Chemical Composition of Turnip Roots Stored or Intermittently Grown at Low Temperature

V.I. Shattuck, Y. Kakuda, B.J. Shelp, and N. Kakuda

University of Guelph, Guelph, Ontario N1G 2W1, Canada

Abstract. Field and greenhouse studies were conducted to investigate the effects of low temperature on the starch, sugar, ascorbic acid, and glucosinolate (GS) concentration in turnip [Brassica rapa ssp. rapifera (Metzg.) Sinsk] roots. Field-harvested roots were stored at 0°C for 2 and 4 weeks. In the greenhouse, plants were grown at 0 to 12°C for parts of 11 days before harvest. Cold-stored roots decreased in both starch and total sugar concentration (sucrose, fructose, and glucose) when compared to freshly harvested roots. Greenhouse-grown plants subjected to low temperature had roots with a similar starch content but with a higher concentration of total sugars than the control. In both experiments, the cold treatments induced a slight but significant increase in root sucrose concentration. The ascorbic acid concentration of roots was not affected by low temperature. In both the field and greenhouse studies, low temperature did not change the total concentration of the eight major GSs identified in peeled root “and peel tissues, but did alter the concentration of specific GSs.

Turnips are popularly believed to retain good eating quality when stored under optimal conditions (0°C, 95% relative humidity). However, preliminary investigations have indicated that long-term storage of large roots of certain turnip cultivars, such as ‘White Lady’, resulted in appreciable flavor change (V.I.S. and Y. K., unpublished data). This observation is not surprising since variation in storage potential commonly exists among cultivars of other horticultural crops (Lidster et al., 1988; Ministry of Agriculture, Fisheries and Food, 1979) and low-temperature-induced quality changes involving carbohydrates and GSs have been shown to occur in various cruciferous crops (Bérard and Chong, 1984; Guffy and Hicks, 1984; Peirce, 1987; Suzuki and Cutchilffe, 1981).

In spite of the economic importance of turnip, no reports have focused on the influence of low temperature on root quality. Low temperatures, besides affecting the sensory characteristics of the root, might also change other compounds that could ultimately lead to poor acceptance by consumers. This study was undertaken to determine the influence of low temperature on the starch, sugar, ascorbic acid, and GSs of a) field-grown turnip roots subjected to short-term refrigerated storage, and b) roots of intact greenhouse-grown plants before harvest. The first experiment was conducted since large turnip roots (>350 g) are occasionally harvested, detopped, and stored for short periods before being marketed. Since we obtained conflicting reports from turnip growers whether low night temperatures before harvest influence the quality of turnip roots, the second experiment was initiated.

Materials and Methods

‘Purple Top White Globe’ turnip (Stokes Seeds, St. Catharines, Ontario), the most widely marketed turnip in North America, was used in the field–storage and in the greenhouse studies.

Field–storage study. The turnips were planted at the Horticulture Research Station, Cambridge, Ontario, on 9 June 1989 on Fox Sandy loam soil. The experiment was set up in a randomized complete-block design with three replications. Plants were grown according to the standard cultural practices of the region (Ontario Ministry of Agriculture and Food, 1989). The roots were harvested on 10 Aug., 59 days after sowing and 1.5 months before the initial frosts in the region. Twenty roots were randomly selected from each replication, then all these roots were combined. From the 60 roots, 24 of uniform size were selected and divided into three groups of eight roots each. The treatments consisted of evaluating roots at harvest and after 2 and 4 weeks of storage at 0°C and 93% relative humidity.

Greenhouse study. Plants were started in the greenhouse in seedling trays, then transplanted after 3.5 weeks into 6-liter pots (one plant/pot) containing vermiculite. The plants were grown in the greenhouse from September to December at 25±3°C. High-pressure sodium vapor lamps yielding a light intensity of 250 µmol·m⁻²·s⁻¹ at the top of the plants were used each day for 14 h to supplement natural lighting. Each plant was normally watered with 1.0 liter of a nutrient solution every second day, but during extremely sunny days, they were irrigated daily with the nutrient solution.

The plants were initially irrigated with a nutrient solution (pH 6.5) containing (in mg·liter⁻¹) Ca, 100; K, 234; Mg, 49; N, 168 (139 as NO₃⁻, and 28 as NH₄⁺); P, 31; S, 96; Cl, 0.2; B, 1.0; Mn, 0.1; Zn, 0.05; Cu, 0.05; Mo, 0.02; Co, 0.01; and Fe, 1.1 (as FeNa ethylenedinitro-tetracetate). When the root/hypocotyl of plants was 2 to 3 cm in diameter, which corresponded to 42 days after sowing, the N, K, and B concentrations in the nutrient solution were reduced to 78 (63 as NO₃⁻, and 15 as NH₄⁺), 116, and 0.5 mg·liter⁻¹ respectively.

Sixty-three days after sowing, the plants were divided into three treatments of uniformly sized roots consisting of six plants per treatment. The experiment consisted of a completely randomized design with each plant comprising a replication. In treatment 1 (T₁), the plants were immediately harvested. For treatment 2 (T₂), the plants were kept in the greenhouse under the conditions previously described, then harvested at the same time as T₁. In treatment 3 (T₃), the plants were subjected to a series of cold treatments for 11 days, then harvested. The plants were kept in the greenhouse during the daytime under the conditions previously described; during the night, they were ex-
posed in a refrigerated room to a sequence of two nights at 12 ± 1C, three nights at 4 ± 1C, and six nights at 0 ± 1C. This temperature was used to acclimate plants to low temperature.

_Ascorbic acid determination_. Harvested roots were peeled, and a representative sample of midcore tissue was pulverized in a food processor with 6% metaphosphoric acid and 0.005 M EDTA. The juice extract was filtered through glass wool and an aliquot was assayed for ascorbic acid content using the 2,6 dichlorophenolindophenol visual titration method (Assn. of Vitamin Chemists, 1966). Duplicate samples were analyzed and the data were averaged to give the values reported.

_Sample preparation for remaining chemical analyses_. Peeled roots were cut into pieces, and random samples were collected, quickly frozen in liquid N2, and stored at – 20C for not longer than 2 weeks. For the greenhouse study, the outer peel was also saved and frozen for GS evaluation. The frozen samples were subjected to freeze-drying, then sealed under vacuum in plastic bags, and stored at 5 to 6C until the chemical analyses were conducted. Tissues were macerated by use of an electric coffee grinder before being sampled.

_Starch determination_. A 1-g sample of freeze-dried material was refluxed in 100 ml of 80% ethanol for 5 h. The aqueous fraction was filtered and retained for sugars, the ethanol-insoluble residue was dried in an oven at 80C for 3 h, and the starch in the residue was solubilized and hydrolyzed (Taylor et al., 1988). The liberated glucose was evaluated by an anthrone assay (Yem and Willis, 1954).

_Soluble sugar determination_. The filtered ethanol fraction from the 5-h reflux was roteovaporated to near dryness and redissolved in 100 ml of water. An aliquot of the resulting solution was filtered through an alumina Sep-Pak cartridge (Millipore Waters, Mississauga, Ontario) and a 0.45-μm nylon membrane 66 filter. The solution was analyzed by high-performance liquid chromatography (HPLC) using a SP 8000 B Liquid Chromatography (Spectra Physics, Santa Clara, Calif.) fitted with an Aminex Carbohydrate HPX 87C column (300 x 7.8 mm) (Bio-Rad, Richmond, Calif.). The column temperature was 85C, and the mobile phase was water at a flow rate of 0.60 ml·min⁻¹. Sucrose, fructose, and glucose were quantified using peak height.

_GS determination_. GSs were extracted from 100 mg of ground tissue using 5 ml of boiling HPLC-grade methanol in a water bath at 60°C for 10 min. A known quantity of benzyl-GS was added to each sample during the extraction as an internal standard. The tissue was re-extracted and combined extracts were evaporated to dryness at 40C on a Speed Vac Concentrator (Model SVC200H-115; Savant Instruments, Hicksville, N.Y.). The residue was defatted by redissolving in 1 ml of hexane then 1 ml of water; the solution was vortexed, centrifuged, and the upper hexane layer discarded. Fifty microliters of a 0.5-M lead and barium acetate solution was added to the lower layer containing the water-soluble GS to remove proteins. The solution was vortexed, then centrifuged. The supernatant was loaded onto prepared ion-exchange minicolumns and the GS desulfated using procedures previously described (Daun and McGregor, 1981; Sang et al., 1984). The desulfoglucosinolate solution was filtered through an aqueous 0.45-μm ACRO LC3A filter, then analyzed by gradient system HPLC (Waters Assoc., Milford, Mass.). The HPLC system consisted of a model 481 gradient programmer and model 45 pumps. Separation was performed using a C18 (5μm) reverse-phase column (25 cm x 4.6 mm) (Alltech, Avondale, Pa.) with a linear gradient (0% to 30%) acetonitrile/H₂O over 70 min at a flow rate of 1 ml·min⁻¹. Values of the various GSs were adjusted according to their response factors (Buchner, 1987). The identity of individual GSs was further confirmed using LC plasma spray mass spectrometry.

_Statistical treatment_. Analyses of variance were performed on the data and means were separated using Dunnett’s test (Steel and Torrie, 1980) and Duncan’s multiple range test.

**Results**

Field-grown roots had a mean fresh weight of 495 ± 119 g. No significant change in dry matter concentration occurred during storage (range 6.3% to 6.5%), and no root growth or sprouting was visually apparent. Roots harvested from greenhouse-grown plants had mean fresh weights for T₁, T₂, and T₃ of 349 ± 34 g, 495 ± 37 g, and 435 ± 25 g, respectively; dry weight was similar for all three groups (6.2% to 7.0%). In both studies, harvested roots were free from brown heart (Shattuck and Shelp, 1985) and other types of internal discoloration. Mineral analyses revealed that the N, P, K, Ca, Mg, Mn, and B concentrations in roots of field and greenhouse-grown plants were similar; the Cu and Zn concentrations were lower in roots from greenhouse than field-grown plants.

During storage, the starch content of cold-stored roots from field-grown plants decreased 17% during the first 2 weeks then leveled off (Table 1). The starch content of greenhouse-grown roots was 22% lower for T₂ than for T₁, and no significant change was detected between treatments T₁ and T₂.

The major root sugars in turnips were glucose, fructose, and sucrose, with glucose and fructose accounting for > 90% of the total identified sugars. Several other neutral compounds were also detected, and although they were assumed to be sugars, they were in trace amounts and not identified. The total sugar concentration in roots was higher in field-grown plants at harvest than in greenhouse-grown T₃ plants (Table 1).

In the field study, the glucose and fructose concentrations decreased proportionally during storage, as indicated by the constant glucose and fructose ratios at 2 and 4 weeks (Table 1). The sucrose concentration increased significantly during the first 2 weeks of storage and remained at a similar concentration thereafter. The total sugar content in roots was highest at harvest and decreased during the 4 weeks of storage (Table 1).

In the greenhouse-grown roots, fructose was the only sugar that increased in concentration between harvest T₁ and T₃ (Table 1). The low-temperature treatment caused an increase in sucrose, which resulted in an increase in total root sugars. The total sugar concentration was higher in T₃ than T₁, which was due to an increase in fructose (Table 1).

The overall means for ascorbic acid content of roots from field and greenhouse plants were 23.4 ± 0.7 and 24.8 ± 0.5 mg/100 g fresh tissue, respectively (data not shown). Storage at 0C did not influence the ascorbic acid content of roots.

The most prevalent GS at harvest was 2-hydroxy-3-butanyl-GS, but 4-pentenyl-GS, 3-hydroxy-4-pentenyl-GS, and 2-phenylethyl-GS were also present at high concentrations (Table 2). These four GSs comprised 86% of the total detected. Cold storage for 4 weeks increased 2-hydro-3-butanyl-GS 34% over freshly harvested roots, while 4-pentenyl-GS reached a maximum amount at 2 weeks before declining at 4 weeks to a concentration similar to that at harvest. During the 4 weeks of cold-storage, the total GS level did not change significantly (Table 2).

The principal GSs in peeled root tissues of greenhouse-grown T₃ plants were 2-hydroxy-3-butanyl-GS and 4-pentenyl-GS (Table 3). Growth in the greenhouse for 11 days (T₁ vs. T₃) resulted

J. Amer. Soc. Hort. Sci. 116(5):818-822. 1991.
In our study, field-harvested roots, when stored at low temperature, showed a decline in total soluble sugars. One of the most notable changes in some, but not all root crops, is low-temperature-induced accumulation of soluble carbohydrates (Rutherford, 1977; Rutherford and Weston, 1968), where complex carbohydrates are hydrolyzed enzymatically to sugars (Dixon and Ap Rees, 1980; Isherwood, 1973; Miller and Langhans, 1990). Our data are similar to those reported for carrots, where total sugars decrease rapidly to about half the initial value after 60 days of cold storage (Phan et al., 1973), but differs from that of mature rutabaga (Brassica napus ssp. rapifera) roots, which accumulate sugars during short-term cold storage (Suzuki and Cutcliffe, 1981). It is noteworthy that turnip appeared to respond differently than rutabaga in as much as the amphidiploid rutabaga evolved from a cross between turnip and Brassica oleracea (Shattuck and Proudfoot, 1990). We noted a 2.7-mmol increase in sucrose in turnip roots during the first 2 weeks of storage; this increase was accompanied by a 0.40-g decline in starch. Since the hydrolysis of 0.40 g of starch would be expected to yield 2.4 mmol of glucose, it is likely that fructose and glucose from endogenous pools were also involved in the increase in sucrose during storage. When plants were exposed to low temperature in the greenhouse, the sucrose concentration in roots increased slightly, but glucose, fructose, and the starch concentrations remained stable. Presumably, the increase in sucrose in roots following the cold treatment arose from the translocation of this sugar from foliage tissues. The greenhouse study allowed for the first time a controlled examination of the unproven assumption that large (> 400 g) field-grown turnip roots increase in sugar content following a series of frosts. Our greenhouse data supports this contention.

During cold storage and the low-temperature treatment, we did not detect any significant change in ascorbic acid content of turnip roots. The ascorbic acid values reported in our studies are consistent with the concentration (25 mg/100 g fresh weight) normally expected for turnip (Buss and Robertson, 1976).

Our HPLC evaluation of turnip root GSs was verified by LC plasma spray mass spectrometry analysis and revealed eight principal compounds (Tables 2 and 3). Fenwick et al. (1983a) list 18 GSs that have been detected in turnip and likely contribute to the distinctive flavor and odor of this vegetable. Of the major GSs presented in our study, 4-hydroxy-3-indolylmethyl-GS, 4-methoxy-3-indolylmethyl-GS, 1-methoxy-3-indolylmethyl-GS, and 3-hydroxy-4-pentenyl-GS have not been pre-
previously reported in turnip. However, it should be noted that 4-methoxy-3-indolylmethyl-GS and l-methoxy-3-indolylmethyl-GS are known to occur in the hypocotyl (Sang et al., 1984) and mature root (Mossoba et al., 1989) of rutabaga. Since the indole-GSs are subject to degradation with gas-liquid chromatography separation, it is not surprising that earlier studies on turnip using GLC may not have detected the first three compounds. The values obtained in the field study for 3-butenyl-GS, 2-hydroxy-3-butenyl-GS, and 4-pentenyl-GS (Table 2) agree well with a previous study involving field-grown 'Purple Top White Globe' peeled turnip (Carlson et al., 1981). However, striking concentration differences are noted for the other GSs when compared to the data of Carlson et al. (1981), which were obtained primarily through GLC analysis. Furthermore, we detected 4-hydroxy-3-indolylmethyl-GS and l-methoxy-3-indolymethyl-GS but not l-methylpropyl-GS, 4-methylthiobutyl-GS, 2-hydroxy-4-pentenyl-GS, and 5-methylthiopentyl-GS. These differences may have been due, in part, to the seed sources and the differences in analytical procedures (Sang et al., 1984) and in the growth environments (Freeman and Mossadeghi, 1972; MacLeod and Pikk, 1978).

During the 11-day interval between the harvests of T1 and T2 of greenhouse-grown plants, the roots increased in size from 349 to 495 g and significantly decreased in the mean GS content of the peeled root and increased in that of peel tissues (Table 3). The size of turnip roots has been shown to be inversely correlated with GS content of peeled roots (Carlson et al., 1981). This difference indicates that the GS content may be subject to change during early sizing of the root, but this association is lost after roots reach a certain size. This finding may explain the marked changes we noted in GS. Since plants subjected to the cold treatment (T3) yielded roots 12% smaller by weight than the control (T2), comparisons involving the GSS between these treatments in our study should be approached with caution. For example, we recognize that it cannot be stated with certainty that the difference in 3-butenyl-GS between T1 and T2 roots resulted from the cold treatment and not from tissue dilution effects. However, it would appear that the cold treatment altered the concentrations of 4-hydroxy-3-indolylmethyl-GS in peeled roots and peel tissues and of 4-pentenyl-GS, 2-phenylethyl-GS, and l-methoxy-3-indolymethyl-GS in peeled roots (Table 3). Our study does not indicate whether the accumulation of the various GSs in peeled roots and peels following the cold treatment resulted from GS translocation from the foliage to roots, from GS biosynthesis in the root, or from a combination of both.

Although GS hydrolysis products have been widely used to quantify GS levels (Chong and Bérard, 1983; Ju et al., 1982b; Mullin and Sahasrabudhe, 1977), we decided in our study to measure the individual GSs present as the corresponding desulfoglucosinolates using HPLC. Data presented in Tables 2 and 3 suggest that storage at low temperature altered or induced the synthesis and degradation of specific GSs in turnip roots. This response was not associated with a change in the total concentration of the principal GSs measured. Several studies (Chong and Bérard, 1983; Guffy and Hicks, 1984) have revealed possible changes in GS levels during cold storage of cabbage by quantifying changes in aglucoside products, but our experiment appears to be the first where individual GSs have been clearly identified to change in response to storage temperature. Our finding is in accordance to unpublished data by Ju (see Chong and Béard, 1983), who noted fluctuation in the hydrolysis products of GS in turnip cultivars during cold storage.

An interesting finding of our field study was that 2-hydroxy-3-butenyl-GS (progoitrin) increased during short-term storage of turnip roots. Progoitrin is typically abundant in the turnip root (Chong et al., 1982; Mullin et al., 1980) and, following tissue disruption, is hydrolyzed to form 5-vinyl-oxazolidine-2-thione, a potent goitrogen (Nishie and Daxenbichler, 1980; Van Etten, 1969), which is also linked to bitterness in tissues (Fenwick et al., 1983b). A decrease in the reducing sugars (glucose and fructose) occurred during cold storage and was accompanied by an increase in progoitrin concentration (Tables 1 and 2). A decrease in the reducing sugars (glucose and fructose) occurred during cold storage and was accompanied by an increase in progoitrin concentration (Tables 1 and 2). Although the biological significance of this relationship is unclear, it may reflect a general stress response of turnip roots. A similar relationship has been reported in B-deficient turnip (Ju et al., 1982a). In view of the concerns of today's health- and quality-conscious consumers, it would be useful to know whether the levels of progoitrin and sugars continue to change in turnip roots when stored beyond 4 weeks.

Our results suggest that low temperature affects both carbo-
hydrate and glucosinolate metabolism in turnip roots and the effects are dependent on the low-temperature treatment. Further studies are required to ascertain if the alteration of carbohydrates and glucosinolates at low temperature could have affected the sensory characteristics of roots.

Literature Cited

Association of Vitamin Chemists. 1966. L-ascorbic acid (vitamin C), p. 287-341. In: M. Freed (cd.). Methods of vitamin assay. Interscience, New York.

Béard, L. S. and C. Chong. 1984. Influence of storage on glucosinolate fluctuations in cabbage. Acta Hort. 157:203-209.

Buchner, R. 1987. Response factors, p. 50-58. In: J.P. Wathelet (cd.). Transform infrared spectrometry to the identification of glucosinolates from brassica vegetables. J. Agr. Food Chem. 37:367-372.

Buss, D. and J. Robertson. 1976. Manual of nutrition. Ministry of Agriculture, Fisheries, and Food. 1979. Refrigerated storage of fruit and vegetables. Her Majesty's Stationery Office, London, England.

Mossoba, M.M., G.J. Shaw, D. Andrzejewski, J.A. Sphon, and S.W. Page. 1989. Application of gas chromatography/mass isolation/fourier transform infrared spectrometry to the identification of glucosinolates from brassica vegetables. J. Agr. Food Chem. 37:367-372.

Mullin, W.J., K.G. Proudfoot, and M.J. Collins. 1980. Glucosinolate content and clubroot of rutabaga and turnip. Can. J. Plant Sci. 60:605-612.

Mullin, W.J. and M.R. Sahasrabudhe. 1977. Glucosinolate content of cruciferous vegetable crops. Can. J. Plant Sci. 57:1227-1230.

Nishie, K. and M.E. Daxenbichler. 1980. Toxicology of glucosinolates, related compounds (nitriles, R-goitrin, isothiocyanates) and vitamin U found in cruciferaceae. Food Cosmetics Toxicol. 18:159-172.

Ontario Ministry of Agriculture and Food. 1989. Vegetable production recommendations. Queen’s Printer for Ontario, Canada.

Peirce, L.C. 1987. Vegetables, characteristics, production and marketing. Wiley, Toronto.

Phan, C. T., H. Hsu, and S.K. Sarkar. 1973. Physical and chemical changes occurring in the carrot root during storage. Can. J. Plant Sci. 53:635-641.

Rutherford, P.P. 1977. Carbohydrate changes in stored vegetables with special reference to red beet and parsnip. Ann. Applied Biol. 85:440-444.

Rutherford, P.P. and E.W. Weston. 1968. Carbohydrate changes during cold storage of some inulin-containing roots and tubers. Photochemistry 7: 175-180.

Sang, J. P., I.R. Minchinton, P.K. Johnstone, and R.J.W. Truscott. 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. Can. J. Plant Sci. 64:77-93.

Shattuck, V.I. and K.G. Proudfoot. 1990. Rutabaga breeding. Plant Breeding Rev. 8:217-247.

Sang, J. P., I.R. Minchinton, P.K. Johnstone, and R.J.W. Truscott. 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. Can. J. Plant Sci. 64:77-93.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill, New York.

Suzuki, M. and J.A. Cutcliffe. 1981. Sugars and eating quality of rutabagas. Can. J. Plant Sci. 61:167-169.

Taylor, D. C., B.J. Shelp, L.M. Nelson, and B. Grodzinski. 1988. Carbon and nitrogen partitioning in young nodulated pea (wild type and nitrate reductase-deficient mutant) plants exposed to NH₄NO₃. Physiol. Plant. 74:593-601.

Van Eeten, C.H. 1969. Goitrogens, p. 103-142. In: I.E. Liener (ed.). Toxic constituents of plant foodstuffs. Academic, New York.

Yem, E.E. and A.J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57:508-514.