CHANGES IN THE CONTENT OF ASCORBIC ACID AND REDUCING SUGARS IN THREE VARIETIES OF STRAWBERRIES DURING STORAGE REGARDING THE WAY OF PLANTING

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Pulse polarography and HPLC methods were used for the determination of vitamin C – ascorbic acid (AA) content in three varieties of strawberries and the content of reducing sugars (RS) determined after Schoorl. There were also investigated changes in the content of AA and RS during 11 months' storage period under deep freezing. The tested strawberries were picked out in two seasons from plants obtained from meristems and planted in classical way. The content of AA was determined by DPP of standard additives using the electrochemical detector EDLC with XY Recorder-4103 and by modified method using Waters 600E Multisolvent Delivery system with 900 series Photodiode Array detector on reverse phase column C_{18}. The content of AA dropped dramatically during the first half of storage period and there was an apparent significant variety dependence. For all three varieties the higher concentration of AA in strawberries from plants cultivated from meristems was evident. RS performed slow increase during storage period.

ascorbic acid and reducing sugars in strawberries; differential pulse polarography; HPLC for determination; influence of variety and origin on content; influence of storage

INTRODUCTION

Vitamin C – ascorbic acid (AA) and dehydroascorbic acid (DAA) – are vital substances for healthy growth and development of many organisms.

Ascorbic acid (AA) is the nutrient most affected by processing fruits and vegetables, therefore its retention is supposed to be a limitation to other nutrients.

Since 1932, when Tillmans et al. (1932a, b) published the determination of AA with 2,6-dichlorindolphenol, several different methods using different techniques have been developed which led to the reproducible and

quantitative analyses of vitamin C. Besides officially issued and accepted AOAC method (1975) as a criterion of accuracy and reproducibility, for instance polarographic method (Kozar et al., 1988), ion-pair chromatography (Dong et al., 1988) and normal and reversed phase HPLC methods (Finley, Duang, 1981; Wimalasiri, Wills, 1983; Dennison et al., 1981; Ashoor et al., 1984; Behreus, Madere, 1987; Berchmans et al., 1989) have found out very important place among analytical methods for the quantification of vitamin C.

The object of our work was to study the changes in the content of ascorbic acid in strawberries of three varieties planted classically and from meristems during storage under standard conditions of the deep freezing. The phenomenon of decreasing of the content of vitamin C during storage and high variety dependence were already described in white cabbage (Čurdová et al., 1991; Mähringová,1992). The samples of strawberries were also analyzed for the content of the reducing sugars.

MATERIAL AND METHODS

All chemicals were of analytical grade, purchased from Fluka, Aldrich and Merck. Solvents were of HPLC grade, degassed by filtration under vacuum, water was redestilled and deionized.

Samples of fully ripened strawberry fruits were obtained from the Agricultural Centre Jesenice in the highest sale quality from the same locality. Varieties Dagmar, Red Gauntlett and Senga Sengana planted both classically and from meristems were chosen for our experiment. Strawberry harvested in June 1990 and 1991 were tested for vitamin C (AA) and reducing sugars concentration during 11 months' storage period under deep freezing in micro-ten bags (–18 °C).

Instrumentation for pulse polarography: The electrochemical detector EDLC (L. P. Praha) connected to XY Recorder-4103 (L. P. Praha) was used for measuring polarographic curves, where the height of the peaks corresponded directly to the concentration of the respective compound. The concentration of AA was determined using the method of standard additives (Čurdová et al., 1991). Samples of strawberries (100 g) were homogenized in a glass homogenizer for 1 min in 100 ml cold 2% metaphosphoric acid. To 40 g of the homogenate water was added to obtain 100 ml of the sample. After centrifugation under cooling to 10 ml of the clear supernatant 2 ml 2.5M sodium acetate was added and the sample was further diluted with water to a final volume 25 ml. Another 10 ml of supernatant was brought to 25 ml volumetric bottle and after the addition of 250 ml 0.1% solution of ascorbic acid as an internal standard additive and 2 ml of 2.5M sodium acetate buffer

the volume was set up to 25 ml with water and then used for polarographic measurement.

Standardization: Serial dilutions containing 0.05 to 1.0 mg of ascorbic acid in 1 ml were prepared by dissolving analytical grade AA in 0.2M aqueous solution of sodium acetate. Standard curve was determined by plotting peak height versus AA amount.

HPLC instrumentation: Waters 600 E Multisolvent Delivery system with 900 Series Photodiode Array detector was used for analyses of AA and DAA. We used a modified method of Dong et al. (1988). 200 g of frozen strawberries were mixed with 50 ml of 3% metaphosphoric acid for 2 min. The cold mixture after filtration (red stripe) was transferred to the volumetric bottle (100 ml) and volume was adjusted with 3% solution of metaphosphoric acid. HPLC analyses were performed on a reverse phase column C_{18} (200 mm x 4 mm i.d., 5 µ; Tessek Praha) at 40 °C in isocratic regime. To avoid the damage of the column a precolumn C_{18} (400 mm x 4 mm i.d.; Tessek Praha) was installed in front of the HPLC column. A composition of a mobile phase: methanol (MeOH) : 5 mM aqueous solution of sodium dodecyl sulphate = 30 : 70; pH = 2.5 was adjusted with orthophosphoric acid; flow rate = 0.6 ml/min; detection UV in the maximum of absorbance; injection 5 ml. AA concentration of the sample extracts was calculated by interpolation on the standard curve and the use of dilution factors. A calibration graph plotted as a peak area of AA (UV detection in maximum of absorbancy at 254 nm) against the amount of AA demonstrated a linear relationship and good correlation

$Y = 580.6\ x + 5{,}827.8$; $r = 0.9997$; $r^2 = 0.9994$; $P < 0.01$

(these dates were calculated from 7 determinations).

A good recovery of AA in strawberries was tested by adding of AA to the homogenate.

For analyses of reducing sugars we used the method of Schoorl.

RESULTS AND DISCUSSION

We tried to elucidate the influence of the origin of the plants (classical and from meristems) and variety on the content of AA and RS during the deep freezing storage. We used two analytical methods for the determination of the content of ascorbic acid in strawberry fruits. A comparison of HPLC and pulse polarographic method showed almost no difference in the results of analyses (Tab. I). Strawberries for sampling were treated during growing and ripening in the same way to diminish the influence of other factors like weather, application of fertilizers or pesticides.

I. HPLC (A) and polarographic (B) analyses of ascorbic acid in strawberries planted clasically and from meristemes during storage

Date of analysis	Content of ascorbic acid (mg/kg of fresh strawberries) in variety											
	Dagmar				Red Gauntlett				Senga Sengana			
	clasically		meristem		clasically		meristem		clasically		meristem	
	A	B	A	B	A	B	A	B	A	B	A	B
16. 9. 1990	737	758	777	820	577	580	599	596	751	749	793	802
20. 8. 1990	726	746	770	805	553	558	590	581	724	730	762	765
20. 11. 1990	633	651	691	707	487	493	512	508	618	631	641	638
20. 1. 1991	543	545	569	573	403	404	462	460	500	606	612	609
20. 3. 1991	512	509	522	519	356	362	412	409	543	570	573	566
20. 4. 1991	488	481	490	502	341	340	400	398	510	515	519	521
20. 5. 1991	478	489	490	505	341	340	400	396	510	511	520	517
14. 6. 1991	833	830	906	912	665	665	724	731	826	823	887	893
19. 11. 1991	701	710	782	802	572	566	612	613	716	716	800	806
15. 1. 1992	653	637	714	711	512	526	556	549	676	674	763	752
16. 3. 1992	628	622	693	690	496	496	529	518	650	652	739	722
16. 4. 1992	613	609	672	678	430	441	501	496	609	613	711	705
16. 5. 1992	596	603	650	658	407	401	489	486	589	582	688	680

II. Changes in the content of reducing sugars (% in fresh fruits) in three varieties of strawberries during the storage regarding the way of planting

Date of analysis	Variety Dagmar		Variety Red Gauntlett		Variety Senga Sengana	
	clasically	meristem	clasically	meristem	clasically	meristem
19. 6. 1990	5.06	5.09	4.11	4.16	4.97	5.01
20. 8. 1990	5.82	5.82	4.72	4.67	5.48	5.63
20. 11. 1990	6.02	6.05	4.86	4.85	5.66	5.71
20. 1. 1991	6.24	6.19	4.97	5.02	5.71	5.83
20. 3. 1991	6.31	6.38	5.06	5.11	5.79	6.05
20. 4. 1991	6.36	6.38	5.14	5.18	5.86	6.21
20. 5. 1991	6.36	6.41	5.13	5.21	6.12	6.22

The changes in the content of AA during 11 months of deep freezing storage for the both tested seasons can be seen from Tab. I. The concentration of AA dropped dramatically during the first half of storage period approximately about 1/3 of the total content of AA. The difference in the content of AA between strawberry fruits picked from plants originally from meristems and planted in classical way varies from 8 to 18% depending on the variety. For all three varieties the higher concentration of AA in strawberries from plants cultivated from meristems is evident. The lower concentration of AA can be explained by the role of ascorbic acid as an antioxidant in the case of less resistant plants. Senga Sengana showed the highest concentration of ascorbic acid during the both tested periods. Decreasing of the content of vitamin C during storage and significant variety dependence is in accordance with the results of Čurdová et al. (1991) and Mähringová (1992) in white cabbage. The highest concentration of AA in strawberries planted from meristems coheres with the fact that these plants are more resistant to certain diseases and in general are more healthier than those planted in classical way.

The results of the analyses of reducing sugars are shown in the Tab. II. The reducing sugars performed the slow and irregular increase during the storage period. The highest difference in their content was found out for the variety Dagmar. No essential influence of the way of planting on the level of reducing sugars in strawberry fruits was found, but the content of RS slightly differs according to the variety. The reason for the increase in RS during storage is hydrolysis of oligosaccharides, predominantly saccharose.

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Změny obsahu kyseliny askorbové a redukujících sacharidů u tří odrůd jahod během skladování v závislosti na způsobu pěstování.

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Vitamin C – kyselina askorbová (AA) a kyselina dehydroaskorbová (DAA) jsou nezbytné látky pro zdraví a dobrý růst a vývoj člověka. Kyselina askorbová (AA) je jako důležitý nutriční faktor nejvíce zastoupena v ovoci a zelenině, které mají jako její zdroj nezastupitelnou úlohu.

V práci byly sledovány změny obsahu AA u tří odrůd jahod (Dagmar, Red Gauntlett, Senga Sengana - Zemědělské středisko Jesenice) pěstovaných klasickým způsobem a z meristému během skladování za standardních podmínek hlubokého zmrazení (–18 °C) po dobu 11 měsíců ve dvou časových řadách. Rovněž byl sledován obsah redukujících sacharidů (RS).

Pro stanovení AA byly použity dvě analytické metody – HPLC a pulsní polarografická metoda – a jejich výsledky se téměř nelišily (tab. I). HPLC analýzy byly provedeny na přístroji Waters 600 E Multisolvent Delivery systém vybaveném detektorem Diode Array (900 series) s použitím kolon s reversní fází C_{18} (200 x 4 mm i.d., 5 µ, Tessek Praha) při teplotě 40 °C v izokratickém režimu s mobilní fází metanol : 5mM vodného roztoku dodecylsulfátu = 30 : 70, pH = 2,5 (upraveno kyselinou trihydrogenfosforečnou). Detekce byla provedena při 254 nm a byla získána dobrá lineární korelace.

Pro pulsní polarografické stanovení byl použit elektrochemický detektor EDLC spojený se zapisovačem XY Recorder-4103 za použití metody standardního přídavku. Vzorky byly homogenizovány ve 2% kyselině monohydrogenfosforečné a pH bylo upraveno 2,5M octanem sodným.

Obsah RS byl stanoven metodou podle Schoorla varem se známým množstvím iontů Cu^{2+}, přebytek nezredukovaných měďnatých iontů byl stanoven jodometrickou titrací.

Z důvodu minimalizace vlivu dalších faktorů (počasí, aplikace hnojiv a pesticidů) byly jahody během růstu a zrání ošetřovány stejným způsobem.

Změny v obsahu AA během 11měsíčního skladování jsou uvedeny v tab. I. Během skladování došlo k úbytku vitaminu C, přičemž koncentrace AA klesla podstatně v první polovině doby skladování, a to téměř o jednu třetinu celkového obsahu. U všech tří odrůd byla nalezena vyšší koncentrace AA u rostlin pěstovaných z meristému než u rostlin pěstovaných klasickým způsobem (rozdíly se v závislosti na odrůdě pohybovaly v rozmezí 8 až 18 %). Nižší koncentrace AA v plodech rostlin pěstovaných klasickým způsobem může být vysvětlena rolí AA jako antioxidantu u méně rezistentních rostlin. Vyšší obsah AA u rostlin pěstovaných z meristémů je v souladu s faktem, že tyto rostliny jsou odolnější vůči chorobám než rostliny pěstované klasickým způsobem. Z porovnání obsahu AA u jednotlivých odrůd vyplývá značná odrůdová závislost – v obou testovaných obdobích byl nejvyšší obsah AA nalezen u odrůdy Senga Sengana a nejnižší u odrůdy Red Gauntlett.

Obsah RS vykazoval během skladování nelineární nárůst (tab. II). Toto zvýšení je důsledkem hydrolýzy oligosacharidů, převážně sacharózy. Nebyly nalezeny podstatné rozdíly v obsahu RS v závislosti na způsobu pěstování, avšak byly zjištěny rozdíly mezi odrůdami. Nejvyšší obsah RS byl nalezen u odrůdy Dagmar.

kyselina askorbová a redukující cukry v jahodách; pulsní polarografie; HPLC pro stanovení; vliv odrůdy a původu; vliv skladování

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