activities seem to be due to two independent enzymes and are not the activity of a nonspecific phosphatase as has been suspected formerly.

The enzyme phytase (EC 3.1.3.8) with an alkaline pH optimum has been reported[1-4] in the intestine of various species including man. Our preliminary studies[5] indicated the presence of an acid phytase, in addition to that of the alkaline phytase in rat intestine. Studies were therefore undertaken to confirm the presence of 2 distinct phytase activities in rat intestine. The attempts were also made to differentiate the phytases from corresponding phosphatases on the basis of their developmental patterns and the response to various inhibitors.

Materials and methods. Method for the preparation of tissue extract and assay of the enzyme activities was as reported by Bitar and Reinhold[4] and modified as described earlier[6]. Protein was estimated by Lowry's method[7] and phosphorus by that of Fiske and Subbarao[8]. For the developmental studies intestines of normal healthy rats (Charles Foster) of different ages (7–10 in each case) were used.

Results and discussion. The initial studies were carried out to determine the optimum conditions for the assay of intestinal phytase and phosphatase activities, using intestinal mucosal extract of adult rat as the enzyme source. The pH curve (figure 1) for phytase showed 2 distinct peaks, a small one in the acid range at pH 4.7 and a large one in the alkaline range at pH 8.0, indicating the presence of 2 phytases. On the other hand, phosphatase showed pH optima at 3.8 and 9.0. The optimum conditions worked out for the acid and alkaline phytases and phosphatases are given in table 1. To confirm the presence of 2 distinct phytases in the intestine, attempts were made to separate acid phytase activity from that of alkaline phytase in intestinal mucosal extract. For this purpose, intestinal mucosal extract (10% w/v) was subjected to acid treatment by bringing its pH to 4.0 with 0.1 M acetic acid and after 1 h the extract was centrifuged at 10,000 × g for 40 min. The pH of so obtained supernatant and residue was readjusted to 7.6 with 0.1 M Na_2CO_3. The acid phytase activity

Table 1. Optimum assay conditions for phytase and phosphatase activities of rat intestine

	Acid phytase	Alkaline phytase	Acid phosphatase	Alkaline phosphatase
Optimum pH	4.8	8.0	3.7	9.0
Substrate concentration (mM)	0.8	0.8	0.8	1.6
Enzyme concentration (mg protein)				
Intestinal mucosa	1.50	1.50	1.50	0.75
Intestine whole	2.2	2.2	2.2	1.1
Optimum temperature (°C)	60	60	60	37
Linearity till (min)	15	15	15	15

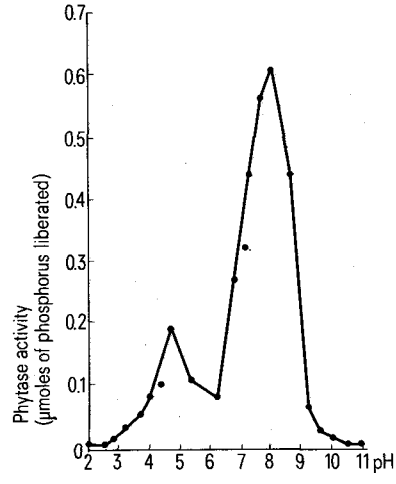

Fig. 1. pH curve of phytase activity of rat intestinal mucosa. Buffers used: glycine-HCl, for 2.0–3.5 pH, acetate for 3.5–6.0 pH tris succinate for 6.0–9.0 pH, and carbonate bicarbonate for 9.0–11.0 pH.