

A Study on Gas Exchange Dynamics and Biochemical Changes during Shelf life of European Plums (*Prunus domestica* 'Hauszwetsche')

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Abstract: Plum fruits belong to Family Rosaceae and form the second largest group of stone fruits. Plums being characterized as climacteric type rapidly undergo profound changes in texture and quality resulting in short storage period. The aim of present study was to obtain a continuous profile of gas exchange processes and related changes in biochemical constitution of plum fruits at 20° C and 50 percent relative humidity for 8 days. Physiological loss in weight increased significantly with increasing period of storage. Significant peaks in O₂ consumption rate (35.36±3.65 and 26.83 ±4.36 ml kg⁻¹h⁻¹) were observed on day 1 and day 5 respectively. The O₂ consumption rate appeared to have a positive correlation (r = 0.448) with CO₂ production and negative correlation with physiological loss in weight (-0.778). Rate of CO₂ production showed a significant decrease on 2nd day of storage and remained more or less constant upto 6th day of storage. A peak in CO₂ efflux (18.39± 0.34 ml kg⁻¹h⁻¹) was observed in 7th day of storage. Respiratory quotient of 0.65±0.07 was observed on day 0 which significantly decreased to 0.52±0.05 on day 2. A significant increase in ethylene production was observed from 5th day onwards reaching to a peak 68.54±8.13 ml kg⁻¹h⁻¹ on 8th day of storage. Acetaldehyde production was observed from 5th day onwards (1.59±0.44 ml kg⁻¹ h⁻¹). Plum fruits exhibited 17.66±1.22% mean dry matter content. A non significant increase in DMC was observed during 8 days of storage at 20°C. TSS in plum fruit increased significantly from 12.20±0.59% (day 0) to 15.33±0.39% (day 7). After an initial decrease from 0.97±0.02% (day 0) to 0.91±0.08% (day 4), increase in acidity (0.98±0.10%) was observed on day 5. Sucrose content showed a significant decrease from 0.701±0.55% (day 0) to 0.480 ± 0.028% (day 7). Glucose content of fruit showed a significant increase on 2nd day of storage. Highest fructose content (1.368±0.053%) was recorded on 5th day which decreased gradually upto the end of storage period. Highest sorbitol content (1.631±0.111%) was obtained on 7th day of storage. Total sugars in plum fruits increased significantly upto 5th day of storage (6.396 ±0.270%). Starch content showed a significant increase from 0.156±0.040% (day 0) to 0.198±0.035% (day 5) and then declined gradually. The results obtained indicate the possible role of stimulated ethylene and acetaldehyde levels in fruit senescence and cell structure break down. Presence of alternative respiratory pathway and mechanism of gluconeogenesis in plum fruits to support the metabolic process is hypothesized.

Key words: Plums, Respiration, Respiratory quotient, Ethylene, Acetaldehyde, Alternative respiration, Gluconeogenesis, Moisture stress

INTRODUCTION

Plum is an important stone fruit of temperate zone and belongs to family Rosaceae. It ranks next in

importance to the peaches so far as its economic importance is concerned. Plum is a rich source of sugars, carotene and dietary fiber. The fruit is used

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mainly for table purpose and to small extent for drying purpose. The plums are also used commercially for preparing jam, chutney and drinks. In European countries, plum cake is very popular. Plums being soft perishable fruits, require careful postharvest handling and storage. Like other stone fruits, plums are considered to have a climacteric ripening pattern, in which ethylene is the hormone responsible for triggering the ripening and senescence processes. One of the main consequences of this behaviour is reduced shelf life and decrease in quality parameters such as fruit turgidity, firmness, coloration and occurrence of decay and off-flavours. The present investigation was undertaken with an aim to obtain continuous outline of various physiological and biochemical changes occurring in fruits during storage at 20°C and to obtain a comprehensive coverage on enhancing shelf life and quality of plums.

MATERIAL AND METHODS

Fresh European plum fruits were procured from Klien Alterndorf research station and were Fruits were transported to postharvest laboratory of Institut für Gartenbauwissenschaft, Bonn. Fruits were pre-cooled for 6 hours at room temperature and then shifted to storage chamber. Fruits were stored at constant temperature of 20°C and 50% RH. The observations were recorded on daily basis. Batches of randomly selected 10 fruits in replicate of four were enclosed on each day in 1.75 liter glass jars fitted with rubber septum (10 fruits per glass jar). After four hours, one ml gas sample was drawn out from the head space of jar for analysis of gases. (O₂, CO₂, ethylene, acetaldehyde). After completing gaseous observation, fruits were used for other biochemical observations. Oxygen consumption was assessed using Paramagnetic oxygen analyzer (Type: OA-55, Servomex Ltd., U.K.). For analysis of carbon dioxide production gas chromatograph (GC-Type : Intersmat) equipped with 80 mesh Porapak 'Q' column and thermal conductivity detector was used. Ethylene and acetaldehyde evolution was estimated by gas chromatograph (GC Type-Carlo-erba 6000 Vega series) with 80 mesh Porapak 'Q' column and FID detector. Respiratory quotient (RQ) for the plums was calculated as ratio of CO₂ respired

to oxygen consumed. Physiological weight loss was expressed as the per cent loss in weight. Total soluble solids (%) in fruit juice were determined by using Zeiss-Optron-Abbe's refractometer. Total sugars were estimated using freeze dried samples analyzed by HPLC (Kontron Autosampler HPLC-360, 515 HPLC pump) with a carbohydrate Ca²⁺ 40 x 8mm refill pre-column (Art. No. 52998130, CS-Chromatographic Service, GmbH). The residue left after extraction process for sugars was used for estimation of starch. Dry matter content (DMC) was calculated as percentage of fresh weight.

RESULT AND DISCUSSION

Physiological parameters

The changes occurring in physiological loss in weight, respiration rate, ethylene and acetaldehyde evolution in European plum cv. Hauszwetschge during storage at 20°C has been presented in Table 1.

Physiological loss in weight

Physiological loss in weight increased significantly with increasing period of storage. Maximum PLW (11.96±0.93%) was obtained on 8th day of storage (Table 1, Fig. 1). Shrinkage was clearly evident in fruits. This could be mainly attributed to increased evapo-transpirational losses in fruits and upto some extent due to utilization of substrates. Fruits lost approximately 12% of weight on fresh weight basis during 8 days of storage and maximum was on 8th day. Similar increase in weight loss was also reported by Hoen (1987) in plums.

Respiration rate

Plum fruits showed major shifts in oxygen consumption during storage. Oxygen consumption rate of plums in beginning was 28.05±3.76 ml kg⁻¹ h⁻¹. The rate of O₂ consumption decreased with increase in storage period (Fig. 2). Significant peaks in O₂ consumption rate (35.36±3.65 and 26.83 ±4.36 ml kg⁻¹h⁻¹) were observed on day 1 and day 5 respectively. Each peak showed a significant decrease on next day and thereafter followed a gradual decline. This might be an indication of that

Table 1
Changes occurring in physiological loss in weight and gaseous exchange rates of European plum cv. Hauszwetschge (clone: Hartmann) stored at 20°C

Days in storage	Physiological loss in weight (%)	Respiration			Ethylene evolution (ml kg ⁻¹ h ⁻¹) ¹	Acetaldehyde evolution (ml kg ⁻¹ h ⁻¹)
		Oxygen consumption (ml kg ⁻¹ h ⁻¹)	Carbon dioxide production (ml kg ⁻¹ h ⁻¹)	Respiratory quotient (RQ)		
0 (initial)	-	28.05±3.76	18.00±0.39	0.65±0.07	24.40±7.20	-ne-
1	1.52±0.12	35.36±3.65	18.20±0.39	0.52±0.05	28.10±3.84	-ne-
2	3.05±0.25	29.71±3.59	15.81±0.34	0.52±0.07	28.02±4.67	-ne-
4	6.69±0.57	20.18±0.98	15.12±0.26	0.75±0.01	22.24±5.92	-ne-
5	7.92±0.65	26.83±4.36	15.50±0.72	0.59±0.08	44.24±8.93	1.59±0.44 (7.19)
6	9.25±0.74	18.59±2.35	14.32±0.76	0.78±0.08	49.80±6.61	8.65±6.45 (16.41)
7	10.68±0.84	21.12±3.72	18.39±0.34	0.89±0.13	62.11±7.13	49.12±8.10 (44.47)
8	11.96±0.93	19.90±1.56	15.41±0.80	0.78±0.06	68.54±6.87	82.38±5.88 (65.36)
Mean	7.29±3.68	24.96±6.32	16.26±1.62	0.68±0.15	40.93±18.01	17.72±29.91
CD at 5%	0.957	4.70	0.75	0.11	9.63	8.71
Correlation coefficient (r)						
Oxygen	-0.778**	-	0.448**	-	-	-
RQ	0.762**	-0.888**	-0.73	-	0.473**	0.486**
Ethylene	0.801**	-0.414**	-0.37	-	-	-
Acetaldehyde	-	-0.556**	-0.534**	-	-	-

Linear regression (Oxygen-RQ): $Y = -0.0204X + 1.1937$

(Where, Constant (X)=oxygen consumption, Variable (Y)= RQ, Standard error=0.0683); $R^2 = 0.7882$

Data are mean ± standard deviation

** Correlation is significant at 1% level of significance (Pearson correlation (2-tailed), SPSS ver. 11.0)

-ne- no evolution of acetaldehyde observed on respective day of observation

Figure in parenthesis are angular transformed values

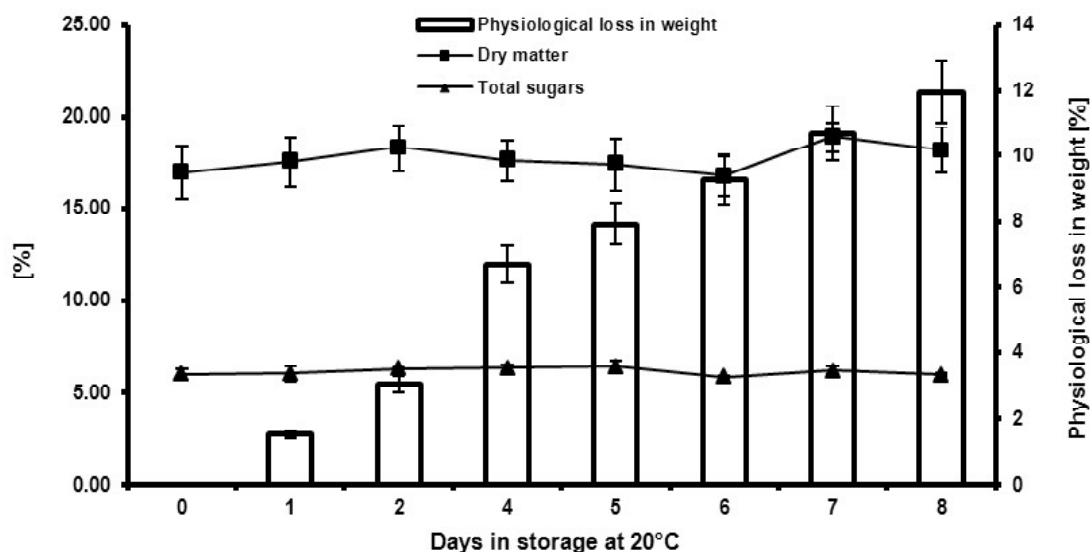


Fig. 1: Changes in physiological weight loss, dry matter and total sugars in European plums during storage

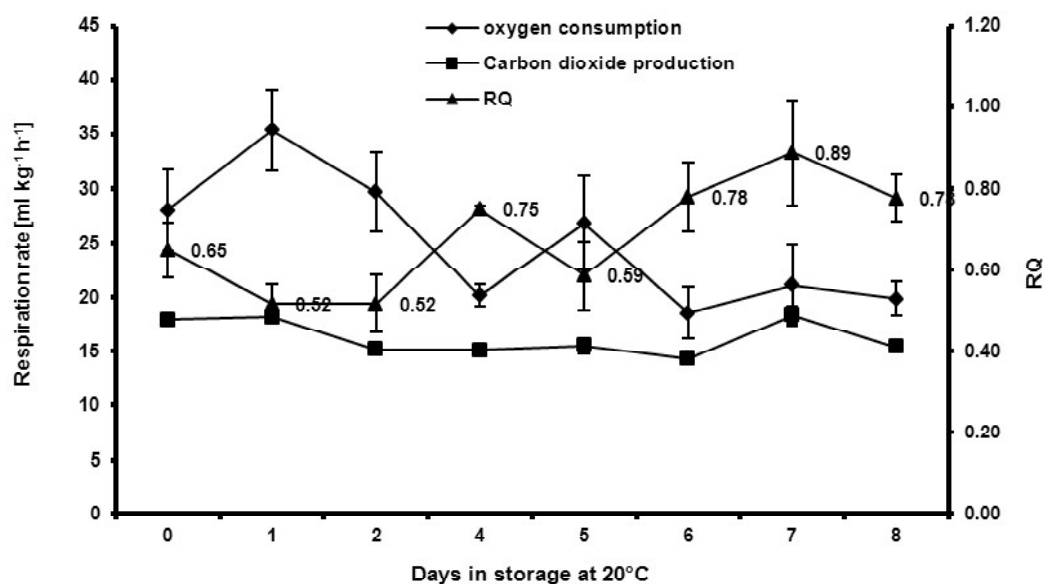


Fig. 2: Changes occurring in respiration rate of European plum fruits during storage

fruit after harvest, was trying to adapt the prevailing conditions and metabolism was shifting from one mechanism to other. The O_2 consumption rate appeared to have a positive correlation ($r = 0.448$) with CO_2 production and negative correlation with physiological loss in weight (-0.778).

Another interesting thing to observe was the pattern of CO_2 evolution. European plum initially (day 0) produced CO_2 at the rate of 18.00 ± 0.39 ml kg^{-1} h^{-1} . A very typical trend of CO_2 production was observed (Fig. 2). Rate of CO_2 production showed a significant decrease on 2nd day of storage and remained more or less constant upto 6th day of storage. A peak in CO_2 efflux (18.39 ± 0.34 ml kg^{-1} h^{-1}) was observed in 7th day of storage. Rate of CO_2 evolution starting from 2nd day of storage depicted no significant variation upto 5th day of storage. Lippert and Blanke (2004) also reported a similar trend of CO_2 production in European plum and proposed the possibility of CO_2 refixation. A similar pattern of CO_2 production and similar probability of CO_2 refixation during storage was reported by MacRae *et al.* (1992) in kiwifruit.

Respiratory quotient of 0.65 ± 0.07 was observed on day 0 which significantly decreased to 0.52 ± 0.05 on day 2. Highest RQ (0.89 ± 0.13) was obtained on 7th day of storage (Fig. 1). It was interesting to

observe that shift in RQ was found related to oxygen consumption and a negative correlation ($r = -0.888$) existed between the two. Linear regression of RQ with rate of O_2 consumption gave regression coefficient (R^2) 0.7882. A week but positive correlation of RQ was also observed with ethylene and acetaldehyde evolution ($r = 0.473$ and 0.486 , respectively).

Shift in RQ is indicative of differential utilization of substrate in metabolic processes. For the observed duration of storage, RQ was found below unity. It has been reported in literature that if RQ is deviating from unity (i.e. below unity), there are possibilities that respiratory substrates are other than carbohydrates and are partially oxidized or CO_2 formed is utilized metabolically instead of being given out (Pandey and Sinha, 2002).

Ethylene evolution

In the beginning, European plum produced ethylene at the rate of 24.40 ± 7.20 ml kg^{-1} h^{-1} . Ethylene evolution showed a non significant variation upto 4th day of stage. A significant increase in an ethylene production was observed from 5th day onwards reaching to a peak 68.54 ± 8.13 ml kg^{-1} h^{-1} on 8th day of storage (Fig. 3). Ethylene evolution showed negative correlation with rate of O_2 consumption ($r = 0.414$).

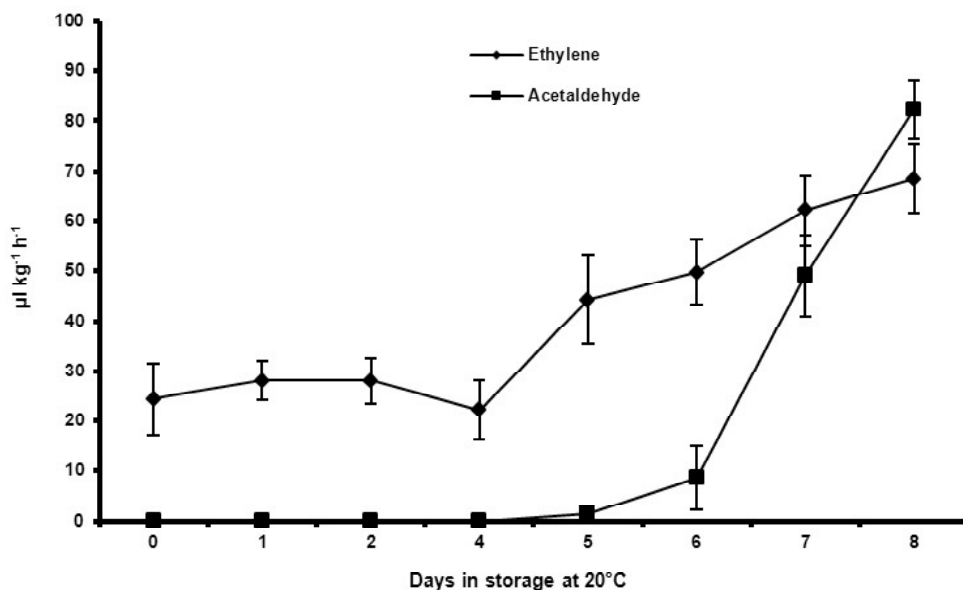


Fig. 3: Changes occurring in ethylene and acetaldehyde evolution in European plum fruits during storage

It was seen that outburst of ethylene on 7th day of storage coincided with rise in rate of respiration (oxygen uptake and carbon dioxide production) on the same day (Table 36) indicating the climacteric behaviour of European plum fruits. Ethylene evolution was also found positively correlated to physiological loss in weight ($r=0.801$). This could further be supported by fact that water stress in plant tissue may stimulate ethylene production (Yang and Pratt, 1978, Grierson and Wardowski, 1978). Increase in ethylene production under water stress has also been reported in banana (Finger *et al.* 1995), avocado (Adato and Gazit, 1974), persimmon (Nakano *et al.*, 2002). So results indicate that apart from autocatalytic ethylene synthesis mechanism existing in European plum fruits, water stress induced ethylene evolution might also be there.

Acetaldehyde evolution

No acetaldehyde evolution was observed upto 4th day of storage (Table 1, Fig. 3). From 5th day onwards ($1.59 \pm 0.44 \text{ ml kg}^{-1} \text{ h}^{-1}$) significant increase in acetaldehyde production was observed. Highest rate of evolution ($82.38 \pm 5.88 \text{ ml kg}^{-1} \text{ h}^{-1}$) was observed on 8th day of storage. Acetaldehyde evolution appeared to have a negative correlation with rate of O_2 consumption and CO_2 production

($r = -0.556$ and -0.534 , respectively). No correlation could be developed between ethylene synthesis and acetaldehyde evolution. Results are in conformity with observations of Salvador *et al.* (2003a) in plums.

Dry matter content

Fruits exhibited $17.66 \pm 1.22\%$ mean dry matter content. A non significant increase in DMC was observed during 8 days of storage at 20°C. This might be because of reason that after harvest it is generally interconversion of one substance into another but total organic content remains the same.

Biochemical parameters

Total soluble solids

TSS in plum fruit increased significantly from $12.20 \pm 0.59\%$ (day 0) to $15.33 \pm 0.39\%$ (day 7) and later declined. Fruits exhibited $13.70 \pm 1.00\%$ mean TSS at end of storage period. This increase could be attributed to increasing physiological loss in weight as fruits were stored under low humidity conditions and upto some extent due to conversion of insoluble carbohydrates to soluble sugars (Singh *et al.* 1981). Decrease in TSS at end of storage could be due to utilization of various sugars as substrates to maintain metabolism of fruit.

Acidity

No specific trend of change in acidity content of fruits was observed (Table 37). After an initial decrease from $0.97 \pm 0.02\%$ (day 0) to $0.91 \pm 0.08\%$ (day 4), increase in acidity ($0.98 \pm 0.10\%$) was observed on day 5. Highest acidity content ($1.00 \pm 0.08\%$) in fruits was observed on 7th day of storage. Changes observed in acidity during storage were statistically non significant. Results are in conformity with findings of Kluge *et al.* (1999) and Dundar *et al.* (1997). Decrease in acidity content could be attributed to utilization of acids in respiratory process whereas increase could be attributed to induction of fermentive pathway or result of stress induced acetaldehyde/ethylene induced mechanisms (Pool *et al.* 1972).

Sugars and starch

In fresh fruit (Day 0), glucose was the major sugar contributing to hexose pool followed by sorbitol, fructose and sucrose. A very small portion of starch was also detected (Table 3, Fig. 4).

Sucrose content showed a significant decrease from $0.701 \pm 0.55\%$ (day 0) to $0.480 \pm 0.028\%$ (day 7). A significant increase in sucrose concentration was observed on 8th day of storage. Sucrose appeared to have negative correlation with glucose ($r = -0.466$) and fructose ($r = -0.588$). The decrease in sucrose content is indicative of its utilization as substrate or breakdown in simple sugars for utilization in respiratory process (Park, 1996). On 8th day of storage sucrose concentration increased significantly which corresponded well to decrease in fructose content on same day.

Glucose content of fruit showed a significant increase on 2nd day of storage. Glucose concentration was found statistically at par on day 2, 4 and 5 (2.886 ± 0.048 , 2.854 ± 0.154 and $2.877 \pm 0.123\%$, respectively). Glucose showed a high positive correlation with fructose ($r = 0.723$) and total sugars ($r = 0.802$). Increase in glucose content was observed on 2nd day of storage which corresponded well to the decrease in sucrose content. Afterwards no major variation in level of glucose was observed.

After a initial decrease in fructose content on 2nd day of storage, fructose concentration

significantly increased upto 5th day of storage. Highest fructose content ($1.368 \pm 0.053\%$) was recorded on 5th day which decreased gradually upto the end of storage period. Fructose appeared to have a positive correlation ($r = 0.674$) with total sugars. A non significant increase in sorbitol concentration was observed upto 4th day of storage from initial of $1.517 \pm 0.150\%$ to $1.621 \pm 0.115\%$ following decrease upto 6th day of storage. Highest sorbitol content ($1.631 \pm 0.111\%$) was obtained on 7th day of storage. Sorbitol showed a positive correlation ($r = 0.735$) with total sugars.

Total sugars in plum fruits increased significantly upto 5th day of storage. Highest total sugars ($6.396 \pm 0.270\%$) were recorded on 5th day of storage. Freshly harvested mature plum fruits (day 0) showed a total sugar composition of 11% sucrose, 42% glucose, 19% fructose and 25% sorbitol. After 8 days of storage mean composite of total sugars remained more or less same except sucrose and glucose content showing some significant decrease and increase, respectively (9% sucrose, 45% glucose, 20% fructose and 23% sorbitol).

Starch content showed a significant increase from $0.156 \pm 0.040\%$ (day 0) to $0.198 \pm 0.035\%$ (day 5) and then declined gradually. Starch content showed a negative correlation with sucrose and sorbitol (-0.434 and -0.364 , respectively). Starch content constituted 3% to total carbohydrates present in plum fruits (Total carbohydrates = sucrose + glucose + fructose + sorbitol + starch).

Some dynamics in sugars was observed after 5th day of storage, the day on which the upsurge in ethylene and initiation of acetaldehyde was observed. The increase in sucrose content on 8th day might be attributed to ability of ethylene to reduce the rate of conversion of sucrose to hexose in fruits (Iglesias *et al.*, 2001). The increased concentration of fructose on 7th day might be attributed to the ability of acetaldehyde to initiate accumulation of fructose (Halinska and Frenkel, 1991). The decrease in fructose content on 8th day might be attributed to its utilization as respiratory substrate or conversion to sucrose via action of sucrose synthase (Yamaki, 1995). No significant variation in total sugar content was observed (Table 38, Fig. 23) upto 5th day of

storage. This might be due to interconversion of sugars from one form to other or a possibility that there was some *de novo* synthesis of sugars under water stress conditions (as evident from increasing physiological loss in weight, ethylene outburst and acetaldehyde evolution) which were utilized as ready substrates for on going metabolic process (Halinska and Frenkel, 1991, Iglesias *et al.*, 2001,

Table 2
Changes occurring dry matter content, total soluble solids and acidity of European plum cv. Hauszwetschge (clone: Hartmann) stored at 20 °C

Days in storage	Dry matter content (%)	Total soluble solids (%)	Acidity (%)
0 (initial)	16.93±1.44	12.20±0.59	0.97±0.02
1	17.50±1.29	12.48±0.50	0.94±0.04
2	18.27±1.30	12.83±0.56	0.92±0.12
4	17.59±1.10	14.08±0.39	0.91±0.08
5	17.38±1.39	14.23±0.43	0.98±0.10
6	16.75±1.07	14.88±0.30	0.96±0.07
7	18.87±0.84	15.33±0.39	1.00±0.08
8	18.16±1.28	14.28±0.60	0.98±0.09
Mean	17.66±1.22	13.70±1.00	0.96±0.08
CD at 5%	NS	0.707	NS

Data are mean ± standard deviation

Table 3
Changes occurring in sugars and starch content of European plum cv. Hauszwetschge stored at 20°C

Days in storage	Sugars (%)					Starch (%)
	Sucrose	Glucose	Fructose	Sorbitol	Total Sugars	
0 (initial)	0.701±0.055	2.601±0.124	1.144±0.062	1.517±0.150	5.962±0.309	0.156±0.040
1	0.652±0.079	2.582±0.237	1.197±0.082	1.569±0.190	6.000±0.435	0.163±0.078
2	0.569±0.025	2.886±0.048	1.275±0.048	1.548±0.068	6.278±0.054	0.173±0.059
4	0.538±0.039	2.854±0.154	1.319±0.029	1.621±0.115	6.309±0.170	0.167±0.020
5	0.555±0.019	2.877±0.123	1.368±0.053	1.571±0.164	6.396±0.270	0.196±0.052
6	0.527±0.018	2.696±0.049	1.352±0.026	1.385±0.058	5.843±0.078	0.198±0.035
7	0.480±0.028	2.763±0.119	1.305±0.085	1.631±0.112	6.178±0.228	0.171±0.051
8	0.551±0.018	2.737±0.022	1.191±0.022	1.372±0.137	5.942±0.074	0.191±0.022
Mean	0.572±0.018	2.749±0.160	1.254±0.088	1.527±0.151	6.113±0.282	0.177±0.049
CD at 5%	0.041	0.147	0.048	NS	0.439	0.024
Correlation coefficient (r)						
Sucrose	-	-0.466**	-0.588**	-0.94	-0.220	-0.434*
Glucose	-	-	0.723**	0.307	0.802**	0.197
Fructose	-	-	-	0.391*	0.674**	0.273
Sorbitol	-	-	-	-	0.735**	-0.364*
Total sugars	-	-	-	-	-	-0.093

Data are mean ± standard deviation

** Correlation is significant at 1% level of significance (Pearson correlation (2-tailed), SPSS ver. 11.0)

* Correlation is significant at 5% level of significance (Pearson correlation (2-tailed), SPSS ver. 11.0)

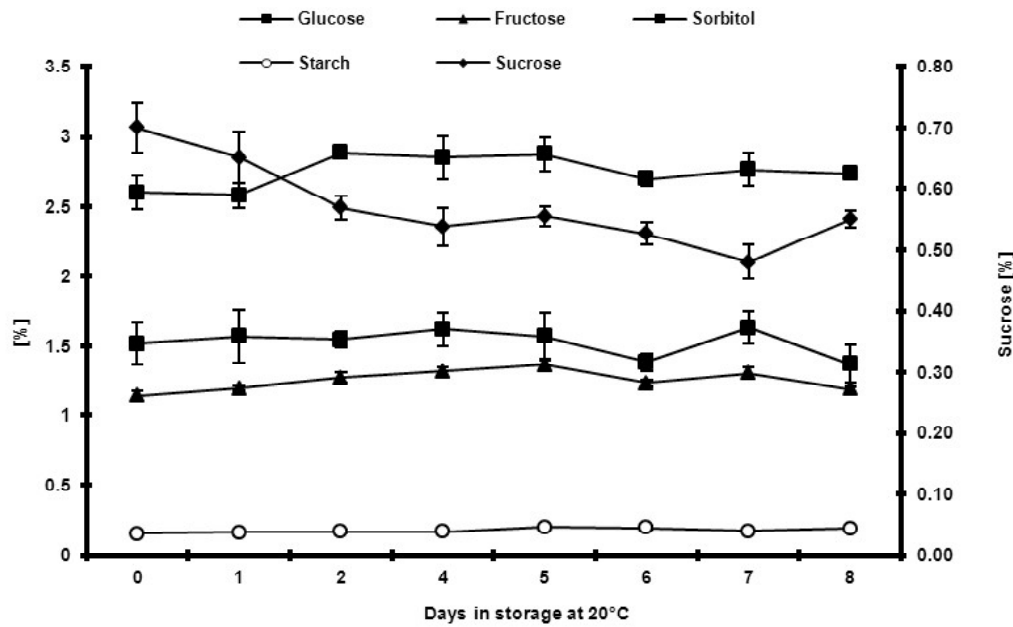


Fig. 4: Changes in sugars and starch content of European plums during storage

Kameli and Losel, 1996). Gross and Sams (1984) also reported lack of net loss of sugars in plums during storage.

The role of respiration in survival of fruit tissue during storage can be considered in terms of demand for respiratory energy and competition for and availability of respiratory substrates. In present study results shows high oxygen consumption during early phase of storage indicating presence of significant oxidative processes other than respiration. Utilization of different metabolic substrates could be understood by shift in RQ values during storage. More or less a constant rate of carbon dioxide evolution was observed. A non-significant shift in total sugars and dry matter content was observed. Simultaneous evolution of ethylene and acetaldehyde was observed in later phase of storage but no correlation could be developed. A continuous increase in physiological loss in weight (largely due to evaporational losses) was observed. As fruits were stored under low relative humidity conditions (50% RH) and also suffered continuous depletion of moisture, water or moisture stress conditions were developed. Such moisture stress conditions are capable of activating stress induced mechanisms like:

- **Formation of stress proteins:** These could serve as “compatible solutes” in biochemical reactions or may get associated with lipid/ lipoprotein to prevent membrane disintegration, dissociation of protein complexes or inactivation of enzymes (Chung *et al.*, 1997).
- **Carbohydrate accumulation:** Water stress can induce production of low molecular weight osmotically active compounds like glucose and fructose. (Kameli and Losel, 1996). Accumulation of carbohydrates, amino acids and ethanol insoluble dry matter has also been reported as water stress induced mechanism (Carl *et al.* 1978, Pheloung and Barlow, 1981, Virgona and Barlow, 1991). In plum, organic acids, sugars, and sorbitol constitute approximately 84% of the dry matter content (Taylor *et al.*, 1993). Anderson *et al.* (1995) reported that water stress could induce 3-fold increase in total organic acids in plums. This could probably support the obtained result of increased accumulation of glucose at the end of storage period and non-significant shift in dry matter content. Role of ethylene in cell structure breakdown cannot be ignored, as it is known to activate many ripening related

enzymes (Saltveit, 1999) like polygalacturonase and pectin methyl esterase (Dong *et al.*, 2001a), cellulase (Fischer and Bennett, 1991, Sexton *et al.*, 1997). This could have further added to substrate pool for respiratory processes.

- **Alternative respiratory mechanism:** Alternative respiratory mechanism might have been activated via activation of alternative oxidase (AOX) under moisture stress conditions (Laties, 1982, Wagner and Krab, 1995) and accumulating carbon dioxide (Nanos *et al.*, 1994, Kader, 1995).
 - o High internal carbon dioxide level has capacity to inhibit cytochrome 'C' oxidase enzyme (Gonzalez-Meler *et al.*, 1996) and can restrict the succinic dehydrogenase activity resulting in accumulation of succinic acid (Williams and Patterson, 1964), i.e. enrichment of substrate pool. Alternative pathway under such conditions has also been reported in litchi (Lin *et al.*, 1990), mango (Reddy and Srivastava, 1998).
 - o Apart from this, it has been reported that less acidic fruits and vegetables has capacity to accumulate carbon dioxide. This accumulated CO₂ is capable of initiating malate metabolism resulting in production of pyruvate, the most favoured substrate for TCA cycle (Davies, 1986, Lencki *et al.*, 2004).
 - o Various reports have indicated that AOX gets activated in presence of organic acids like pyruvate (Miller *et al.*, 1993), succinate and malate (Liden and Akerlund, 1993, Wagner *et al.*, 1995), citrate (Vanlerberghe and McIntosh, 1997), isocitrate (Vanlerberghe *et al.*, 1995), NADPH generated by intra-mitochondrial malate dehydrogenase and isocitrate dehydrogenase (Vanlerberghe *et al.*, 1995, Rasmusson and Moller, 1990) and oxyglutarate and hydroxy pyruvate (Miller *et al.*, 1993).
- **Acetaldehyde evolution:** Acetaldehyde evolution also plays important role in anabolic

and catabolic processes of fruits during ripening and senescence leading to increased membrane permeability, damage to lipid bilayers (Podd and Staden, 1998). Acetaldehyde production also influences kreb cycle, electron transport (Cederbaum *et al.*, 1974), lipid metabolism (Cederbaum *et al.*, 1975) and sugar accumulation (Halinska and Frenkel, 1991). Production of ethanol can take place under moisture stress conditions (Saltveit, 1989). This may lead to acetaldehyde evolution via ethanol by action of alcohol dehydrogenase enzyme in presence of NADH (Monk *et al.*, 1987).

- **Gluconeogenesis:** Acetaldehyde is capable of stimulating sugar accumulation in fruits (Janes *et al.*, 1978, Paz *et al.*, 1981). Acetaldehyde is able to initiate gluconeogenesis process by attenuation of the fructose 2,6-biphosphate level (gluconeogenesis inhibitor) (Halinska and Frenkel, 1991). As mentioned earlier, plum fruits are rich in amino acids, enzyme phosphoenolpyruvate carboxykinase (PEPCK) may play a key role in amino acid metabolism by conversion of carbon skeleton of asparagine/aspartate (oxaloacetate) to glutamate/glutamine (2-oxoglutarate). PEPCK catalyses the reversible decarboxylation of oxaloacetate to yield phosphoenolpyruvate (PEP) (Lea *et al.*, 2001). Glyoxylate and beta-oxidation are two pathways occurring in glyoxysomes involved in gluconeogenesis (initiated by degradation of structural and non structural lipids) by enriching malate pool in cytosol and transporting succinate to mitochondria for conversion into malate. (Eschel and Widmer, 1997)

It is hypothesized that these processes viz., glycolysis, gluconeogenesis and alternative respiration do not operate independently as they share common substrates like pyruvate, succinate and malate. Decarboxylation of pyruvate results in formation of acetaldehyde (Beaulieu *et al.*, 1997), which is capable of degrading structural and nonstructural lipids (Cederbaum *et al.*, 1975) and provides substrates for beta-oxidation and glyoxylate cycle. Malate released from glyoxysome

in cytosol is converted to PEP via oxaloacetate and contributes to gluconeogenesis. PEP also produces pyruvate via action of pyruvate kinase which acts as substrate of mitochondrial tricarboxylic acid (TCA) cycle and acetaldehyde formation in cytosol. Excess of malate in cytoplasm could be transported to mitochondria to join TCA cycle where it can generate pyruvate via malic enzyme and succinate coming from glyoxysome joins TCA cycle to replenish malate in mitochondria. Excess of pyruvate in mitochondria

serve as substrate for alternative oxidase that prevents over reduction of cytochrome chain and fermentation of accumulated pyruvate. Alternative respiratory pathway increases availability of sucrose as substrate for vacuolar acid invertase resulting in accumulation of reducing sugars (Duplessis *et al.*, 1996). The generated carbon dioxide has possibility to get refixed via phosphoenolpyruvate carboxylase enzyme in respiratory pathway and thus completing the cycle (Fig. 5).

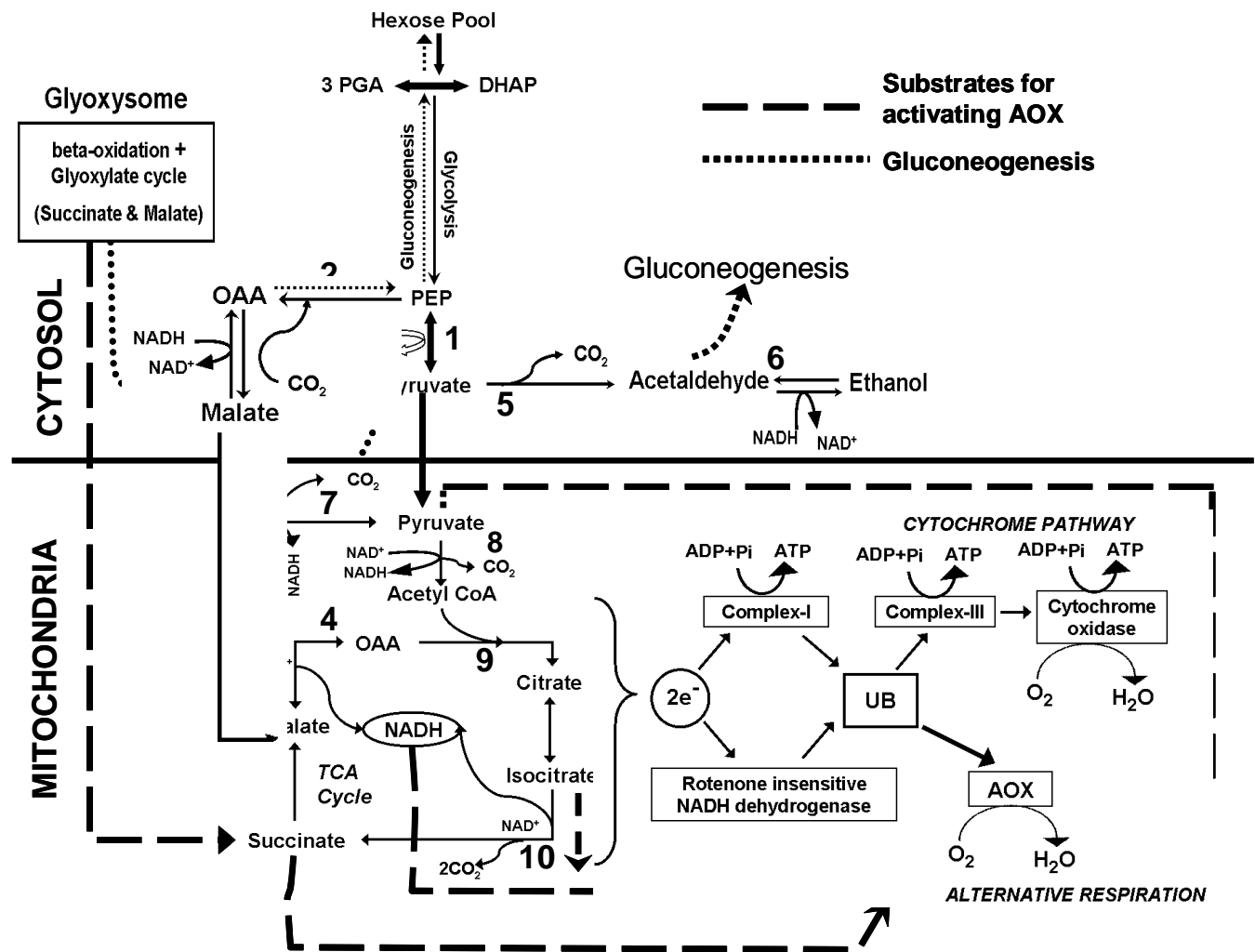


Figure 5: Schematic representation of gluconeogenesis and alternative respiration occurring in stored plum during storage period as proposed in hypothesis.

Enzymes: 1. Pyruvate kinase, 2. Phosphoenolpyruvate carboxy kinase, 3. Phosphoenolpyruvate carboxylase, 4. Malate dehydrogenase, 5. Pyruvate decarboxylase, 6. Alcohol dehydrogenase, 7. Malic enzyme, 8. Pyruvate dehydrogenase, 9. citrate synthase, 10. Isocitrate dehydrogenase.

Abbreviations: PGA-Glyceraldehyde-3-phosphate, DHAP-Dihydroxyacetone phosphate, PEP-Phosphoenolpyruvate, UB- Ubiquinone, AOX- Alternative oxidase

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