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Proc. Fla. State Hort. Soc. 105:160-162. 1992.

COMPARISON OF OLIGOSACCHARIDES GENERATED DURING SUCROSE INVERSION AND CITRUS JUICE FERMENTATION

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Abstract. **Pure citrus juices contain few oligosaccharides (OS), which are mostly disaccharides (group I). During fermentation a second group of OS (II), predominantly trisaccharides, is generated. Concentrated juice reconstituted to 11.8° Brix with water was kept at 25°C for up to 144 hr and allowed to ferment without the introduction of extraneous microorganisms. OS were formed after 30 hr, at about the same time as the major sugars began to be utilized significantly. OS formation reached a maximum at about 50 hr, at which time they began decreasing. All saccharides were eliminated after 72 hr. During fermentation, the concentration of the preexisting Group I OS increased significantly. One of the OS belonging to group II showed a major increase while the others increased moderately or remained unaffected. Previous studies have shown that group II OS are generated during acid sucrose inversion and can be used to quantitate the addition of medium invert sugar (MIS) to juices. Fermentation generated OS interfere with those originating from added MIS. However, differences in OS composition, as well as the production of alcohol and inisitol, should allow differentiation between acid and fermentation induced OS.**

During concentration, when citrus juice is subjected to a temperature of 99°C for about 10 sec, most microorganisms are destroyed (Faville et al., 1951). However, a microbial population of up to 10000 counts per ml can still be found in juices reconstituted from concentrate (McAllister, 1980). They can eventually induce spoilage, particularly after reconstitution (Faville and Hill, 1951). During fermentation, sucrose is first hydrolyzed by invertases before being metabolized with the other sugars. These enzymes have been shown to have a transfructosidase activity that catalyses the synthesis of various OS (Hassid and Ballou, 1957). Previous HPLC studies of MIS and citrus juice OS (Cancalon, 1992a; Swallow et al., 1991; White and Cancalon, 1992) have revealed two groups of peaks. Group I eluded between 15 and 22 min, contains a mixture of di and trisaccharides, and is found in both juices and MIS. In juices, the origin of these peaks is very complex and may be due to fruit enzymes, microbial activity or acid hydrolysis within the fruit (Echeveria, 1990). The second group, mostly trisaccharides, eluded between 22 and 30 min, is largely absent from fresh juice, but contains the OS generated by keeping sugars in acidic solutions. The presence of Group II OS has been used to monitor the addition of exogenous MIS to citrus juices (Cancalon, 1992a; Swallow et al., 1991); however, OS formed during microbial activity have been shown to interfere with the analysis (Cancalon and Bryan, 1991).

In this study, OS generated during citrus juice fermentation were examined and compared with those produced during acid inversion.

Materials and Methods

Sample Preparation. Concentrated orange juice (50.8 °Brix) was diluted with HPLC grade water to 11.8°Brix and allowed to ferment at 25°C. Samples were examined at various times during fermentation. Preparation was the same as described previously (White and Cancalon, 1992). Orange juice samples were diluted to 6°Brix and centrifuged at 10000 g for 15 min. The supernatant was passed successively through Dowex AG50W-X8 (100-200 mesh) H^+-form resin, Dowex AG 1-X4 (100-200 mesh) (Bio-Rad, Richmond, CA) formate-form resin, a Sep-Pak C18 cartridge (Millipore Co., Milford, MA) and finally filtered through a 0.45 m nylon Acrodisc membrane (Gelman Sciences, Inc., Ann Arbor, MI).

HPLC Conditions. Samples were analyzed with a Waters (Milford, MA) HPLC system consisting of an Ultra WISP Model 715, a Model 625 LC pumping system, and a Model 464 metal-free electrochemical detector, set at a range of 50 A. Control of the system and data acquisition were performed with a Waters 820 Maxima 386SX work station.

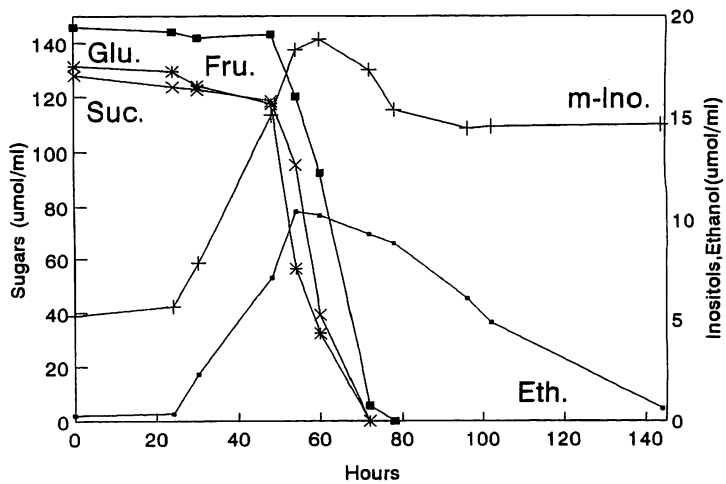

Figure 1. Changes in juice composition during fermentation. Glu: glucose, Fru: fructose, Suc: sucrose, m-Ino: meso-inositol, Eth: ethanol.

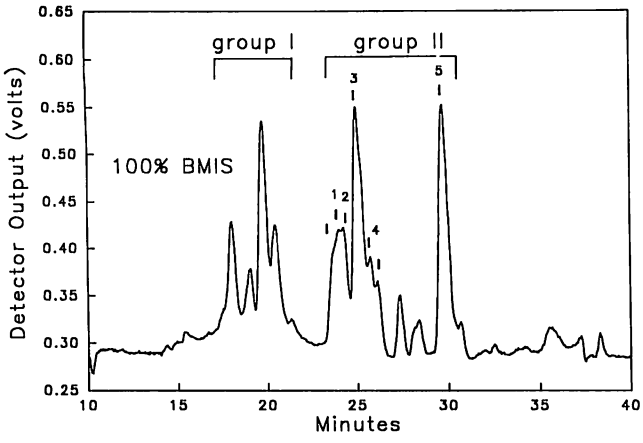

Figure 2: Chromatogram of beet medium invert sugar (BMIS) oligosaccharides. Group I OS are found in both citrus juice and MIS. Group II OS are specifically generated in acidified sugar solutions.

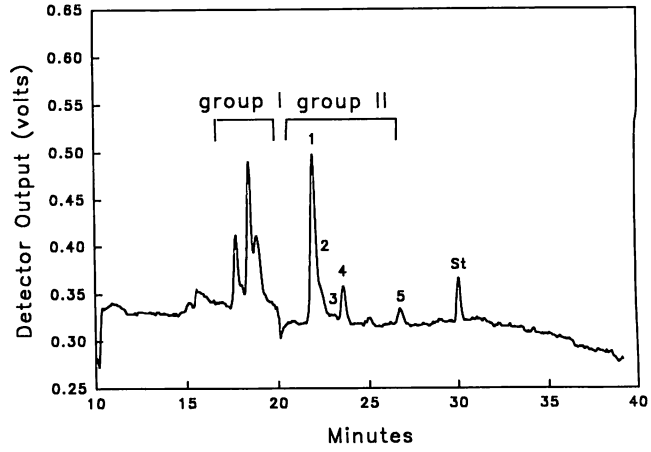

Figure 4: Chromatogram of orange juice OS after 48 hr of fermentation. The prominence of Peak 1 can be seen. Standard: maltotetraose.

The columns (Dionex Corp., Sunnyvale, CA) consisted of an ATC-1 anion trap, a CarboPac PA1 guard column, and two CarboPac PA1 analytical columns.

The HPLC mobile phase A was 0.1 M NaOH. Mobile phase B was 0.1 M NaOH containing 0.2 M sodium acetate. Mobile phase C and the post-column reagent were 0.3 M NaOH. The conditions for OS analysis were: initially 80% A, 20% B; then 0-20 min concave gradient number 7 to 70% B; 10 min at 70% B followed by 100% C for 60 min and 30 min of equilibration under initial conditions. Fifty µl were injected for each analysis. Mono- and disaccharides were eluted to waste within the first 10 minutes, at which time the switching valve was activated to elute the oligosaccharides onto the second column and the detector.

Glucose, fructose and sucrose were analyzed by the same methods with minor modifications as described previously (Cancalon, 1992a).

Results and Discussion

During fermentation, sugars decreased only very slowly during the first 48 hr. Sugar concentrations fell very rapidly during the next 24 hr and all saccharides disappeared from the solution after 3 days. HPLC analysis of the sugars has revealed several extraneous smaller peaks indicating the presence of other compounds (Cancalon, 1992b). Two were present in all citrus juices and were identified as meso- (MI) and epi-inositol (EI). While EI was unaffected by fermentation, MI started rising after 24 hr, peaked when half the sugar was used and plateaued at later times. The last peak identified as ethanol behaved similarly as inositol until a maximum was reached, then subsequently decreased to almost zero by day six (Fig. 1). The microbial population increased vigorously during the first 48 hr from 2.77 log CFU/ml to 7.6, but remained relatively constant thereafter.

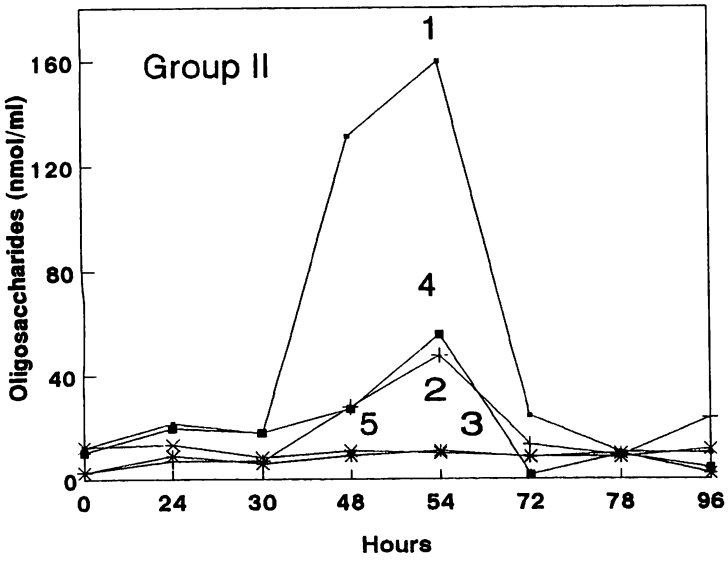

Figure 3: Changes in the concentration of Group II OS during fermentation. The OS are expressed as nmol/ml of maltotetraose.

During the acid hydrolysis of sucrose (Cancalon, 1992a), both group I and II OS were generated. Group I OS were rapidly produced at the begining of the inversion, but group II OS did not appear simultaneously, and their concentrations increased and decreased independently of each other during the entire process. Each OS pattern was shown to depend on the sugar concentration at the time of analysis. Figure 2 shows the OS composition of 50% inverted sucrose.

Both groups of peaks were affected by fermentation, and the OS concentrations varied in a synchronous manner. They appeared after 30 hr when sugars started to be utilized significantly. Their concentration peaked at about 50 hr and decreased to almost zero by 72 hr when all mono, di and oligosaccharides were utilized (Fig. 3). In Group I, apparently no new peaks were created during fermentation.

Most Group II peaks seen during sucrose hydrolysis were also produced during fermentation (Fig. 4). Although up to seven OS were revealed during sucrose inversion, only five major peaks were followed (Cancalon, 1992a). Peaks 3 and 5 were present only as traces and were largely unaffected by the process. Peaks two and four showed a moderate increase during fermentation but peak one showed an extremely large increase. It was found that OS profiles characterized by large peak 1 and 4 are typical of fermented juices. This profile was reported previously in pulp wash (Cancalon and Bryan, 1991). Therefore, these peaks are not specific of pulp wash, but reflect a low level of fermentation in a product that may not be treated with the same care as that for pure juice.

Conclusions

Citrus juice fermentation induced the formation of significant amounts of OS. However, their composition was found to be significantly different from that of OS generated by acid oligomerization. Furthermore, the formation of ethanol and meso-inositol should distinguish between fermentation OS and those due to the addition of medium invert sugar. Nevertheless, care should be taken to assess the freshness of a juice when quantitating beet medium invert sugar in citrus juice.

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Proc. Fla. State Hort. Soc. 105:162-168. 1992.

ANTICARCINOGENIC ACTIVITY OF PHYTOCHEMICALS IN CITRUS FRUIT AND THEIR JUICE PRODUCTS

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Additional index words. Flavonoids, limonoids, phenolic acids, vitamin C, vitamin A.

Abstract. **Dietary components present in citrus juices have been shown to exert protective effects against the induction and spread of cancer in animals and humans. The components with the most potent anticarcinogenic activities are mainly naturally occurring secondary metabolites and include flavonoids, limonoids, phenolic acids and vitamins. These phytochemicals react by different mechanisms, namely, by maintaining cellular oxidation-reduction balance and protecting cells against free-radical mechanisms, direct detoxification of xenobiotics, control of membrane permeability and by other unknown mechanisms. The chemical structures of citrus phytochemicals with known anticarcinogenic activities and various aspects of cancer and anticancer mechanisms are noted in this review.**

Cancer

Initiation

The agents of cancer are many, but most act by damaging cellular DNA (deoxyribonucleic acid). This step is termed "initiation" and is a genotoxic event. Many genotoxic carcinogens have been identified. Carcinogens may be of a chemical or viral nature, or may be induced by radiation (as occurs primarily in skin cancer).

From this initial damage, mutation, duplication or translocation of normal cellular genes involved in growth control may portend the development of cancer. By one mechanism or another, damage to diverse proto-oncogenes (normal cellular genes that may become cancer producing) has been implicated in the genesis of human tumors. Many oncogenes encode proteins with key roles in controlling normal growth and development.

Promotion

While a genotoxic carcinogen can initiate or alter the genetic material of a cell, this event is only the first step in an elaborate sequence of events leading to neoplastic growth. Cancers are populations of cells that have acquired the ability to multiply and spread without normal restraints. An abnormal cell population needs to achieve a selective growth advantage in the presence of surrounding normal cells that are regulated by growth-controlling fac-

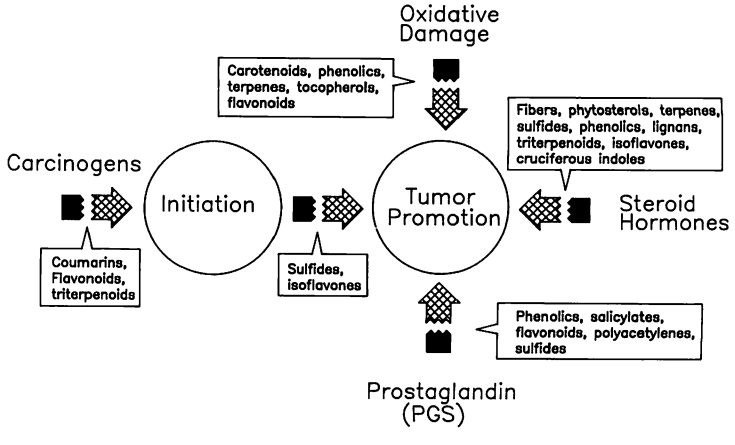

Figure 1. The effects of phytochemicals on metabolic pathways associated with breast cancer (Pierson, 1992).

Florida Agricultural Experiment Station Journal Series No. N-00736.