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Biodegradation of Oxalic Acid from Spinach Using Cereal Radicles

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A high level of oxalate intake constitutes a health risk for infants and metabolically disposed adults. Spinach, acclaimed for its many health benefits, is among the vegetables richest in oxalate. Blanching reduces oxalate unsatisfactorily and unspecifically. An alternative, biological method is proposed on the basis of rye seedlings or radicles (also barley and wheat) containing an oxalate-specific oxalate oxidase by nature. Dissolved oxalate (0.25 mM) was rapidly degraded in the presence of radicles (e.g., 70% within 100 min). With commercial deep-frozen spinach, near-complete degradation of soluble oxalate was achieved at pH 3.5. The total level of oxalate was reduced by half. Similarly high rates occurred from 18 to 35 °C. Even at 55 °C appreciable rates were observed. The seedling as a whole is effective, too, and enrichment with cereal-specific healthy components would occur. Removal of oxalate from other vegetables, juices, cycled process waters, or feeds is conceivable with fresh or heat-dried cereal seedlings or radicles.

KEYWORDS: Oxalate; biodegradation; spinach; urinary stone prevention; infant nutrition

INTRODUCTION

In human and animal nutrition, oxalic acid, denoted "oxalate" hereafter, is considered an "undesired compound". It has been suggested that oxalate is often the culprit for the great many unspecific cases of digestive disorders occurring with young children. Diarrhoea may be elicited by oxalate crystals (*1*, *2*) which can irritate the intestinal mucosa (*3*). The low dissociation constant of Mg or Ca oxalate has led to the proposal that oxalate may decrease the bioavailability of metal ions essential for human health (*4*, *5*). However, information about the effects in vivo of oxalate on the bioavailability of metal ions such as Mg, Ca, or Fe is scarce (*5*, *6*). Especially rare are in vivo studies (*7*, *8*). It is worth noting that oxalate has been suggested to play a role in the detoxification of aluminum in plants, buckwheat, and spinach (*9*, *10*). For animals or humans, similar information is lacking to our knowledge.

In humans or animals, oxalate is removed by excretion through the urinary system where it can precipitate Ca and other ions to form renal stones. It has been shown that there is considerable variety among humans as far as oxalate metabolism, oxalate excretion, and the susceptibility to suffering from oxalate-type renal stones are concerned (*11*, *12*). Although there is a good deal of data suggesting that endogenous oxalate is more critical in this respect than oxalate from nutrition, there is consensus that oxalate from the diet poses an additional risk, especially for infants or genetically disposed adults (see ref *13* for references).

Moreover, oxalate removal is desirable also for sensorial reasons to prevent the formation of oxalate crystals during freezing. Cold-induced oxalate crystals have been blamed for the unpleasant mouth feeling that can occur with frozen spinach products (personal communications).

Among plant families, polygonaceous plants reportedly have high levels of oxalic acid (e.g., refs *14* and *19*). It is important to note that spinach, which is consumed by many on a regular basis because of its incontestable health benefits (e.g., secondary plant compounds), contains more oxalic acid than, for example, rhubarb stems, which is generally perceived as the major source of oxalic acid in nutrition because of its acerbity (*19*). With respect to starchy foods, pseudocereals contain substantially more oxalic acid than "true" cereals (*20*).

Plants can synthesize oxalate from different precursors in leaves, stems, and storage tissues (see ref *14* for a review). It is a general observation that there is a negative correlation between the level of plant nitrogen fertilization, which, among other factors, determines the concentration of nitrate in the plant, and the leaf oxalate concentration (*14*−*18*). This implies a dilemma: a low level of oxalate would generally occur in tandem with a relatively high nitrate level in spinach, or vice versa.

Because oxalate is an undesired compound, efforts have been made to reduce the level of oxalate by blanching in the course of the industrial spinach processing for the frozen food sector (*17*). The blanching process extracts water soluble compounds such as oxalate but also health protective compounds such as water soluble vitamins, secondary plant compounds, and minerals. If a near-complete removal of oxalate is desired for dietary reasons, intense or even excessive blanching is required which would leach out healthy components to an unacceptable extent.

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To avoid such losses, a method of specifically reducing oxalate content in vegetables, spinach, to a low level has been sought. If such a method were available, it would be possible to reduce the time or temperature of the blanching procedure to the minimum required for reasons of hygiene and preservation.

One approach is to degrade oxalate by enzymic biodegradation utilizing a low-cost source of an oxalate degrading enzyme. Cereal seedlings could serve the purpose. During the germination of the cereal grain, proteins denoted germins are expressed in the differentiating and extending cells of the growing radicles (*21*), where they are located in the apoplastic space (cell wall). Because germin expression is so closely linked to the onset of germination, it has been proposed that germin be used as a marker for sprouting-dependent quality deterioration of cereal grains (*22*, *23*). With respect to food science, germin's most important asset is its enzymic activity: germin can rapidly catalyze the oxidation of oxalate, with carbon dioxide and hydrogen peroxide as products (*24*, *25*). The affinity of isolated barley germin oxalate oxidase for oxalate is high [$K_m = 0.27$ mM (*26*, *27*)]. It should be noted that germins are special, featuring extraordinary stability under hot or acidic conditions (*28*).

The rationale behind the work presented here was that cereal seedlings, especially radicles that contain oxalate oxidase by nature, would degrade spinach oxalate if present during spinach processing. To investigate this, radicles (seedling rootlets) or entire seedlings from different cereal species were added to aqueous oxalate solutions. Optimum conditions for the enzyme in situ were so determined. To probe the application potential of cereal radicles to rid spinach of oxalate, frozen spinach was combined with radicles, and oxalate content was determined in different fractions under different incubation conditions.

Possible applications of the biological method for removal of oxalate from foods are discussed before the background of improving the nutritive value of spinach or other vegetables combined with cereal seedlings. The oxalate−nitrate relationship is considered with respect to the environment.

MATERIALS AND METHODS

Spinach. Commercial deep-frozen spinach was generally used so we could work with a constant and near-uniform material. If fresh spinach was used, it was purchased from the local market.

Seedlings. Surface-sterilized grains of rye (commercial blend), barley (var. Alpaca), wheat (var. Zentos), spelt (commercial blend), and oats and maize (no specification) were used. The grains were thoroughly rinsed, soaked overnight in water, rinsed again, and placed on several layers of wet paper towels. The seeds were germinated while being protected from light in plastic bins covered with perforated cardboard boxes at 20−23 °C. In the standard experiments, 4−7-day-old seedlings were used. Fresh or heat-dried radicles were used for oxalate degradation. Rye radicles were excised using a razor blade. For drying, a temperature regime from the malting industry was adopted: 55 °C for 16 h, with the temperature increased in 5 °C steps (1 h each) to reach 75 °C. The dried radicles were reduced to pieces of ∼0.5 mm with a household-type mill (rotating blades).

Oxalate Degradation. To determine oxalate degradation rates with dissolved oxalate, fresh radicles were sliced and, if not noted otherwise, 0.1 g fw (fresh weight or as is) was added to 20 mL of 0.25 mM oxalic acid adjusted to pH 3.5. The suspension was stirred gently at 30 °C. Aliquots were taken at time intervals given in the text or figures.

To determine pH dependence, succinate-K^+ buffer (50 mM) was used throughout. The temperature and incubation time were 45 °C and 30 min, respectively.

To determine oxalate degradation rates with frozen spinach and cereal radicles or entire seedlings, 10 g of spinach was thawed and, still ice-cold, combined with 120 mL of distilled water and 5 g of sliced rye radicles or seedlings cut in pieces with a mincing knife. After a short treatment with an Ultra-Turrax instrument, the resulting spinach/radicle mixture, denoted "suspension", was adjusted to pH 3.5 with citric acid for the temperature dependence experiments, or other pH values to determine pH dependence. The incubation temperature was 25 °C. The suspension was then incubated with stirring for 240 min. After centrifugation (2000*g* for 10 min), the level of oxalate was determined in the supernatant, denoted the liquid phase, and in the residue, denoted the solid phase. Oxalate content in the suspension was calculated from these values. The liquid phase was boiled for 15 min to inactivate enzymes. After the sample had cooled to room temperature, the pH was readjusted to 3.5 before analysis. To extract oxalate from the solid phase, it was resuspended in a mixture of 75 mL of H_3PO_4 (1 mol/L), 45 mL of deionized water, and 7.5 mL of EDTA solution (5%, w/v) and boiled for 30 min under reflux. After the sample had cooled to room temperature, the pH was adjusted to 3.5. Water was added to a final volume of 250 mL. The solution was paper-filtered and used for analysis.

Total oxalate content in fresh, blanched, or commercial spinach was determined using the extraction procedure as described for the "solid phase".

Analytical Methods. Oxalate was quantified enzymatically using a commercial kit (Sigma 591-C). To assess hydrogen peroxide formation in oxalate solution with added radicles (see above), a 0.2 mL sample was combined with 1.7 mL of peroxidase (2 units/mL in 0.05 mol/L acetate buffer) and 0.1 mL of 1,2-phenylenediamine [0.8% (w/v) in methanol]. After incubation for 5 min at 25 °C, the absorption was read at 445 nm.

Nitrate and nitrite levels were determined using commercial kits (Boehringer, Mannheim, Germany).

Oxalate oxidase activity was calculated from the disappearance of oxalate in a solution (0.5 mM in this experiment) as described in Oxalate Degradation.

To demonstrate by histochemical dying the presence of active oxalate oxidase in situ, seedlings were incubated in an oxalate solution containing 4-chloro-1-naphthol which reacts with hydrogen peroxide yielding an insoluble purple-colored product (*21*).

Statistics. The standard deviation was exemplarily estimated by assessing the results obtained with independent batches of the same spinach. The standard deviation was 4% for oxalate content ($n = 4$) and between 4 and 5% in the oxalate degradation experiments with spinach and radicles ($n = 3$).

RESULTS

Degradation of Dissolved Oxalate. *Species Dependence of Oxalate Degradation*. Radicles from 3-day-old seedlings from rye, wheat, oat, maize, spelt, and barley were incubated in an oxalate solution (0.25 mM). A rapid decline of the oxalate concentration was observed with all species (**Figure 1**). Having reached a level of ∼30%, oxalate degradation slowed slightly. Rye radicles were most effective (**Figure 1**). With oat or maize radicles, little or negligible oxalate degradation was observed, respectively (results not shown). Therefore, the examination of oxalate degradation by cereal seedling radicles was done with rye seedlings, except where stated otherwise.

Seedling Development. Seedlings of different developmental stages, i.e., time of germination, were examined for their potential to degrade oxalate. Oxalate degradation was slow but appreciable with soaked seeds, and the rate increased rapidly during the first 4 days of germination (**Figure 2A**). The seedling-based oxalate degradation rate (complete seedling with radicles) was similarly high for 4−8-day-old seedlings (∼1.6 nmol of oxalate min^{-1} $seedling^{-1}$). Near-equimolar amounts of oxalate were removed, and hydrogen peroxide formed (results not shown). The presence of oxalate oxidase was also demonstrated using histochemical staining of the radicles for oxalate oxidase activity (**Figure 2B**). Heavily stained radicle sections alternated with less stained sections alongside the radicles. In the elongated

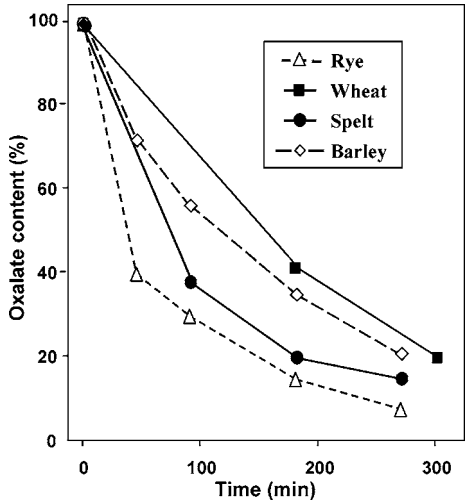

Figure 1. Species dependence of the oxalate degradation velocity catalyzed by radicles from different cereals. The assay medium contained dissolved oxalate (0.25 mM, pH 3.5, 30 °C) and 0.1 g (fw) of cereal radicles from seedlings of approximately the same developmental stage.

radicles of older seedlings, the proportion of heavily stained sections was decreased. This histochemical observation matches the results of oxalate degradation rates (**Figure 2A**) which showed that seedling-based oxalate oxidase activity leveled off after germination for 5 days. Microscopic examination revealed that the colored product of the histochemical reaction was accumulated in the apoplastic space (cell wall), as reported previously (*21*, *23*) The coleoptile and the seed coat, not the embryo (*22*), were also stained, suggesting considerable oxalate oxidase activity also in these sections of the embryo (**Figure 2B**). Similar patterns were obtained with barley and wheat.

These results show that seedlings of different developmental stages, as a whole or the excised radicles, have a high potential to degrade oxalate enzymatically. This implies that no strict control of germination is required for the proposed technological process, biodegradation of oxalate, that resorts to cereal seedlings or radicles.

Oxalate Degradation, Time Courses, Radicle Drying, and Storage. The rate of oxalate degradation was nearly proportional to the amount of radicles added to an oxalate solution during 100 min. Oxalate degradation then slowed if the radicle concentration was high, probably because of substrate limitation of the enzyme. With regard to the effect of heat drying, **Figure 3B** shows that either 1.0 g of fresh radicles or 0.1 g of dried radicles was required to obtain the same oxalate degradation rate. It is important to note that the radicles were heat-dried under relatively harsh conditions similar to those used in the commercial barley malting process. Our results show that oxalate biodegradation can be carried out with heat-dried radicles, even though heat drying reduces the rate of oxalate degradation activity by approximately half (deduced from the results in **Figure 3B**). Possible reasons are enzyme deactivation and/or reduced accessibility for oxalate due to shrinking. Modifying the drying process is expected to bring about improvement. Deep-freezing the radicles to −20 °C did not lead to any loss of oxalate degradation activity within 4 weeks of storage (results not shown).

Effects of pH and Temperature. Maximum activity occurred in the range between pH 4.0 and 4.5 (**Figure 4**). The results observed with radicles, i.e., oxalate oxidase in situ, thus match those reported for isolated oxalate oxidase (*27*).

High temperatures occur during commercial spinach processing. Oxalate degradation in an oxalate solution was nearly equal between 30 and 60 °C, and the ratio of oxalate degradation obtained within 30 or 60 min was similar in this range of temperatures (**Figure 5**). These results obtained with oxalate oxidase in situ confirm the remarkably high heat resistance of isolated oxalate oxidase (*23*, *28*).

The observed flat temperature dependence curve (**Figure 5**) is untypical for kinetics of an isolated enzyme. It suggests that, at the different temperatures, multiple factors variably codetermine oxalate oxidase activity in situ to different degrees.

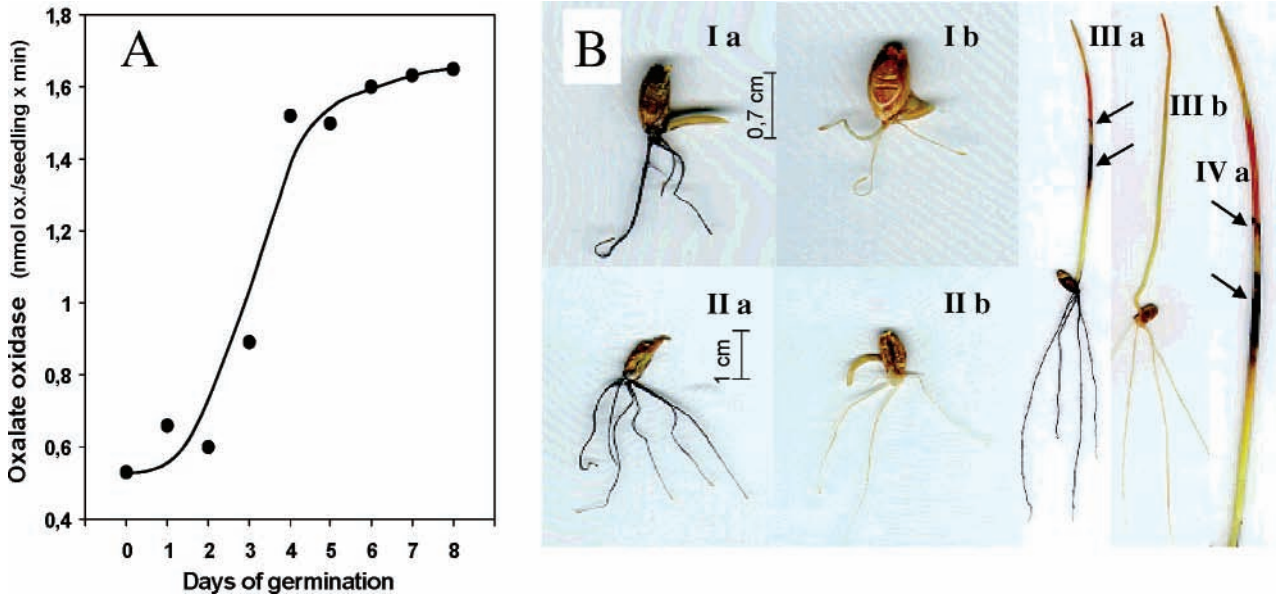

Figure 2. Oxalate degradation by entire rye seedlings (sliced), and visualization of oxalate oxidase activity, depending on the developmental stage of the seedlings. (A) Dependence of the rate of oxalate degradation on the time of germination. The assay medium (20 mL of succinate-K^+ buffer at pH 4.0) of this particular series of experiments contained dissolved oxalate (0.5 mM, pH 4.0, 45 °C, incubation time of 30 min) and 15 soaked seeds (time 0) or 5–15 seedlings. (B) Histochemical staining (purple) of seedlings for the presence of oxalate oxidase: (Ia and Ib) 2-day-old seedlings with and without oxalate, respectively, (IIa and IIb) 4-day-old seedlings with and without oxalate, respectively, (IIa and IIIb) 8-day-old seedlings with and without oxalate, respectively, and (IVa) coleoptile magnified. Note the alternation of stained and unstained sections (arrows) in the coleoptile (IIIa and IVa).

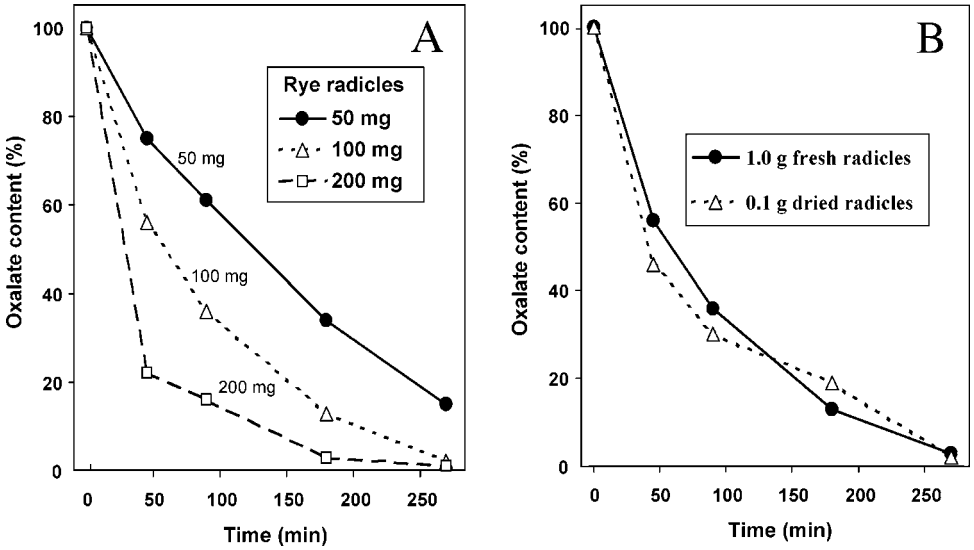

Figure 3. Dependence of oxalate degradation on radicle concentration (A) and requirement of fresh or heat-dried radicle material to obtain the same oxalate degradation rate (B). The assay medium contained dissolved oxalate (0.25 mM, pH 3.5, 30 °C). For panel A, different amounts (fw) of fresh radicles were added. For panel B, 1.0 g of fresh radicles on a fresh weight basis or 0.1 g of heat-dried radicles on a dry weight basis was added.

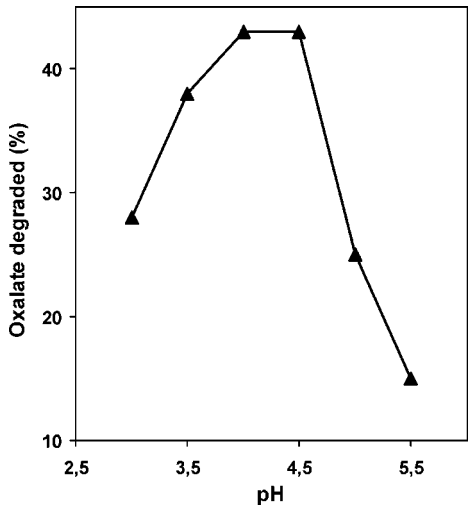

Figure 4. Dependence on pH of the oxalate degradation rate with fresh rye radicles. The assay medium (50 mM succinate-K^+ buffer, 20 mL) contained dissolved oxalate (0,25 mM oxalate, 25 °C, incubation time of 30 min) and 0.1 g (fw) of cereal radicles.

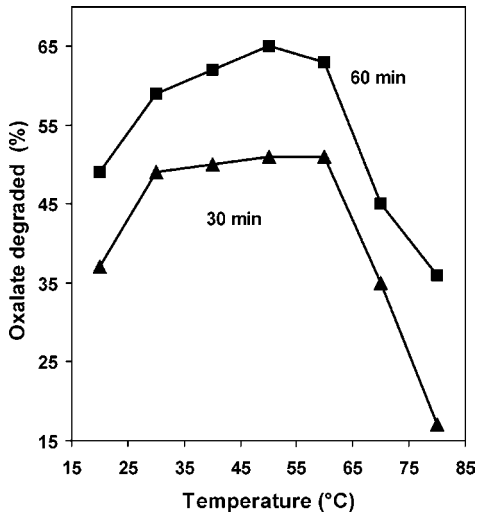

Figure 5. Temperature dependence of oxalate degradation at two different time intervals of incubation. The assay medium (50 mM succinate-K^+ buffer, 20 mL) contained dissolved oxalate (0.25 mM oxalate, pH 3.5, incubation time of 30 min) and 0.1 g (fw) of cereal radicles.

Probable factors are the temperature dependence of the enzyme's activity, its stability, and of the extent of limitation by substrate diffusion.

Our results obtained with oxalate solution altogether demonstrate that radicles from a variety of cereal seedlings of different developmental stages can effectively remove dissolved oxalate over a wide range of temperatures. A moderately acidic pH is required which, in an envisaged technical process with spinach, would allow for readjustment toward neutral pH without inflicting a heavy salt load. Fresh radicles or radicles dried under conditions similar to those of the malting industry may be used.

Degradation of Oxalate in Spinach. *Oxalate Solubility and Content*. The total oxalate content of fresh spinach (**Table 1**, stringent extraction; see Materials and Methods) was at the lower end of published values ranging from 440 to 1570 mg/100 g fw (from 61 to 218 mM; see ref *14*). The proportion of oxalate that was solubilized by water from fresh spinach at room temperature increased with time, but a considerable portion of the oxalate resisted extraction for incubation periods as long as 17 h (**Table 1**). This observation confirms that there are at least two oxalate pools, one of which probably is the oxalate dissolved in the vacuole of the spinach leaf cells (*29*). The pool resistant to water extraction may comprise intracellular Ca or Mg oxalate crystals and oxalate trapped in the cell wall (*1*, *14*).

Table 1. Cold and Hot Extraction (blanching) of Oxalate from Fresh Spinach[a]

extraction condition	amount solubilized (liquid phase) (%)	amount resistant (solid phase) (%)	total amount (mg/100 g as is)
cold			
extraction for 4 h	53	47	844 ± 51
extraction for 17 h	63	37	831 ± 36
hot			
blanching for 4 min	28	72	693 ± 30
spinach after blanching	–	–	498 ± 30

[a] The number of separate extractions was three to four.

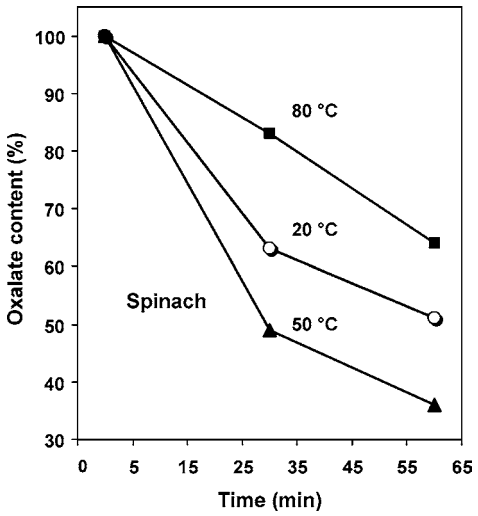

Figure 6. Time kinetics of oxalate content in the liquid phase ("soluble" oxalate) in a spinach preparation at a cool, a medium, and a hot temperature. The pH was adjusted to 3.5; for details, see Materials and Methods. A value of 100% is equivalent to 539 mg of oxalate/100 g of spinach in the liquid phase.

Blanching is commonly used to reduce oxalate content in spinach. To estimate the efficacy of blanching, spinach was subjected to hot extraction for 4 min. This blanching treatment reduced oxalate content by a mere 28% (**Table 1**). The total oxalate concentration after blanching was similar to that of the commercial frozen spinach (certainly blanched) that was used in the degradation experiments (539 mg of oxalate/100 g fw). The moderate effect of blanching on oxalate content indicates that blanching, as the sole treatment and if not applied excessively, is not sufficient to obtain a spinach product compatible with a low-oxalate diet (**Table 1**).

The high oxalate degradation rates obtained with dissolved oxalate and radicles prompted us to try the method with spinach. It should be noted at this point that, with dissolved oxalate and radicles, oxalate needs to pass only one obstacle, the radicle's cell wall, to reach the oxalate oxidase located therein. Fortunately, the cell wall is equivalent to the so-called "free space" of plant cells where small molecules can move freely. In a spinach/radicle system, by contrast, the cell membranes of the leaf tissue constitute specific diffusion barriers (see **Table 1**). Because heat renders membranes porous, one can expect that a short heat treatment (blanching) makes the endogenous spinach oxalate available for the radicle's oxalate oxidase, at least to some extent. Elevated temperatures are uncritical for oxalate oxidase (**Figure 5**), but certain endogenous compounds of the spinach leaves could inhibit oxalate oxidase (*26*, *27*). Experimental evidence was therefore required to show whether oxalate biodegradation can occur at a satisfactory rate in a spinach/radicle or seedling system.

The lab-scale experiments that were conducted were meant to provide the basis for the development of industrial processes for reducing oxalate levels in spinach or other oxalate-containing foods. For practical reasons, commercial frozen spinach was used except in one set of experiments.

Temperature. Over time periods of 30 min and 1 h, the highest oxalate degradation rate in the liquid phase was observed at 50 °C by comparison with that obtained at 20 or 80 °C (**Figure 6**). It is worth emphasizing that even at 80 °C, i.e., near the boiling temperature, oxalate degradation proceeded linearly at an appreciable rate over a time period of as much as 1 h. Substrate limitation of the enzyme may be responsible for the deviation from linearity at the lower temperatures (**Figure 6**).

Because heat treatment is a general feature of any spinach processing (blanching), oxalate degradation with suspended spinach leaf slices was assessed over a wide temperature range. **Figure 7A** shows that, during 4 h, the rate of oxalate degradation in the spinach suspension (total oxalate) remained similarly high in the temperature range between 18 and 35 °C. If the temperature was increased to 55 °C, the rate of oxalate degradation declined to a still appreciable value of 20%. Even in the cold, at 5 °C, some oxalate degradation occurred. The very flat temperature dependence kinetics obtained with the complex spinach/radicles system (**Figure 7A**) supports the hypothesis that multiple kinetics overlap probably describing the oxalate oxidase's temperature optimum, deactivation, substrate diffusion, and probably other limiting factors.

To gain more information, the level of oxalate was determined separately in the liquid phase and in the solid phase of the spinach preparation. In the liquid phase, the rate of oxalate degradation remained high if the temperature was decreased to below 18 °C (**Figure 7A**). By contrast, the rate of oxalate degradation in the solid phase declined considerably. It appears feasible to propose that one reason for this decline is the low solubility and diffusion rate of oxalate in the cold. The result that reducing the size of the tissue pieces (using a blender; see Materials and Methods) gave approximately one-third higher degradation rates (results not shown) supports the hypothesis that, in the spinach/radicle system, solubilization and diffusion of oxalate to the enzyme are major limiting factors for total oxalate degradation.

This complexity notwithstanding, our results indicate that substantial oxalate removal from spinach can be achieved over a wide range of temperatures using rye radicles.

Effects of pH. With spinach, the pH dependence of oxalate degradation in the solid phase differed from that recorded with dissolved oxalate (**Figure 7B**). There was a marked shift toward acidic pH. In contrast, the pH dependence of oxalate degradation for the liquid phase was similar to that for oxalate solution. Likewise, the curve for the spinach suspension, calculated from the values for the liquid and solid phase, was intermediate. The requirement for a more acidic pH to obtain maximum oxalate degradation in the solid phase probably reflects the higher solubility and leaching of oxalate from the spinach leaf tissue at acidic pH.

As far as practical application is concerned, it is an important finding that moderate acidification of the spinach is sufficient to achieve substantial oxalate degradation rates.

Other Experiments. If the amount of added radicles was reduced to 1 g instead of 5 g, the rate of oxalate degradation was reduced by only one-third. With spelt radicles, results similar to those obtained with rye were obtained (data not shown).

Different parts of the fresh rye and wheat seedlings (germination for 8 days) were tested for soluble oxalate degradation, and sprout, grain, radicle, and entire seedling. With the same amount of fresh weight added to spinach suspensions, 36−65% oxalate degradation (liquid phase) was obtained in these particular experiments (two replicas). It is concluded that, if desired, each portion of the seedling, radicle, sprout, grain, or entire seedling can be used to reduce spinach oxalate content. It is deemed appropriate to recognize at this point the work of Zalesky and Reinhard who reported, in 1911, that the wheat grain (meal) contains some "catalyzer that executes the oxidation of oxalic acid" (*30*).

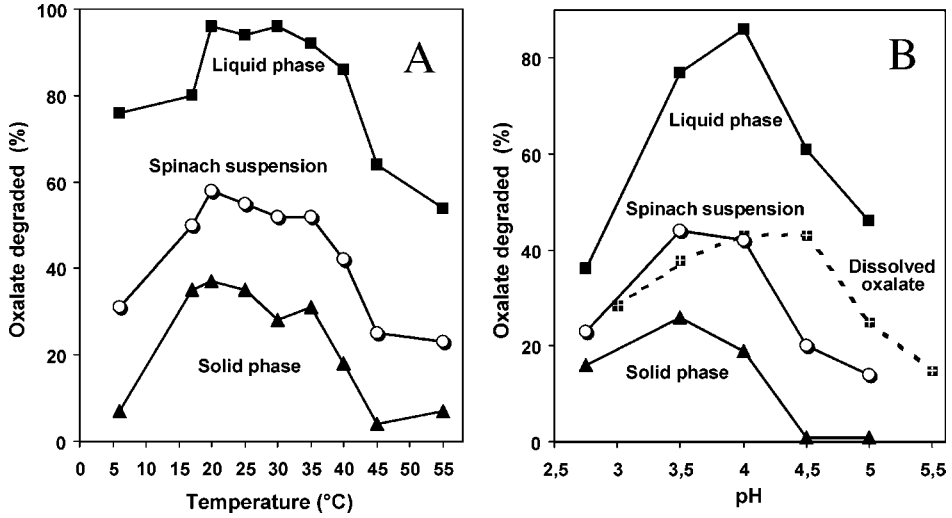

Figure 7. Temperature dependence (A) and pH dependence (b) of oxalate degradation in a spinach suspension, and in the liquid phase (soluble oxalate) and the solid phase (insoluble oxalate) obtained thereof by centrifugation. The incubation time was 240 min. The pH and temperature were pH 3.5 and 25 °C, respectively. See Materials and Methods for details. The gray line in panel B is the pH curve obtained with dissolved oxalate (**Figure 4**). The total amount of oxalate in the spinach suspension was calculated from the values for the liquid and solid phases.

Heat-Dried Radicles. The malting process includes the production of heat-dried cereal radicles as waste. With 2 g of rye radicles similarly heat-dried in the laboratory, oxalate degradation at 45 and 55 °C was like that obtained with 5 g of fresh radicles (results not shown). It is concluded that heat-dried radicles, which are stable and readily available at a cheap price, can generally replace fresh radicles to biodegrade oxalate from spinach.

Fresh Spinach. If fresh, sliced spinach was used without any heat treatment, little or negligible oxalate degradation was detected in the liquid phase or solid phase, respectively (results not shown). These observations confirm that some heat treatment, or any other treatment that alleviates the containment of the spinach oxalate, is required for effective oxalate degradation by the radicles' oxalate oxidase.

Nitrite. One set of experiments was carried out to check for the possible reduction of nitrate to nitrite during incubation. If incubation was conducted at pH 3.5, up to 20% of the nitrate in the mixture disappeared. The level of nitrite remained below 1 mg/100 g of spinach. At near-neutral pH (water was used instead of buffer), nitrate degradation was more rapid, but no nitrite was accumulated (results not shown). Because of the heat sensitivity of nitrate reductase, it is reasonable to assume that even less, if any, nitrite is formed with heat-dried radicles and blanched spinach. These results show that nitrite accumulation is not critical for the applicability of the biotechnological procedure for removal of oxalate from spinach.

DISCUSSION

In human nutrition, the bulk of oxalic acid intake comes from vegetables. **Table 2** shows oxalate intake rates if preferably either oxalate-rich or oxalate-poor vegetables are consumed (*31*). One can conclude from these data that a more effective technique than blanching for reducing the oxalate content in oxalate-rich vegetables, e.g., spinach, would have a marked effect on total oxalate intake through nutrition, and therefore on oxalate-associated health risks (**Table 2**).

Accepting on the current basis of knowledge that oxalate content should be low in foods, especially in infant formulas or in dietary foods for consumers metabolically prone to oxalate renal stone formation, the development of low-oxalate products is considered to be a reasonable goal (see ref. *12*, *32*, *33*). Because of the oxalate oxidase's high substrate specificity, the proposed biological approach would remove oxalate while preserving health-protective endogenous compounds. Moreover, if the entire young cereal seedling was used, novel food products could be created that would be enriched with cereal-specific health-protective ingredients such as unsaturated fatty acids and lipophilic vitamins. As for possible health risks from products of the oxalate oxidase reaction, only carbon dioxide and hydrogen peroxide would be formed. The latter is most probably eliminated by catalase and nonenzymic catalyzers present in the spinach/seedling mixture. As far as hygiene is concerned, the use of heat-dried radicles or cereal seedlings may be especially advantageous.

In the malting industry, the radicles of the dry barley seedlings are discarded as waste. As a consequence, vast amounts of this oxalate oxidase-containing material should be available at no cost. It is probable that directed modifications of drying process preparation would help to preserve even more of the radicle's or seedling's oxalate oxidase. It is clear that the malting industry, or the processes used there, could also provide fresh seedlings at a reasonable price.

In addition to health effects, indirect environmental benefits are also expected from the proposed biodegradation process. To minimize the leaching of nitrate to the groundwater, growing vegetables at the economically lowest possible level of nitrogen

Table 2. Estimation of the Daily Oxalate Intake through the Consumption of Different Combinations of Vegetables and of Fruits[a]

vegetables or fruit	rate of oxalate intake (mg day^{-1} person^{-1})	oxalate concentration (mg/100 g as is)
all vegetables, 33 species	159	70
high-oxalate vegetables, 5 species	908	400
low-oxalate vegetables, 28 species	23	10
fruit, 13 species	12	10

[a] The rate of oxalate intake was calculated from the average oxalate concentration in and the average consumption per capita of vegetables in Germany, 227 g day^{-1} person^{-1}. Vegetable intake means intake from either of the three hypothetical vegetable baskets as alternatives. The rate of oxalate intake from fruit was calculated on the basis of 116 g of fruit consumed per day per person. Data sources are refs *19* and *31*.

fertilization is desirable. It is unfortunate, however, that low nitrogen levels tend to produce high oxalate levels in leafy vegetables (see Introduction). Aside from yield, this is another reason spinach is generally grown at a relatively high level of nitrogen supply. The proposed biodegradation method for removing oxalate has the potential to allow for the use of low-nitrate spinach in a low-oxalate diet, despite its initially elevated oxalate content in the field. It is interesting in this respect that nitrogen fertilization levels are generally relatively low in organic agriculture, and in conventional agriculture, if an integrated crop management concept is applied.

It has been proposed that oxalate oxidase would play a role in the defense against certain plant pathogens (*21*, *34*). Therefore, oxalate oxidase genes have been cloned and expressed in plant tissues expecting the introduction of oxalate oxidase-conferred resistance against pathogens (*35*). To our knowledge, this technology has not been used so far to reduce oxalate levels in the plant for reasons of human or animal nutrition and health.

As far as optimization of oxalate biodegradation using cereal seedling or radicles is concerned, the lab-scale experiments have demonstrated that oxalate is effectively biodegraded in the liquid phase of spinach preparations, while there is room for improvement for oxalate in the solids. On the other hand, the oxalate pool that was not not accessible for the radicle's oxalate oxidase is probably under containment (e.g., located within the spinach cell wall). In human nutrition, this pool may be bioavailable only to a low extent. Research on this topic appears to be justified.

It is a matter of fact that, before a novel process is introduced to the industrial practice, analyses of the potential for improving the overall efficiency of the process, its practicability, and chances of success of the resulting produce in the marketplace are an absolute requirement. The presented results are encouraging in this respect: oxalate degradation by the radicles from seedlings of different ages occurred (i) at a high rate over (ii) a wide temperature range for (iii) prolonged time spans, and also (iv) with heat-dried radicles. Moreover, the proposed biodegradation method requires neither enzyme preparation or purification nor gene technology or any microorganism.

Further innovative applications of cereal seedlings or radicles with regard to oxalate are envisaged: removal of oxalate from other vegetables or from respective fluids such as cycled blanching waters, other process waters, or vegetable or fruit juices.

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