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SHORT COMMUNICATION

# Relationship between the activity of the orotic acid pathway and glucose content of roots of *Cucurbita pepo*

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**Abstract.** Apical 3-cm root segments excised from 2-d-old squash seedlings (*Cucurbita pepo* L. cv. Early Prolific Straightneck) that were germinated and grown between sheets of paper towelling moistened with $H_2O$, incorporated $144 \pm 10$ nmol ($\bar{x} \pm$SE, $n = 15$) $NaH^{14}CO_3$ into uridine nucleotides (ΣUMP) per gram intact roots during the 3-h incubation period (Lovatt, Albert & Tremblay, 1979). Continued culture of squash seedlings in this manner for an additional 24 or 48 h had no effect or slightly increased (30%) the activity of the orotic acid pathway. However, transfer of 2-d-old seedlings to Shive's nutrient solution reduced the activity of the orotic acid pathway within 15 h to 2.3 nmol $NaH^{14}CO_3$ incorporated into ΣUMP per gram apical 3-cm root segments during the 3-h incubation period. The observed decrease in capacity to synthesize pyrimidine nucleotides *de novo* paralleled the reduction in glucose content of the roots and was reversed by supplying glucose during hydroponic culture in Shive's nutrient solution. Root elongation was not affected by the reduced activity of the orotic acid pathway nor by the decreased level of available glucose.

*Key-words: Cucurbita pepo;* Cucurbitaceae; squash; pyrimidine biosynthesis; glucose content.

Previous investigations which assessed the activity of the orotic acid pathway in intact roots excised from 2- and 6-d-old summer squash seedlings, *Cucurbita pepo* L. cv. Early Prolific Straightneck (Lovatt, Albert & Tremblay, 1979, 1981), revealed a 60-fold difference in the relative capacities of these two tissues to synthesize uridine nucleotides *de novo*. Apical root segments, averaging 3 cm in length, excised from 2-d-old seedlings that were germinated and grown in the dark at 30 °C, between sheets of paper towelling moistened with 25 $cm^3$ $H_2O$, incorporated $144 \pm 10$ nmol ($\bar{x} \pm$SE, $n = 15$) $NaH^{14}CO_3$ into uridine nucleotides (ΣUMP) per gram root segments during the 3-h incubation period. Transfer of 2-d-old seedlings grown in this manner to hydroponic culture in Shive's nutrient solution under continuous illumination at 310 μmol $m^{-2}$ $s^{-1}$ at 30 °C until the plants were 6-d-old reduced the incorporation of $NaH^{14}CO_3$ into ΣUMP per gram apical 3-cm root segments to $2.4 \pm 0.1$ nmol ($\bar{x} \pm$SE, $n = 11$) during the 3-h incorporation period. Marked differences in the sensitivity of the orotate pathway to exogenous uridine and sucrose supplied during the incubation period were also observed in these two tissues. The incorporation of $NaH^{14}CO_3$ into ΣUMP in 2-d-old roots was inhibited 80% by the addition of 0.5 mol $m^{-3}$ uridine while 6-d-old roots were insensitive to added uridine (Lovatt *et al.*, 1981). In contrast, added sucrose (0.1 kmol $m^{-3}$) stimulated the incorporation of $NaH^{14}CO_3$ into ΣUMP 8-fold in 6-d-old roots but had little effect on pyrimidine synthesis in 2-d-old roots; stimulation of less than 1.5-fold was observed (Lovatt *et al.*, 1981). The cause of the marked differences in the capacities of 2- and 6-d-old roots, grown as described above, to synthesize pyrimidine nucleotides *de novo* is not known. The present study was undertaken to determine: (1) the influence of environmental parameters, e.g. hydroponic culture, nutrient availability and light on the activity of the orotic acid pathway; (2) if the loss in capacity to synthesize pyrimidines *de novo* is a consequence of the natural processes associated with maturation as germinating seeds develop into seedlings; and (3) the relationship between glucose availability, the activity of the orotic acid pathway and the rate of root growth. In this communication, the results of these investigations and the aetiology of the observed differences in the capacities of 2- and 6-d-old roots of *C. pepo* to synthesize uridine nucleotides *de novo* is reported.

Summer squash seeds (*Cucurbita pepo* L. cv. Early Prolific Straightneck), supplied by courtesy of the Joseph Harris Company, Inc., were imbibed in distilled $H_2O$ for 24 h at room temperature. They were then spread evenly on one sheet of paper towelling placed in a plastic box ($33 \times 23 \times 9.5$ cm) and moistened with 25 $cm^3$ $H_2O$. The box was covered and sealed with clear plastic wrap and placed in a growth chamber where the seeds germinated at 30 °C for specified periods of time,

either in the dark or under continuous illumination at 310 $\mu$mol $m^{-2}$ $s^{-1}$. When net root elongation was to be determined, 30 seedlings were selected randomly at the end of each 24-h period and total root length was measured. Average net elongation was calculated.

After 48 h (excluding imbibition), seeds germinated as described above were inserted into holes in the lid of a polyurethane breadbox (38 × 14 × 13 cm, 5.5 $dm^3$) covered with aluminum foil and containing either distilled $H_2O$ or Shive's nutrient solution [5 mol $m^{-3}$ $Ca(NO_3)_2$, 2 mol $m^{-3}$ $MgSO_4$, 2 mol $m^{-3}$ $K_2SO_4$, 1 mol $m^{-3}$ $KH_2PO_4$, 1 mg Fe $dm^{-3}$, 1 mg Mn $dm^{-3}$, 0.13 mg Cl $dm^{-3}$, 0.1 mg Zn $dm^{-3}$, 0.1 mg B $dm^{-3}$, 0.01 Cu mg $dm^{-3}$, 0.01 mg Mo $dm^{-3}$, and 0.01 mg Na $dm^{-3}$] (pH 4.7). The box was transferred to a growth chamber where the plants were hydroponically cultured in aerated solution at 30 °C in the dark or under continuous illumination at 310 $\mu$mol $m^{-2}$ $s^{-1}$ for specified periods of time. At the end of this time, the apical 3 cm of the primary root was excised. The activity of the orotic acid pathway for the *de novo* biosynthesis of uridine nucleotides was assessed in this tissue by measuring the incorporation of $NaH^{14}CO_3$ (10 mol $m^{-3}$, 1650–6600 dpm nmol$^{-1}$) into ΣUMP as described previously (Lovatt *et al.*, 1979, 1981).

Apical 3-cm root segments were also extracted by the method of Richmond *et al.* (1981), and the glucose content of the extract and starch content of the insoluble fraction were determined by a modification of the glucose oxidase-peroxidase-*o*-dianisidine method (MacRae, 1971; Hassig & Dickson, 1979).

The differences in capacities for *de novo* pyrimidine synthesis observed for apical 3-cm root segments excised from 2-d-old seedlings germinated in the dark on paper towels and those excised from 6-d-old seedlings hydroponically cultured in the light are not a function of age or exposure to light. Apical 3-cm root segments excised from 3- and 4-d-old squash seedlings germinated and grown in the dark or in the light at 30 °C on a sheet of paper towelling moistened with distilled water, incorporated $NaH^{14}CO_3$ into ΣUMP at a rate equal to or slightly greater (30%) than that of 2-d-old roots (Table 1). However, 24 h after transfer of these 2-d-old squash seedlings to hydroponic culture in Shive's nutrient solution in the dark or in the light at 30 °C, roots exhibited all the characteristics previously observed with 6-d-old roots grown in the light in Shive's nutrient solution for 4 d: (1) activity of the orotic acid pathway was reduced 98% to only 2.3 nmol $NaH^{14}CO_3$ incorporated into ΣUMP per gram apical 3-cm root segments during the 3-h incubation period (Table 1); (2) activity of the orotic acid pathway was insensitive to uridine added to the incubation medium; and (3) glucose supplied during the pre-incubation and incubation periods significantly stimulated the incorporation of $NaH^{14}CO_3$ into ΣUMP.

Taken together, these results provide strong evidence that hydroponic culture in Shive's nutrient solution was the primary cause for the reduced capacity of squash roots to synthesize uridine nucleotides *de novo* and for the altered sensitivity of the orotate pathway to the exogenous supply of both uridine and glucose. To test this possibility and to examine the relationship between the activity of the orotate pathway and the observed stimulation by glucose added during the incorporation period to plants cultured in Shive's, 2-d-old squash seedlings were hydroponically cultured in Shive's nutrient solution in the dark for increasing lengths of time up to 30 h. To determine the effect of Shive's nutrient solution from that of hydroponic culture itself, seedlings were initially transferred to aerated distilled $H_2O$ and subsequently transferred to Shive's nutrient solution to complete the 30-h treatment. Glucose content and capacity to incorporate $NaH^{14}CO_3$ into ΣUMP were determined in apical 3-cm root segments. The 2-h pre-incubation and 3-h incubation in Shive's nutrient solution routinely employed to assess the activity of the orotic acid pathway were considered as 5 h of Shive's treatment.

The capacity of 3-d-old *C. pepo* plants to synthesize uridine nucleotides *de novo* decreased as the duration of hydroponic culture in Shive's nutrient solution increased (Fig. 1a) and paralleled a

**Table 1.** Influence of culture conditions and plant age on the capacity to synthesize pyrimidine nucleotides *de novo* and the starch content of apical 3-cm root segments excised from *Cucurbita pepo* and on root elongation during the 24 h prior to root excision. Squash seeds were imbibed for 24 h at room temperature and germinated on paper towels for 3 d or transferred after 2 d to hydroponic culture in aerated Shive's nutrient solution at 30 °C. When grown in the light, continuous illumination of 310 $\mu$mol $m^{-2}$ $s^{-1}$ was provided

| Plant culture conditions | Paper towels moistened with $H_2O$ | | Hydroponic culture in Shive's nutrient solution | |
|---|---|---|---|---|
| | 2* | 3 | 3 | 6 |
| | nmol $NaH^{14}CO_3$ incorporated into ΣUMP $g^{-1}$ apical 3-cm root segments 3 $h^{-1}$† | | | |
| Dark | 144 ± 10 (15) | 179, 140 | 2.5, 1.3 | |
| Light | | 123 | 1.7 | 2.4 ± 0.1 (11) |
| | mg starch $g^{-1}$ fr wt | | | |
| Dark | 1.0 ± 0.02 (4) | 1.0, 1.0 | 0.3, 0.0 | |
| Light | | 2.9, 3.1 | 0.7, 0.5 | 0.1 ± 0.04 (4) |
| | mm root elongation 24 $h^{-1}$ | | | |
| Dark | 36 ± 1 (4) | 26, 30 | | |
| Light | | | 33, 30 | 37 ± 6 (10) |

*Plant age (d).

†Results of individual experiments or the average of several experiments ± standard error with number of experiments given in parentheses are presented. $NaH^{14}CO_3$ was provided at a final concentration of 10 mol $m^{-3}$ and specific radioactivity of 1650–6600 dpm nmol$^{-1}$.

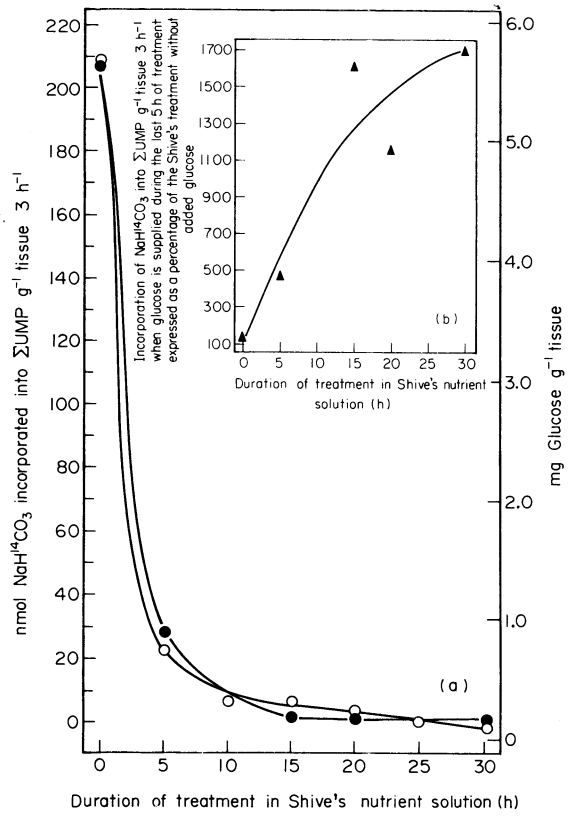


**Figure 1.** (a) Incorporation of $NaH^{14}CO_3$ into ΣUMP (●) and glucose content (○) of apical 3-cm root segments excised from 3-d-old *Cucurbita pepo* seedlings hydroponically cultured in Shive's nutrient solution for increasing periods of time. Squash seedlings germinated in the dark on paper towels moistened with $H_2O$ were transferred at age 2 d to aerated solution culture in $H_2O$. Plants were subsequently transferred to Shive's nutrient solution so that they completed the indicated treatment period in Shive's immediately prior to determination of glucose content or activity of the orotic acid pathway. The 2-h pre-incubation and 3-h incubation in Shive's nutrient solution routinely employed to assess the activity of the orotate pathway was considered part of the Shive's treatment. Roots excised from 2-d-old plants comprised the no-Shive's treatment; the pre-incubation and incubation were carried out in $H_2O$. All plants were cultured in the dark at 30 °C. (b) The effect of adding glucose (0.1 kmol $m^{-3}$ final concentration) during the pre-incubation and incubation periods on the incorporation of $NaH^{14}CO_3$ into ΣUMP in roots excised from *C. pepo* seedlings grown as described in (a). Results are expressed as a percentage of the Shive's treatment without added glucose for the treatment period indicated (▲). Roots excised from 2-d-old plants pre-incubated and incubated in Shive's plus glucose comprised the no-Shive's treatment.

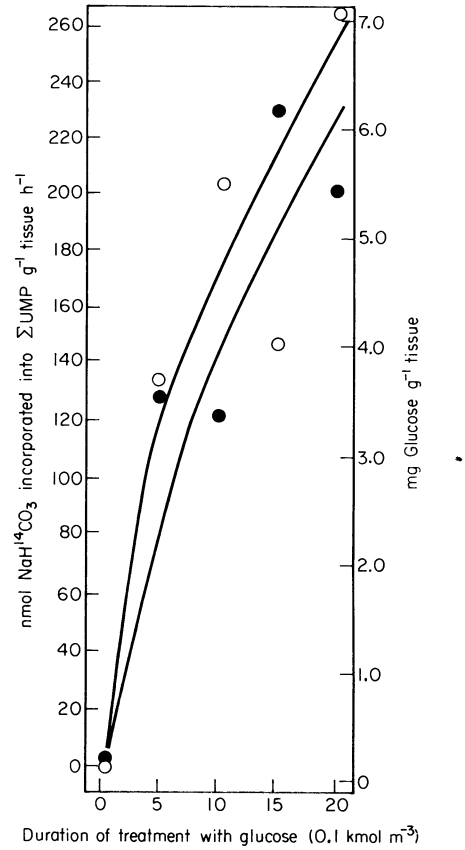


**Figure 2.** Effect of supplying exogenous glucose during hydroponic culture of *Cucurbita pepo* in Shive's nutrient solution on the incorporation of $NaH^{14}CO_3$ into ΣUMP (●) and glucose content (○) of apical 3-cm root segments excised from 3-d-old plants. Two-day-old dark-germinated squash seedlings were transferred to hydroponic solution culture in the light (310 μmol $m^{-2}$ $s^{-1}$) at 30 °C in Shive's nutrient solution for 30 h. Glucose was added to a final concentration of 0.1 kmol $m^{-3}$ at specified times such that culture in the presence of glucose was completed immediately before assessing the activity of the orotate pathway or glucose content of the roots. Glucose (0.1 kmol $m^{-3}$) was added to both the pre-incubation and incubation periods which constituted 5 h of plus-glucose treatment.

reduction in glucose content of the roots (Fig. 1a). The changes in metabolism observed were related partly to hydroponic culture itself and partly to the presence of Shive's nutrient solution. A 30-h treatment in aerated $H_2O$ reduced both the incorporation of $NaH^{14}CO_2$ into ΣUMP and the glucose content of the roots by 66%. However, it can be seen in Fig. 1a that a 15-h treatment in Shive's reduced these two parameters by 98%. In contrast, the glucose content of roots excised from 3-d-old plants grown on paper towels remained high: $5.2 \pm 0.5$ mg ($\bar{x} \pm$ SE, $n = 4$) per gram apical 3-cm root segments. Evidence that the activity of the orotic acid pathway is regulated by changes in glucose content was demonstrated when: (1) exogenous glucose supplied in the pre-incubation and incubation media was more effective in stimulating *de novo* pyrimidine biosynthesis as the endogenous glucose content of the roots decreased (Fig. 1b); and (2) glucose provided at a final concentration of 0.1 kmol $m^{-3}$ in the Shive's nutrient solution during hydroponic culture of 2-d-old seedlings in the light resulted in increased incorporation of $NaH^{14}CO_3$ into ΣUMP as the duration of the glucose treatment increased (Fig. 2). Although the results in Fig. 2 are variable, probably due to excessive washing of the roots to remove residual glucose from the hydroponic culture medium that might be adhering to the roots, it is

clear that the activity of the orotate pathway increases in parallel with the increase in the glucose content of the roots.

Hydroponic culture in Shive's nutrient solution also reduced the starch content of roots regardless of whether the seedlings were grown in the light or in the dark (Table 1). Despite the significantly lower content of both glucose and starch and the dramatic reduction in the activity of the orotic acid pathway in roots excised from plants hydroponically cultured in Shive's nutrient solution, the rate of root growth in these plants was not affected (Table 1). This observation suggested that the reduction in *de novo* pyrimidine biosynthesis caused by decreased glucose and starch availability was not simply due to carbohydrate depletion and loss of energy. Consistent with this observation, pyruvate and malate added to the pre-incubation and incubation media did not stimulate the incorporation of $NaH^{14}CO_3$ into ΣUMP in apical 3-cm root segments excised from 6-d-old plants hydroponically cultured in Shive's nutrient solution. Stimulation of *de novo* pyrimidine biosynthesis by added pyruvate or malate would be expected if the stimulation by added glucose was through its use as a substrate for respiration. Studies employing radiolabelled pyruvate and malate demonstrated that these compounds were taken up by both 2- and 6-d-old roots and metabolized at rates similar to the metabolism of [$^{14}$C] glucose in each tissue: 25 and 9 μmol $^{14}CO_2$ released per gram apical 3-cm root segments during the 3-h incubation period for 2- and 6-d-old roots, respectively. Despite the dramatic effect of added glucose, 6-d-old roots metabolized all three compounds at about one-third the rate of 2-d-old roots.

The results of this study clearly demonstrated that hydroponic culture of young squash seedlings, especially in a complete nutrient solution, e.g. Shive's, reduced the glucose and starch content of the roots and effected a concomitant decrease in their capacity to synthesize uridine nucleotides *de novo*. The result obscured demonstration of end product inhibition as a mechanism regulating the activity of the orotate pathway in *C. pepo* seedlings hydroponically cultured in Shive's nutrient solution for 15 h or longer. Evidence to support the causal relationship between root glucose content and the activity of the orotate pathway was provided by experiments which demonstrate that the addition of glucose to Shive's nutrient solution during hydroponic culture reversed the effect of the Shive's treatment and restored the capacity of roots to incorporate $NaH^{14}CO_3$ into ΣUMP commensurate with the amount of glucose taken up by the tissue. In young squash seedlings (up to 6 d old), the activity of the orotic acid pathway was not related to age and was independent of available light. However, the parallel between glucose content and *de novo* pyrimidine biosynthesis suggests that light availability might influence the activity of the orotate pathway in older plants depending on their nutrient status.

It is clear that a nutrient or combination of nutrients in the Shive's is responsible for the loss of carbohydrate in the roots. Only 15 h hydroponic culture in Shive's nutrient solution reduces the root glucose content by 98% but 30 h of hydroponic culture in water is necessary to reduce the glucose content of the roots by 66%. It is well known that nitrogen availability influences the metabolism of glucose. In preliminary studies, transferring 2-d-old dark-germinated seedlings to hydroponic culture in 5 mol $m^{-3}$ $Ca(NO_3)_2$ for 25 h in the dark and subsequently employing $Ca(NO_3)_2$ instead of Shive's in the pre-incubation and incubation periods reduced the incorporation of $NaH^{14}CO_3$ into ΣUMP to 7 nmol per gram apical 3-cm root segments during the 3-h incubation period. However, a similar experiment replacing the $Ca(NO_3)_2$ in Shive's with $CaSO_4$ failed to significantly increase the activity of the orotic acid pathway; the rate of incorporation increased from 2.3 to 13 nmol $NaH^{14}CO_3$ incorporated into ΣUMP per gram apical 3-cm root segments in 3 h.

The aetiology of the previously observed differences between 2-d-old (Lovatt *et al.*, 1979) and 6-d-old (Lovatt *et al.*, 1981) seedlings of *C. pepo* can now be explained; it is apparently the result of the hydroponic culture of the latter in Shive's nutrient solution. In roots excised from 2-d-old seedlings germinated in the dark on paper towels moistened with $H_2O$, glucose content [5.6 ± 0.8 mg ($\bar{x} \pm$ SE, $n = 4$) per gram fresh weight roots] and activity of the orotic acid pathway (144 ± 10 nmol $NaH^{14}CO_3$ incorporated into ΣUMP per gram roots during the 3-h incubation period; this value is approximately 40% greater when $H_2O$ is employed instead of Shive's during the pre-incubation and incubation periods) are 60–90-fold higher than in 6-d-old plants. Because the root glucose content is high adding more glucose or sucrose has little effect on the activity of the orotate pathway. Similarly, because the activity of the orotic acid pathway is high, end product inhibition of the orotate pathway by added uridine (0.5 mol $m^{-3}$) can be demonstrated. In roots excised from 6-d-old plants, 4 d of hydroponic culture in Shive's nutrient solution reduced the level of available glucose to only 0.06 ± 0.01 mg ($\bar{x} \pm$ SE, $n = 4$) per gram apical 3-cm root segments and, concomitantly, the capacity of these roots to synthesize uridine nucleotides *de novo* to only 2.4 ± 0.1 nmol $NaH^{14}CO_3$ incorporated into ΣUMP. In this tissue, the glucose content and the activity of the orotate pathway are so low that added uridine is without effect, while the effect of added glucose is dramatic. Despite the 60–90-fold difference in glucose content and incorporation of $NaH^{14}CO_3$ into ΣUMP, the rate of root elongation is the same for both tissues. Thus, though low, the glucose

content and rate of *de novo* pyrimidine synthesis observed in 6-d-old roots must be adequate for normal growth.

Since substrates for respiration, other than glucose, are without effect in restoring the capacity of 6-d-old roots to synthesize pyrimidine nucleotides *de novo* but are metabolized to the same degree as glucose by this tissue, the influence of glucose availability on the incorporation of $NaH^{14}CO_3$ into ΣUMP must not be one of simply supplying energy. The possibility that glucose influences the pool of 5-phosphorylribose-1-pyrophosphate (PRPP) available for *de novo* pyrimidine biosynthesis is currently being investigated. It is possible that when glucose is low, the reduced capacity to synthesize uridine nucleotides *de novo* is offset by the ability of hydroponically grown plants to meet their needs for pyrimidine nucleotides by the re-utilization of existing pyrimidine nucleosides and bases through the activity of salvage enzymes (especially uridine phosphorylase and uridine kinase if PRPP were limiting).

The hydroponic culture of plants for biochemical experiments is common. In light of the results of this investigation, care should be used in interpreting the results of studies of plant metabolism and metabolic regulation when tissues from hydroponically cultured plants are employed.

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## References

Hassig, B.E. & Dickson, R.E. (1979) Starch measurement in plant tissue using enzymatic hydrolysis. *Physiologia Plantarum*, **47**, 151–157.

Lovatt, C.J., Albert, L.S. & Tremblay, G.C. (1979) Regulation of pyrimidine biosynthesis in intact cells of *Cucurbita pepo*. *Plant Physiology*, **64**, 562–569.

Lovatt, C.J., Albert, L.S. & Tremblay, G.C. (1981) Synthesis, salvage, and catabolism of uridine nucleotides in boron-deficient squash roots. *Plant Physiology*, **68**, 1389–1394.

MacRae, J.C. (1971) Quantitative measurement of starch in very small amounts of leaf tissue. *Planta*, **96**, 101–108.

Richmond, M.L., Brandao, S.C.C., Gray, J.I., Markakis, P. & Stine, C.M. (1981) Analysis of simple sugars and sorbitol in fruit by high-performance liquid chromatography. *Journal of Agriculture and Food Chemistry*, **29**, 4–7.