Microflora and Invert Sugars in Juice from Healthy Tissue of Stored Sugarbeets

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Bacterial populations increased in juice of healthy tissue of sugarbeet roots stored at 5 C. Average counts showed a sixfold increase after 150 days of storage. Invert sugar levels increased over threefold in “American 4 Hybrid A” and remained fairly constant in “Mono-Hy D-2.” The former cultivar also had significantly higher bacterial colony counts than the latter before 90 days of storage. Of 36 isolates identified, 16 were Pseudomonas spp. including P. chlororaphis; 6 Bacillus spp. including B. subtilis; 5 Arthrobacter spp. including A. globiformis; 4 yeasts; 2 Erwinia spp.; 2 Flavobacterium spp. including F. aquatile; and Streptomyces longisporus. Isolates of all genera except S. longisporus were able to hydrolyze sucrose in vitro.

The survival of bacteria in healthy storage tissue of potato, red beet, turnip, carrot, and kohlrabi has been established (4). MacDonald et al. (3) aseptically excised sugarbeet root tissue, and plate counts of tissue homogenates averaged 10^4 bacteria/g of tissue before incubation. Counts increased to 10^7 to 10^9/g after 4 days of incubation in aerated washing at room temperature.

Sugarbeets are harvested and stored outdoors for 60 to 120 days in regions where freezing temperatures could prevent winter harvest. Extractable sucrose decreases during this storage period. The metabolism of microbial inhabitants, particularly the production of invertase, might contribute to this decrease in sucrose content and juice quality. The objectives of these studies were to (i) measure bacterial populations of healthy sugarbeet roots during storage at 5 C, (ii) identify the most prevalent microorganisms, and (iii) study the relationship of bacterial populations with invert sugar levels.

MATERIALS AND METHODS

Bacterial populations were measured in two cultivars, American 4 Hybrid A (4A) and Mono-Hy D2 (D2). Roots were stored at 5 C in perforated plastic bags (45 by 80 cm). Humidity in the storeroom was 90 to 95%. Cores 18 mm in diameter were removed with a cork borer from each of 50 roots for each cultivar. The same roots were sampled at 30-day intervals during a 150-day storage period. The epidermal ends of the cores were removed (2 to 5 mm), and the cores were trimmed to 10 g. Each core was disinfected by suspension in 1% sodium hypochlorite for 15 s and then rinsed once in sterile distilled water. Juice was extracted from each core with a vegetable juicer fitted with a paper filter. The juice was flushed with 70% ethanol after each extraction. Sterile water was used instead of a core after 25 extractions to determine the effectiveness of the 70% ethanol wash. Filters were replaced after three extractions. Juice samples were diluted and plated in triplicate by the pour plate technique with nutrient agar (Difco) as the medium. Incubation was at 26 C for 4 to 5 days. Identification was based on Bergey (2).

Invert sugar levels in the extracted juice were measured by the 3,5-dinitrosalicylic acid reagent (1).

RESULTS AND DISCUSSION

During this experiment, 42% of sterile water checks through the juicer remained sterile. The average colony count was 1.4/plate on the remaining checks. This count was considered tolerable because of the amount of plant material sampled and the number of plates poured at each sample date. Colony counts of the checks were not used to adjust test counts.

The bacterial counts at harvest were 0.72 × 10^5/ml of juice for 4A and 0.5 × 10^5/ml for D2. This difference was statistically significant (Fig. 1). The bacterial numbers increased up to a maximum between 90 and 120 days of storage. Maximum average counts were 3.7 × 10^5/ml of juice for D2 and 4.5 × 10^5/ml for 4A. The cultivar 4A had significantly more bacteria per
m milliliter of raw juice than D2 up to 60 days storage. Thereafter, 4A continued higher than D2, but considerable variability in both cultivars removed statistical significance.

The invert sugar levels varied less than the bacterial counts. Cultivar 4A had significantly more invert sugar than D2 at harvest and throughout storage (Fig. 2). The invert levels in 4A increased sharply after 90 days, whereas those in D2 remained constant.

The higher bacterial count in 4A, plus the higher invest level, suggests that the microflora could contribute to a storage loss of sucrose by inversion and use. Further, it is not known why 4A supported a greater population of bacteria than D2. The sucrose contents of both cultivars was 11.2% after storage for 150 days. Roots of both cultivars used in these experiments were produced in the same field plot.

The following genera (with numbers of cultures) were represented in the 36 colonies that were isolated and purified: *Pseudomonas*, 16; *Bacillus*, 6; *Arthrobacteri*, 5; *Erwinia*, 2; *Flavobacterium*, 2; and *Streptomyces*, 1. Five of the isolates were identified to species: *P. chlororaphis* (Guignard & Sauvageau) Bergey et al.; *Bacillus subtilis* (Ehrenberg) Cohn; *Arthrobacter globiformis* (Conn Conn and Dimmick (duplicates); *F. aquatile* (Frankland and Frankland) Bergey et al.; and *Streptomyces longisporus* (Krasilnikov) Waksman. Four colonies of yeast were isolated but not identified.

Of special interest because of the potential economic importance was the ability of 20 of the 36 cultures to hydrolyze sucrose in vitro. All genera except *S. longisporus* showed this ability. Experiments are needed to determine if microbial hydrolysis of sucrose in vivo contributes toward sucrose loss and invert sugar accumulation.

**LITERATURE CITED**

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