CHANGES IN BIOPHYSICAL AND BIOCHEMICAL PROPERTIES OF CRANBERRY (*Vaccinium macrocarpon* Ait.) FRUIT DURING GROWTH AND DEVELOPMENT

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Abstract

'Searles' cranberry fruits were harvested at various stages of maturity from July to September and sorted according to their color and size. Fruits were analyzed for their ethylene production, respiration, dry weight, cell wall content, anthocyanin content, chlorophyll content, soluble proteins, membrane proteins, polar lipid fatty acid composition and freeing stress resistance (FSR).

There was no climacteric rise in respiration at the time of an increase in ethylene production and an increase in anthocyanin content. The % dry weight was fairly constant throughout the sampling period. The % cell wall decreased sharply in the beginning then at slower rate. Soluble and membrane proteins decreased in parallel to the loss of chlorophyll. FSR level increased, as the fruits matured, to reach its maximum level at the red stage. The changes in fruit color and FSR were related to an overall decrease in the 18:1 and a simultaneous increase in the 18:2 polar lipid fatty acids and a decrease in chlorophyll content.

Our data suggest that (a) cranberry is a nonclimateric fruit, (b) the changes in the fatty acid composition are highly related to the increase in anthocyanin content, (c) the increase in the FSR of the fruit is related to the loss of chlorophyll content and the increase in the unsaturation level of the fatty acid composition.

1. Introduction

Fruit ripening involves metabolic shift in the fruit and two major theories have been proposed to explain this shift. The first theory "organizational resistance" was proposed by Blackman and Parija (1928) and revised by Sacher (1973). It considers ripening as a catabolic event, senescence, caused by the increase in cell membrane permeability that breaks down the compartmentalization exposing enzymes to their substrates, co-factors, ions...etc. That leads to the increase in respiration rate and subsequent ripening events in the climateric fruits. Several studies have presented evidence in support of this theory (Sacher, 1973; Wade and Bishop, 1978; Legge et al., 1986). The second theory considers ripening as a genetically programmed process. The evidence that supports this theory were based on the newly produced cell wall degrading enzymes such as cellulase in avocado (Bennett and Christoffersen, 1986) and polygalaturonase in tomato fruits (Grierson et al., 1985). But there is no conclusive evidence on specific genes that may be expressed early or at the ripening initiation time.

Most studies have utilized climacteric fruits such as bananas, apples, pears, avocados, and tomatoes and data on nonclimacteric fruits are rare. In the present study we studied the respiration and the ethylene production by the cranberry fruits during ripening. We simultaneously studied the biophysical and biochemical changes during the ripening period. Since cranberry fruits ripen in the fall and therefore subject to the frost hazards, we also investigated the changes in freezing stress resistance of the fruit in relation to the other parameters.

2. Materials and Methods

'Searles' cranberry fruits were harvested at various intervals during the growing seasons of 1986 and 1987. Fruit samples were chosen according to visual color and size that resemble the majority of the fruit at the time of each harvest. For the chemical analyses, the seeds were removed and the rest of the fruit tissue was stored at -20°C (lipid samples were stored at -20°C under nitrogen gas to prevent oxidation). These samples were used to determine protein contents (soluble and membrane), crude cell wall, anthocyanin, and polar lipid fatty acid composition.

Soluble and membrane proteins were analyzed by modified Lowry assay (Markwell et al., 1978). For this purpose half a gram of fruit pericarp was ground in Tris buffer (pH 7.8), centrifuged at 25000 g for 30 minutes. The pellets were washed twice with buffer. The supernatant was collected for soluble proteins and the pellet was used for membrane proteins.

Crude cell wall was extracted from 2g of tissue using the procedure of Hatfield and Nevins (1986). Membrane polar lipids from 2g of tissue were extracted using a modified procedure of Bligh and Dyer (1959). The polar lipid fraction was then alkaline methylated to obtain fatty acid methyl esters. Fatty acid composition was measured using a Shimadzu GC-9AM gas chromatograph. During lipid washing with KCl, we were able to recover all the anthocyanin present in the tissue in the salt phase. That fraction was used to determine anthocyanin contents by reading absorbance at 525 nm (optimum absorbance identified by scanning).

For chlorophyll contents and dry weight measurements, the fresh fruit tissue after removing the seeds was used. Chlorophyll was extracted from 1g of tissue using 96% ethanol and absorbance was read at 654 nm (Wintermans and De Mots, 1965). Dry weight % was determined after drying 1g of tissue at 60°C for 5 days.

For freezing stress resistance, berries still attached to plant uprights were placed in test tubes in a glycol bath and subjected to slow freezing (1°C/hr). Ice nucleation was initiated at -1°C. The berries were observed for loss of turgidity and irreversible water soaking as post thawing viability assays (Palta et al., 1978).

For gas (ethylene and CO_2) evolution measurements, 5g of intact fresh fruits were placed in 70 ml test tubes sealed with serum stoppers. The tubes were then placed in a growth chamber at 24°C for 24 hours. Ethylene and CO_2 evolution were determined by

sampling the gas in the tubes. The analysis of the gas was made by using the same gas chromatograph as used in lipid analysis.

3. Results and Discussion

3.1.Cranberry appears to be a nonclimacteric fruit

Respiration rate was the highest at the green stage and declined to reach a low and steady state level at the time of the appearance of anthocyanin (Figure 1). Ethylene production was low throughout the sampling period, but there was a noticeable peak of ethylene production at the time of fruit ripening. The increase in ethylene production preceded the increase in anthocyanin formation suggesting that ethylene might play a role in cranberry fruit ripening. These results are in agreement with the observations of Forsyth and Hall (1969) and of Hawker and Stang (1985). Our results show that 'Searles' cranberry is a nonclimacteric fruit since it does not exhibit a rise in respiration during fruit ripening which is a criterion for climacteric fruit (Watada et al., 1984).

3.2.Lipid changes relate to fruit ripening

Anthocyanin contents, a criterion of ripening, increased as the fruits matured (Table I). The most pronounced changes were observed in the polar lipid fatty acid composition (Figure 2). These changes were evident at all stages of growth and development particularly during the period of ripening initiation (between 5 August and 19 August in 1986). Fatty acids 16:0 (palmitic) remained constant and 18:3 (linolenic) decreased slightly. Saturated fatty acid 18:0 (stearic) and partially unsaturated 18:1 (oleic) decreased drastically. Unsaturated 18:2 fatty acid (linoleic), the major fatty acid in fruits, increased dramatically. Same results were obtained in 1987 with the following exceptions: (a) Ripening initiation was delayed by 10 days in 1987. (b) The fatty acid 18:0 was constant throughout the sampling period. These differences could be due to the different environments in the two seasons.

Several possible speculations can be made to explain the effect of membrane lipid changes on the ripening initiation. Changes in fatty acid composition can bring about (i) Changes in membrane lipid "fluidity". Such changes have been proposed to cause perturbation of the metabolite distribution during ripening (Brady, 1987). (ii) Changes in membrane permeability. These changes have been proposed to result in change in the compartmentalization within the cell during ripening (Sacher, 1973). (iii) Changes in the lipid environment of the membrane proteins (Lynch et al., 1987). Such changes could either directly affect the activity of the Ethylene Forming Enzyme as a membrane bound enzyme (Yang and Hoffman, 1984) or alter the tissue sensitivity to ethylene.

3.3.Chlorophyll contents relate to freezing stress resistance

Freezing stress resistance (FSR) increased as the fruit matured and reached to the maximum level when fruits became completely red

(Table I). In 1987 (data not shown) FSR was much less than in 1986. Consistent in two years FSR was related to the decrease in chlorophyll contents rather than to the increase in anthocyanin contents.

3.4.Other chemical changes

The % dry weight was fairly constant in both years and that allowed us to present our data on fresh weight basis. The reason of the difference between our data and the increase in the dry weight in Hawker and Stang (1985) work is probably due to the different methods used to determine the dry weight. Furthermore, we excluded the seeds in our case which was not done in the study by Hawker and Stang (1985). Soluble and membrane proteins decreased in parallel to the decrease in chlorophyll content. There was no significant decrease in the % cell wall at the time of ripening initiation indicating that cell wall degrading enzymes may not play a role in ripening initiation in cranberries.

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Table I - Some physical and chemical changes in the cranberry fruit during growth and development in 1986.

Data & fruit color	Time (Weeks)	Freezing* stress resistance (°C)	Anthocyanin contents (at 525 nm)	Total chlorophyll (ug/g fresh weight)
22 July (Green)	0	- 1.0	0.0	23.03
5 Aug (White)	2	- 1.5	0.0	7.05
19 Aug (B 10)	4	- 1.5	0.1	2.38
2 Sept (B 30)	6	- 3.5	0.5	0.97
16 Sept (Red)	8	- 8.0	1.1	0.74
30 Sept (Red)	10	- 8.0	2.0	0.75

(*) : Lowest temperature for 100% survival.

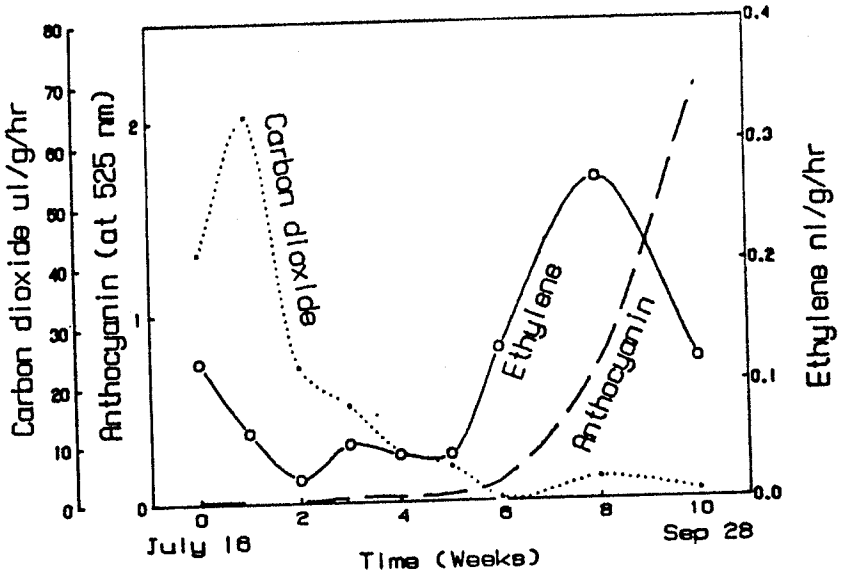

Figure 1- Changes in ethylene and carbon dioxide evolution in relation to anthocyanin formation in 'Searles' cranberry fruit during 1987 season.

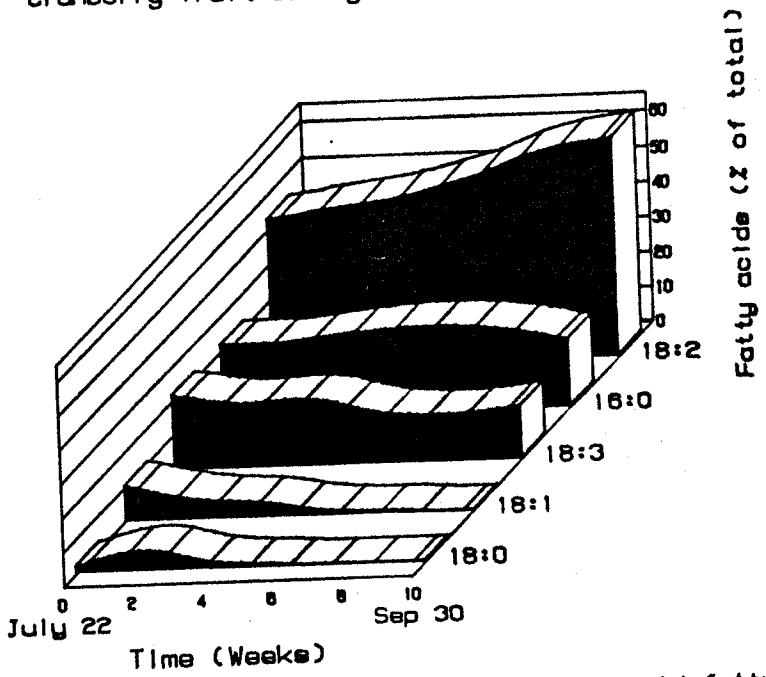

Figure 2. Changes in the composition of polar lipid fatty acids in 'Searles' cranberry fruit during growth and development in 1986 season.