African Journal of Plant Science Vol. 3 (6), pp. 133-138, June, 2009
Available online at http://www.academicjournals.org/AJPS
ISSN 1996-0824 © 2009 Academic Journals

Full Length Research Paper

Physiological and anatomical comparison between four different apple cultivars under cold-storage conditions

Saleh A. M. Ghafir[1], Suliman O. Gadalla[1], Benissa N. Murajei[1] and Mohamed Fathi El-Nady[2]*

[1]Department of Horticulture, Faculty. of Agriculture, Omar AlMukhtar University, EL-Beida, Libya.
[2]Department of Agriculture. Botany, Faculty of Agriculture, Kafrelsheikh University, Egypt.

Accepted 4 June, 2009

The work was carried out in two successive seasons (2005 and 2006) to investigate the storability of four apple cultivars viz. Golden Delicious, Starking Delicious, Star Cremson and Gala, which were grown under El Jabal El Akhdaer conditions, in Libya. Fruits were harvested and stored at 85-90% relative humidity and 0ºC for 0, 30, 60, 90, 120, 150 and 180 days. The obtained results exhibited the significant differences between the tested cultivars in the studied parameters comprising physiological and anatomical parameters. Star Cremson cv. had a potentially high fruits storability trend, as they showed less fruit weight loss, while cv. Gala fruits had the highest level of weight loss. On the other hand, cv. Golden Delicious gave the highest values of fruit firmness, while the lowest values were obtained in fruits of cvs. Starking Delicious and Star Cremson. Fruit weight loss percent increased, while other studied parameters decreased gradually by extending storage period. Anatomical data demonstrated that fruit surface of cvs. Gala and Star Cremson were smooth, while undulate and ripples surfaces were obtained in cvs. Starking Delicious and Golden Delicious, respectively. Gala fruits had the thick cuticle layer compared to the other cultivars. Crushed parenchymatous cells (cpc) were found in storage tissue in Starking Delicious and Golden Delicious. It may be concluded that obtained results indicated a great variability among cultivars. All studied parameters of four apple cultivars reduced during cold-storage periods. The highest fruit storability was achieved in cv. Star Cremson. This study gives useful information to use the suitable cultivar for reducing the quality losses which result from postharvest diseases.

Key words: Apple cultivars, cold-storage, cuticle, crushed parenchymatous cells, storability.

INTRODUCTION

Apple (*Malus domestica* L.) is a member of family Rosaceae and distributed worldwide. The growth period and development of fruit is not only important as a basic problem in plant physiology, but it is also of economic importance during transportation and storage (Ben-Arie and Lurie, 1986). During this period, different biochemical and physical changes take place associated with changes in different characters (Hulme and Rhodes, 1971). Several observations reveal relationship between fruit characters and temperature. This is an important point, because of the frequent use of low temperature during the post-harvest storage of fruit. One of the roles of temperature is its regulatory effect on the rate of chemical reactions, resulting in a slowing of the rate of metabolism when the temperature falls. The phenolic compounds can be affected by cold as a result of slowing of natural ripening (Macheix et al., 1990).

Fruit skin in apples consists of the cuticle, epidermis and several layers of hypodermis (Babos et al., 1984). Both skin and its waxy coat are important during transportation and storage. Its mechanical resistance is directly related to apple fruit damage (Pierzynowska-Korniak et al., 2002). The skin thickness is not uniform in apples, it differs considerably between cultivars and between different years within the same cultivar, (Homutova, 2005; Homutova and Blazek, 2006). Skin characteristics influence fruit bruising to a large extent. The skin thickness influences the penetration of pathogen into fruits (Mourichon and Bompeix, 1979). Also, the apple skin structure and its thickness clearly affect the storage ability of fruits (Ruffa, et al., 1992). The thickness of the epidermis and the surface wax are the main skin characteristics that are related to weight losses during

*Corresponding author: E-mail: mfmelnady@yahoo.com.

cold-storage (Bebic, 1972 and Veraverbeke, et. al., 2003)

Therefore, the aim of this study was to quantify the physiological and anatomical differences in apples between selected cultivars under cold storage conditions.

MATERIALS AND METHODS

This work was carried out in two successive seasons (2005 and 2006) at Horticulture. Department Faculty of Agric., Omar Al Mukhtar University in Libya and Department of Agric. Botany, Faculty of Agric. Kafrelsheikh University in Egypt. Four grown apple cultivars: Golden Delicious, Starking Delicious, Star Cremson and Gala under El Jabal El Akhdaer conditions, in Libya, were used in the experiments. Fruits were harvested at commercial maturity and chosen as well as were stored in controlled chamber at 85-90% relative humidity and 0ºC for 0, 30, 60, 90, 120, 150 and 180 days. Experiment was designed as split plot design with four replicates, where main plots assigned for cultivars and subplots for storage periods. Differences between the tested apple cultivars by the use of physical (fruit weight loss percent and fruit firmness (FF) %) and chemical (TSS%, starch concentration and acidity) as well as anatomical parameters of fruits stored under cold conditions were investigated monthly.

Physiological parameters

The physiological parameters were measured to the subsequent criteria:

Fruit weight loss (FWL) %: Ten fruits of each treatment were selected and assigned with code number for each fruit and each of them was weighed before and after the storage period and then weight loss percentage was calculated as follows:

$FWL\ \% = FW_1\text{-}FW_2/FW_1 \times 100$

Where FW_1 is fruit weight before cold storage and FW_2 is fruit weight after cold storage periods.

Fruit firmness rate (kg/cm^2): Fruit firmness was measured by Ham-held magness-pressure tester of (5-15) inch plunger, according to A.O.A.C (1981).

Chemical parameters

Total soluble solid (TSS)%: was determined by the use of a hand refractometer (Carl-Zeiss).

Titratable acidity: values were measured by malic acid estimation in apple fruits according to Rangana (1979).

Starch concentrations percentage values: were measured by using iodine staining (2.5 g iodine + 10 g potassium iodide in 1 L distilled water) according to Teskey and Shoemaker (1978).

Anatomical parameters

For preparing transfer-sections, the fruit specimens were taken 90 days after cold storage from the surface parts (about 1/2 cm^3) at the maximum fruit diameter of different studied cultivars. Specimens were fixed in Formalin Alcohol Acetic Acid mixture (FAA, 1:18:1 v/v), washed and dehydrated in alcohol series. The dehydrated specimens were infiltrated and embedded in paraffin wax (52-54°C m.p.). The embedded specimens were sectioned on a rotary microtome at a thickness of 10-12 µm. Sections were mounted on slides and deparaffinised. Staining was accomplished with safranine and light green, cleared in xylol and mounted in Canada balsam (Ruzin, 1999). Slides were microscopically examined and measurements and counts were taken and averages of 10 readings from 3 slides were calculated.

Statistical analyses

The obtained data were subjected to the proper statistical procedures for analysis of variance according to that outlined by Gomez and Gomez (1984) using Genstat software as an analytical tool.

RESULTS AND DISCUSSION

Physical and Chemical Parameters

Effect of cultivars: Fruit weight loss percent showed that, there were significance differences between four apple cultivars in the present study. Star Cremson had a high storability tendency, as they showed less fruit weight loss percentage while cv. Gala had a low storability during all cold storage periods (Table 1). It is clear that, fruit storability correlated with fruit firmness rate (FFR) and anatomical analysis (Table 4 and Figure 1). The thickness of main skin, cuticle and surface wax characteristics are related to weight loss percentage in cold-stored apples (Bebic, 1972; Ruffa et al., 1992). Several previous works indicated there was a correlation between different cultivars and fruit weight losses which were stored under cold conditions (Guleryuz et al., 2000; Bandaravicius et al., 2001; Ugolik et al., 2001; Tavares et al., 2002; Baneh et al., 2003).

Starking Delicious and Star Cremson had the highest fruit firmness values in both seasons while the lowest values were recorded in Golden Delicious. These results are in harmony with the anatomical data (Figure 1 and Table 4). FFR values are directly related to cuticle layer thickness. Several observations reveal relationship between Ca content in fruits and FFR values (Zheng et al, 2005, Sasnaukas, 2006).

Concerning, total soluble solid (TSS) percentage, no significant differences were obtained between cvs Gala, Star Cremson and Starking Delicious. The highest and the lowest TSS% values were recorded in Gala and Golden Delicious, respectively. Clearly, TSS% associated with fruit storability, it increased gradually by increasing in fruit weight loss percent. Saquet and Streif (2000) revealed the relationship between TSS% and ethylene production and fruit ripening in Gala cultivar. These results coincided with those obtained by Naseri et al. 2001); Baneh et al. (2003); Hamedi and Mila (2003); Song et al. (2003); Ventura and Sansavini (2005).

Results of starch concentration measurements, however, showed that no significant differences were recorded between the four apple cultivars in both seasons. It was also observed that significant differences between cultivars in acidity values were found after cold-storage pe-

Table 1. Differences between the tested apple cultivars by using some physical and chemical parameters under cold-storage conditions.

Apple varieties	Weight loss %		Firmness rate kg/ cm^2		T.S.S %		Starch concent.		Acidity %	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Golden Delicious	6.89b	2.80b	2.51c	2.48c	13.07b	14.07b	3.50	3.53	0.16c	0.15c
Starking Delicious	6.12c	2.47c	3.16a	3.19a	13.57ab	14.57ab	3.50	3.57	0.19a	0.19a
Star Cremson	5.63d	2.42c	3.14a	3.23a	13.46ab	14.46ab	3.39	3.39	0.17b	0.17b
Gala	8.7a	4.13a	2.74b	2.85b	13.94a	14.91a	3.46	3.46	0.18a	0.19a
F test	**	**	**	**	**	**	NS	NS	**	**

Mean values within a column followed by different letter are significantly different at P≤0.5 level.

Table 2. Effect of storage periods on some physical and chemical characters of cold-stored fruits.

Storage periods	Weight loss %		firmness rate kg/ cm^2		T.S.S %		Starch concent.		Acidity %	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
0 day	0.00f	0.00g	4.47a	3.63a	14.25a	15.25a	5.18a	5.21a	0.23a	0.24a
30 day	6.05e	2.26f	2.44d	2.75d	13.87b	14.88b	5.00a	5.06a	0.22a	0.22b
60 day	7.16d	2.71e	3.57a	2.89c	13.68c	14.69bc	3.68b	3.75b	0.21b	0.21c
90 day	8.06c	3.12d	2.64c	2.81c	13.56bc	14.56cd	3.25c	3.43b	0.19c	0.19c
120 day	8.38d	3.61c	3.00b	2.74d	13.34cd	14.34de	3.25c	2.93c	0.16d	0.16e
150 day	9.23a	4.23b	273c	3.11b	13.09de	14.09ef	3.06d	2.37d	0.12e	0.11f
180 day	9.03a	4.77a	2.44d	2.63e	12.78e	13.72f	1.68e	1.68e	0.11f	0.10g
F test	**	**	**	**	**	**	**	**	**	**

Mean values within a column followed by different letter are significantly different at P≤0.5 level.

riods. The highest and the lowest acidity were recorded in cvs. Starking Delicious and Golden Delicious, respectively.

Effect of cold-storage periods

Mean values of fruit weight loss percent of four apple cultivars in both seasons are presented in Table (2). Analysis of variance reveals no significant differences between mean values of studied cultivars. Mean values enhanced with the increasing of storage periods. Several observations revealed that relationship between apple fruit weight losses stored under cold conditions and checked. This could be attributed to increase in transpiration, respiration rates and decline in fruit firmness (Blampired, 1981). Similar results were noted by El-Shennawi (1989) on Anna cultivar and Gavalheiro et al. (2003) on Bravo de Esmolfe and Erturk (2003) on Jonagold, Elesta and Granny Smith cultivars. It can be also noted that, mean values of fruit firmness rate were decreased gradually with extending storage period. The highest values were recorded at beginning and the lowest at the end of storage periods. These results support several previous works (Gavalheiro et al., 2003; Erturk et al., 2003; Buchanan and Bork, 2005; Kvikliene et al., 2006)

Concerning TSS% data in Table (2) indicated that, mean values decreased with extending storage period. At the beginning of storage, the highest values were produced, whereas the lowest mean values were at the end. These results may be due to respiration process (Rhodes, 1970) and conversion of starch into monosaccharides (Duque et al., 1999). Such results are in harmony with those obtained by Kvikliene et al. (2006). For starch concentration data indicated that, there were significant differences between cultivars. Mean values were reduced with the increasing in fruit ripening and storage period in both seasons. This may be due to starch conversion to simple sugars (El-Shennawi, 1989). In addition, acidity values were decreased in four tested apple cultivars with the increasing in storage periods. This may be due to fruit ripening and respiration processes (Phillps et al, 1954; Knee and Sharples, 1981; El-Shennawi, 1989; Gavalhero et al., 2003; Kvikliene, 2006).

Effect of apple cultivars and storage periods interactions

Data in Table (3) indicated that all studied parameters

Table 3. Effect of apple cultivars and storage periods interactions on some physical and chemical characters of fruits stored under cold storage conditions

Varieties	Storage periods	Weight loss %		Firmness rate kg/ cm^2		T.S.S %		Starch concent.		Acidity %	
		2005	2006	2005	2006	2005	2005	2005	2006	2005	2006
Golden Delicious	0 day	0.00g	0.00g	3.81bc	5.35d-f	13.62	14.62	5.50	5.50	0.28a	0.28
	30 day	6.03mn	1.88p	1.97n	2.75h-k	13.37	14.38	4.75	5.00	0.22cd	0.21d-f
	60 day	6.86kl	2.57m-o	3.11fg	2.63i-k	13.00	14.00	3.75	6.25	0.19fg	0.18h
	90 day	7.97gh	2.66k-m	2.23k-n	2.30lm	13.50	14.50	3.25	3.75	0.16i	0.15i
	120 day	8.56f	3.48g-i	2.47jk	2.14m	13.12	14.12	3.00	3.25	0.12j	0.12jk
	150 day	9.32e	4.37de	2.07l-n	2.08m	12.50	13.50	2.50	3.00	0.08k	0.07l
	180 day	9.50de	4.86c	1.93n	2.12m	12.37	13.38	1.75	2.50	0.05l	0.04m
Starking Delicious	0 day	0.00g	0.00g	3.17e-g	3.89a	14.50	15.50	5.25	1.75	0.21d-f	0.22de
	30 day	5.57po	1.98op	2.82h	3.61b-d	13.87	14.88	5.00	5.25	0.20e-g	0.20f-h
	60 day	6.27m	2.50l-n	3.93ab	3.27ef	14.00	15.00	3.50	5.00	0.24bc	0.23cd
	90 day	7.23j	2.54l-n	3.09fg	2.92h	13.5	14.50	3.25	3.50	0.20d-g	0.20e-h
	120 day	7.12jk	2.94j-l	3.21ef	2.77h-k	13.37	14.38	2.75	3.75	0.16i	0.16i
	150 day	8.29fg	3.39h-j	3.28ef	3.23fg	13.00	14.00	2.75	2.75	0.15i	0.15i
	180 day	8.56f	3.95ef	2.84h	2.63ik	1275	13.75	2.00	2.00	0.16i	0.16i
Star Cremson	0 day	0.00g	0.00g	3.31ef	3.49c-f	14.25	15.25	4.75	4.75	0.20g-e	0.20e-h
	30 day	5.25p	1.69p	2.03mn	3.13m	13.62	14.62	5.00	5.00	0.21g-d	0.21e-g
	60 day	5.83no	2.09n-p	4.12a	3.62a-d	13.50	14.50	3.75	4.00	0.20g-e	0.20f-h
	90 day	6.33m	2.76k-m	2.74hi	3.52b-e	13.37	14.38	3.25	3.50	0.22c-e	0.21d-f
	120 day	6.76l	3.06i-k	3.73de	3.23fg	13.00	14.00	3.25	2.75	0.17hi	0.16i
	150 day	7.61i	3.51f-i	3.30ef	3.63a-c	13.37	14.38	2.25	2.25	0.13j	0.13j
	180 day	7.68hi	3.83f-h	3.14e-g	2.99gh	13.12	14.12	1.50	1.50	0.11j	0.10k
Gala	0 day	0.00g	0.00g	3.61cd	3.79ab	14.62	15.62	5.25	5.25	0.22cd	0.24bc
	30 day	7.35ij	3.47g-i	2.93gh	2.52j-l	14.62	15.62	5.25	5.25	0.26b	0.25b
	60 day	9.67d	3.87fg	3.12fg	2.03m	14.25	15.25	3.75	3.75	0.21d-f	0.21d-f
	90 day	10.72c	4.53b	2.50ij	2.50kl	13.87	14.88	3.25	3.25	0.16gh	0.19h
	120 day	11.08b	4.96cd	2.93gh	2.80hi	13.87	14.88	3.25	2.00	0.19f-h	0.19g-h
	150 day	10.57c	5.63b	2.30j-l	3.51c-e	13.50	14.50	2.00	3.25	0.11j	0.11jk
	180 day	11.69a	6.43a	2.84n	2.78h-i	12.87	13.62	1.50	1.50	0.11j	0.10k
F test		**	**	**	**	NS	NS	NS	NS	**	**

Mean values within a column followed by different letter are significantly different at P≤0.5 level.

Table 4. Means of cuticle thickness of fruit of four apple cultivars under cold storage conditions.

Cultivars	Means of cuticle thickness (µm)
Gala	20. 6a
Starking Delicious	13.8b
Star Cremson	12.3c
Golden Delicious	14.3b

Mean values within a column followed by different letter are significantly different at P≤0.5 level.

were reduced in four cultivars and all storage periods during the both seasons. Analysis of variance shows significant differences between mean values of fruit weight loss percent and fruit firmness rate values. For fruit weight loss percent, cv. Gala gave the highest values at the end of the storage period (180 days). On the other hand, cvs. Gala and Golden Delicious caused the lowest fruit firmness rate. No significant differences were observed in TSS% and starch concentration in both seasons. For acidity percent, significant differences were recorded in both seasons. Golden Delicious gave the highest and the lowest values at the beginning and at the end of cold storage, respectively

Regarding anatomical structure of the surface of apple fruits after 90 days cold-storage, Figure (1) and Table (4) illustrate that cvs. Gala and Star Cremson have smooth fruit surface while fruit surfaces in cvs. Starking Delicious and Golden Delicious were undulate and rippled, respectively. Fruit surface shaped related to weight losses per-

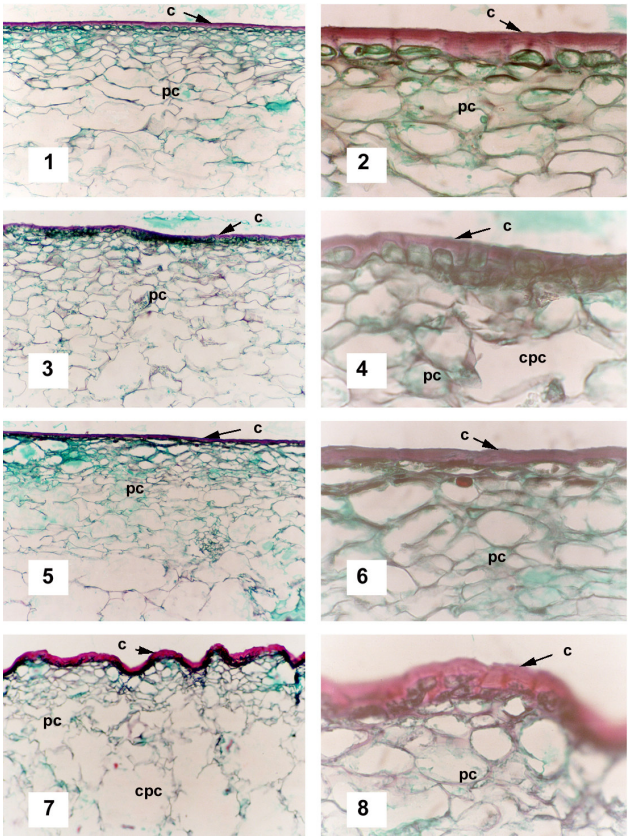

Figure 1 Transfer-sections in surface parts of apple cultivars fruits. 1-2: Gala 3-4: Starking Delicious, 5-6: Star Cremson, 7-8: Golden Delicious. Cuticle (c), parenchyma cells (pc), crushed parenchyma cells. X 100 (1,3,5 and 7), X 400 (2, 4, 6 and 8).

centage. Thickness of cuticle ranged from 12.3 µm in Star Cremson to 20.6 µm in Gala. No significant differences were found between cvs. Starking Delicious and Star Cremson. Crushed parenchymatous cells were found in storage parenchyma tissue in Starking Delicious and Golden Delicious fruits only. The thickness of cuticle and surface wax characteristics are related to weight loss percentages in cold-stored apple (Bebic, 1972; Ruffa et al., 1992). To the authors knowledge it appear that such anatomical study has not been previously investigated. Therefore, this study may be considered the first report on anatomical character of the tested cultivars and a key step for further studies on these cultivars and their susceptibility to post-harvest diseases under similar conditions.

From the foregoing results, it may be concluded that, all studied parameters of four apple cultivars were reduced during cold-storage periods. Higher fruit storability was achieved in cv. Star Cremson.

From the foregoing results, it may be concluded that, all studied parameters of four apple cultivars were reduced during cold-storage periods. Star Cremson cv. achieved a high fruits storability.

REFERENCES

AOAC (1981). Official Method of Analysis Agricultural Chemists11 th Ed. Published by the A. O. A. C., P. O. Box : 540, Washington.

Babos K, Sass P, Mohacsy P (1984). Relationship between the peel structure and storability of apple. Acta Agronomica Academiae Scientiarum-Hungaricae, 33: 41-50.

Bandaravicius A, Gelvonauskiene D, Sasnauskas A (2001). Investigation of biologically and economically important traits of Introduced apple cultivars. Sodininkyste ir Darzininkyste. 20(1): 3 –15.

Baneh HD, Hassani A, Majidi A, Zomorodi S, Hassani G, Malakooti MJ (2003). Effect of calcium chloride concentration and the number of foliar application on the texture and storge quality of apple (Red Delicious) in Orumia Region. Agricult. Sci. Tabriz. 12(4): 47– 54.

Bebic D (1972). Uticaj osobina pokozice ploda jabuke na gubitke u tezini za vreme skladistenja. Zbornik Radova Poljoprivredong Fakulteta, 20(546): 1-8.

Ben-Arie R, Lurie S (1986). Prolongation of fruit life after harvest. In: Monselise SP (ed) Handbook of fruit set and development. CRC, Boca Raton, Fla, p 493.

Blampired GD (1981). A relationship between water loss and storage breakdown of "Mcintosh" apple .Hortscience 16 (4):525- 526.

Buchanan R, Brock B (2005).Optimum harvst maturity and cold storge potential of new ARC Infruitec Nietvoorbij plum varieties Sunbreeze, Lady west and 4A-35-14-SA Fruit. J. 4(5):19-21.

Duque P, Barreiro MG, Arrabaca JD (1999). Respiratory metabolism dipping cold storage of apple fruit. I-sucrose metabolism and glycollysis. physiolgia plantarum .107(1):14 – 23.

EL-Shennawi MRM (1989). Effect of post harvest calcium chloride fruit dipping on quality and storability of "Anna" apple during cold storage. M. Sc. Thesis Alexandria university.

Erturk U, Akbuclak B, Ozer MH (2003). Quality changes of some apple cultivars stored in normal atmosphere for long Period . Acta Horticulture . 599 : 665 – 672 .

Gavalheiro OJ, Santos A, Recasens I, Larrigancliere C, Silvestre A (2003). Quality of the portuguese "Bravo de Esmolfe" apple after normal cold storage or controlld atmosphere and two shelf life periods. Acta Horticultwe. 1: 395 – 400.

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research. 2nd ed. John Wiley and Sons, pp. 229-308.

Guleryuz M, Ercisli S, Erkan E (2000). Physical and chemical changes during fruit development in apple cultivars in Erzincan plain and their interrelationships. Ziraat Fakultesi Dergisi, Ataturk Universitesi. 32(1) : 51 – 59.

Hamedi M, Milani JMZ (2003). Susceptibility of five apple cultivars to browning.Iranian J. Agric.l Sci. 34(1): 91- 96.

Homutova I (2005). Zmêny anatomicke stovby exokarpu plodu u vybranych odrud jabloni. In: ZIMA M., BOLRCEK P., OMELKA R. (eds.) 4. biologicke-progres v bilogii, 8. -9.9. Nitra, UKF: 214-215.

Homutova I, Blazek J (2006). Differences in fruit skin thickness between selected apple (*Malus domestica* Borkh.) cultivars assessed by histological and sensory methods. Hort. Sci. (Prague), 33 (3): 108-113.

Hulme AC, Rhodes G (1971). The biochemistry of fruits and their products.Vol. 2 Academic press. London.

Knee M, Sharples R (1981). The infiuence of controlled atmosphere storage on the ripening of apples in relation to quality p:341-352. In P.W. Goodenough and P.K.Atkins (eds) quality in stored and processed vegetables and fruit. A Cademic Press London and Newyork.

Kvikliene N, Kviklys D, Viskelis P (2006). Changes in fruit quality during ripening and storage in the apple cultivar. auksis. J. of fruit and ornamental. plant. Research. 141: 195 – 202. Kvikliene, N; D. Kviklys and P. Viskelis (2006). Changes in fruit quality during ripening and storage in the apple cultivar. auksis. J. Fruit ornamental Plant. Res., 141: 195 – 202.

Macheix JJ, Fleuriet A, Billot J (1990). Regulation of phenolic metabolism in fruit temperature. In: Fruit phenolics. CRC, Boca Raton, Fla, p 199.

Mourichon Z, Bompeix G (1979). Mise en evidence des voies de penetration du Phytophthora cactorum (L. et C.)Schroeter dans les pommes Golden Delicious. Etude realisee en microscopie electronique a balayage. Fruits, 34: 761-766.

Naseri L, Babalar M, Askary MA, Talaei AR (2001). Effects of foliar sprays of some micronutrients and cold storage on fruit quality of Golden Delicious apple. Iranian J. Agricult. Sci., 32(3): 591-597.

Phillips WR, Poapst PA, Rheaume BJ (1954). The Effect of temperature near 32 degree F. on storage behavior of "Mcintosh" apple. Amer. Soc. Hort. Sci. 65: 214 – 222.

Pierzynowska-kornika G, Zadernowski R, Fornal J (2002). The microstructure of selected apple varieties. J. Polish Agricult. Universities, Food Sci. Technol., 5(2) Available Online: Http://www.ejpau.media.pl/series/volume5/issue2/food/art-13.html.

Rangana S (1979). Manual of analysis of fruits and vegetables products. Mc – Graw Hill Pub. Company.

Rhodes G (1970). The climacteric and ripening of fruit.P.521-533.In A.C. Hulem (ed) The biochemistry of fruit and their products.Vol.1.A Cadmic press, London and New York.

Ruffa E, Me G, Dore B (1992). Histochemical study of some apple cultivars pericarp application of the crystabilization technique in ethylene glycol (SO_3). Histochem. J., 24: 549-550.

Ruzin SE (1999). Plant microtechnques and microscopy. First Ed. Oxfod University press, USA.

Saquet AA, Streif J (2000). Investigations on the respiration and the ethylene production of some new apple cultivars. Erwerbsobstbau. 42(4): 109 – 112.

Sasnaukas A, Gelvonauskiene D, Duchovskis P, Siksnianien JD, Sabajeviene G (2006). Investigation of introduced biological traits of apple tree cultivars. Sodininkyste ir Darzininkyste. 25(1): 3 – 12.

Song K, Hwang J, Ahn SY (2003). Seasonal changes of enzyme activity related to sugar metabolism in leaves of apple cultivars differing in maturty. J. Korean Society for Horticult. Sci. 44(2): 211 – 214. Resources. 6 (4): 67 – 77 .

Tavares JT, Silva CL, Cardoso RL Silva MA, Carvalho LA, Santos CMG (2002). Postharvest application of calcium chloride in fruits of the mountain apple (Eugenia malaccensis L). Magistra. Vol. 14 (2): article l.

Teskey BJ, Shoemaker JS (1978). Tree fruit production AVI: publishing. Co. Inc. Westport Connecticut.

Ugolik M, Zydlik Z, Kantorowicz MB Pacholak E (2001). Yield and fruit quality of selected apple cultivars. Prace z Zakresu Nauk Rolniczych. 91: 181–186.

Ventura M, Sansavini S (2005). Typology of fruiting branches and quality of apple cultivars Braeburn, Golden Delicious and Jonagold. Rivista di Frutticoltura e di Ortofloricoltura. Vol.67(11):52 - 60.

Veravrbeke EA, Verboven P, Oostveldt P, Nicolai BM (2003). Predication of moisture loss across the cuticle of apple (*Malus sylvestris* supsp. *Mitis* (Wallr.)) during storage: part 2. Model simulations and practical applications. Postharvest Biol. Technol. 30: 89-97.

Zheng WW, Zhai H, Yuehua H, Zhang J, Wang X (2005). Analysis of the changes of calcium content of several apple cultivars in developing season. Scientia Agricultura Sinica. 38(11): 2296 - 2300.