Phytochemistry, Vol. 26, No. 9, pp. 2481–2484, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

CHANGES IN CHEMICAL COMPONENTS OF RIPENING OLEASTER FRUITS

FUKIKO SAKAMURA and TAKAYUKI SUGA*

Department of Food, Oshimo Women's College, Gion, Asaminami-ku, Hiroshima, 731-01, Japan; *Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima, 730, Japan

(*Revised received* 28 *January* 1987)

Key Word Index—*Elaeagnus multiflora*; *E. umbellata*; Elaeagnaceae; oleaster; olive; fruits; ripening; L-ascorbic acid; dehydroascorbic acid; polyphenols; associated tannins; sugars.

Abstract—Changes in the chemical components of the ripening fruits of *Elaeagnus multiflora* var. *gingantea* and *E. umbellata*, were examined. In the pulp of the fruits, the sugars present, glucose and fructose, increased remarkably with a concomittant decrease in the titrable acidity. Polyphenols, predominantly composed of associated tannins, markedly diminished. In the stone, the contents of the sugars and polyphenols remained constant. L-Ascorbic and dehydroascorbic acids in the pulp diminished. The fall in L-ascorbic acid is unusual and is paralleled by a fall in the tannin materials.

INTRODUCTION

The ripe fruits of oleasters (olives) such as *Elaeagnus multiflora* var. *gingantea* (Japanese name: Daio-gumi) and *E. umbellata* (Aki-gumi) taste better than those of other species of the Elaeagnus genus. The unripe fruits of the above oleasters are used as pickles and for the production of alcoholic beverages in Japan. The plants belonging to this genus are known to contain mono- and disaccharides in the leaves [1], fructose and L-ascorbic acid in the fruits [2], fatty acids and phytosterols in the seed [3] and the leaves and stems [4] and carotenoids in the fruits [5, 6]. Marked improvement in taste, especially in sweetness and astringency, occurs during ripening of the fruits. However, the changes in the chemical components responsible for improvement in taste of the ripening fruits have not been studied. The present paper describes changes in such components as sugars, organic acids, polyphenols and L-ascorbic and dehydroascorbic acids in the ripening fruits of *E. multiflora* var. *gingantea* and *E. umbellata*.

RESULTS AND DISCUSSION

Five stages in the ripening fruits of *E. multiflora* Thunb. var. *gingantea* Araki and *E. umbellata* Thunb. were defined on the basis of the surface colour of the fruit (see Experimental).

The fresh weight of the pulp per fruit in both species of oleasters increased with ripening of the fruits, but the fresh weight of the stone per fruit remained constant during ripening (Fig. 1). The ratio of the fresh weight of the pulp to that of the stone increased with ripening, and the ratio at the final stage of ripening of *E. multiflora* and *E. umbellata* was 1.7 and 3.6 times the ratio at the initial stage, respectively.

The sugars present in the pulp and stone of the fruits of both oleasters at the various stages of ripening were found to be glucose, fructose and sucrose (TLC analysis). The first two were predominant in the pulp, and the ratio of glucose to that of fructose was about unity during ripening. The glucose and fructose gradually increased during ripening, especially at the later stage (Fig. 2). The content of both glucose and fructose at the final stage of ripening were 2.4 (9.3%) and 1.7 (11.4%) times higher than those at the initial stage of ripening. The sugars present in the stone of *E. multiflora* were also found to be glucose and fructose (Fig. 3). In contrast to the stone of *E. multiflora*, sucrose in the stone of *E. umbellata* predominated over glucose and fructose. Besides these three sugars, the presence of a trace amount of anthrone and naphthoresorcinol negative compounds, probably oligo-

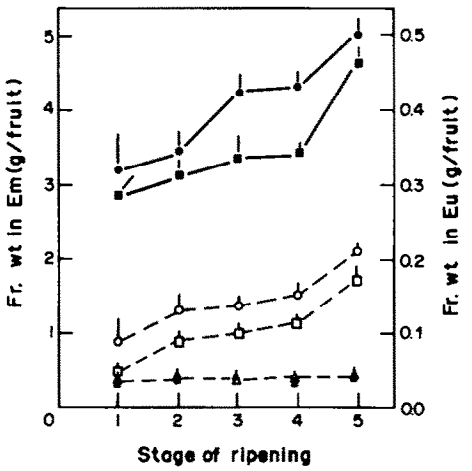

Fig. 1. Changes in the fresh weight of the ripening fruits of *E. multiflora* var. *gingantea* (*Em*) and *E. umbellata* (*Eu*). ● — ● : whole fruit of *Em*; ■ — ■ : pulp of *Em*; ▲ — ▲ : stone of *Em*, ○ --- ○ : whole fruit of *Eu*; □ --- □ : pulp of *Eu*; △ --- △ : stone of *Eu*.

* Author to whom correspondence should be addressed.

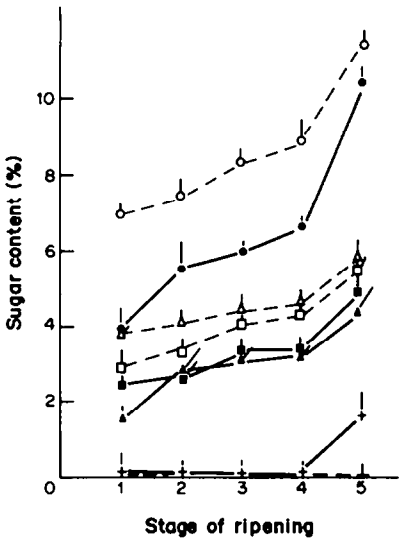

Fig. 2. Changes in the sugar content in the ripening fruit pulp of *E. multiflora* var. *gingantea* (*Em*) and *E. umbellata* (*Eu*). ●—●: total sugar content in *Em*; ■—■: glucose content in *Em*; ▲—▲: fructose content in *Em*; +—+: sucrose content in *Em*, O --- O: total sugar content in *Eu*; □--□: glucose content in *Eu*; △--△: fructose content in *Eu*; +—+: sucrose content in *Eu*.

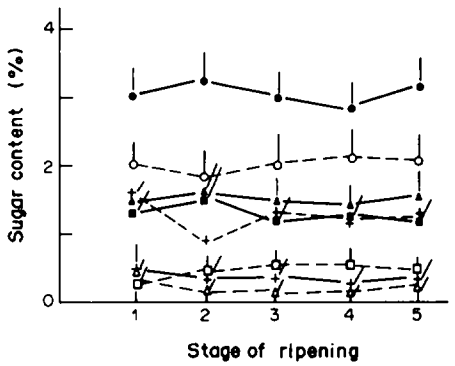

Fig. 3. Changes in the sugar content in the ripening fruit stone of *E. multiflora* var. *gingantea* (*Em*) and *E. umbellata*(*Eu*). ●—●: total sugar content in *Em*; ■—■: glucose content in *Em*; ▲—▲: fructose content in *Em*; +—+: sucrose content in *Em*, O---O: total sugar content in *Eu*; □---□: glucose content in *Eu*; △---△: fructose content in *Eu*; +---+: sucrose content in *Eu*.

saccharides, were found in the stone of both oleasters. However, identification of these oligosaccharides was unsuccessful.

The content of organic acids increased to a maximum at the early stage of ripening of the pulp of both oleasters and then the acid content was gradually reduced to *ca* 80% of the maximum content at the final stage (Fig. 4). The ratio between the content of sugars and that of organic acids increased to 5.6 from 1.8 and to 6.3 from 3.7 in the ripening fruit pulp of *E. multiflora* and *E. umbellata*, respectively. Here, the content of organic acids was calculated as that of citric acid.

Polyphenols in the pulp and stone of both ripening oleaster fruits were found to be mainly composed of associated tannins (proanthocyanines) using the method of ref. [7]. The highest content of polyphenols in the pulp of the fruits was observed in the youngest fruits of both oleasters (Fig. 5). The polyphenol content decreased remarkably with ripening of the fruits, being reduced to two-fifths and one-fifth in the fruits of *E. multiflora* and *E. umbellata* at the final stage, respectively. The fall in the content of the polyphenols was paralleled by a fall in the content of the associated tannins. On the other hand, the content of the polyphenols in the stone remained almost constant during ripening of the fruits of *E. multiflora* and *E. umbellata*, though a slight reduction was observed at the early stage of the ripening for the latter plant, as shown in Fig. 6. The content of the associated tannins in the stones remained constant during ripening for both the plants (Fig. 6).

L-Ascorbic acid and dehydroascorbic acid in the pulp of

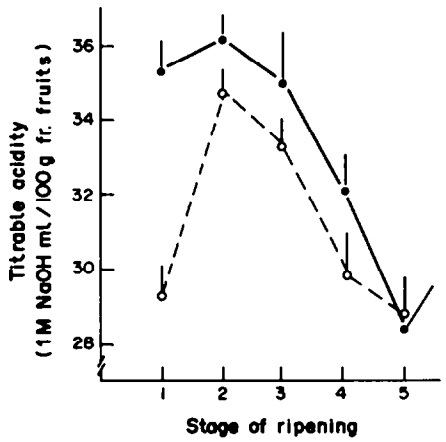

Fig. 4. Changes in the organic acid content in the ripening fruit pulp of *E. multiflora* var. *gingantea* (*Em*) and *E. umbellata* (*Eu*). ●—●: acid content in *Em*, O---O: acid content in *Eu*.

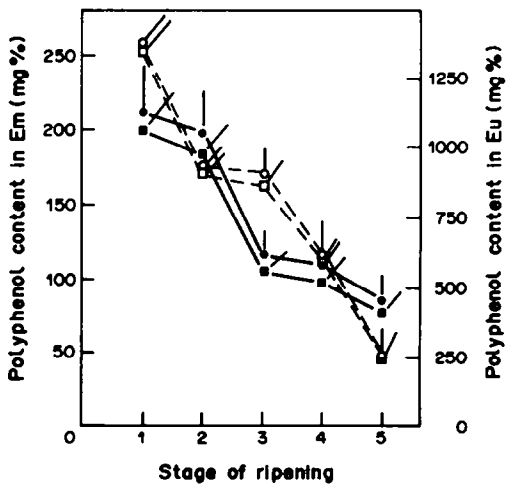

Fig. 5. Changes in the polyphenol content in the ripening fruit pulp of *E. multiflora* var. *gingantea* (*Em*) and *E. umbellata* (*Eu*). ●—●: total polyphenol content in *Em*; ■—■: associated tannin content in *Em*, O---O: total polyphenol content in *Eu*; □---□: associated tannin content in *Eu*.

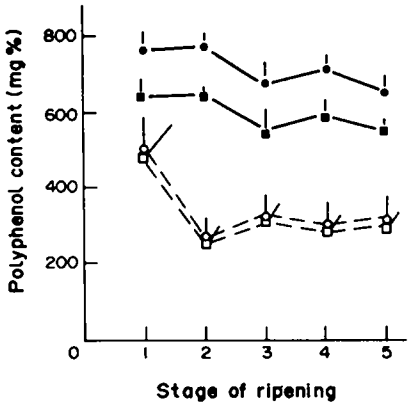

Fig. 6. Changes in the polyphenol content in the ripening fruit stone of *E. multiflora* var. *gingantea* (*Em*) and *E. umbellata* (*Eu*). ● — ● : total polyphenol content in *Em*; ■ — ■ : associated tannin content in *Em*, O—O : total polyphenol content in *Eu*; □- - - □ : associated tannin content in *Em*.

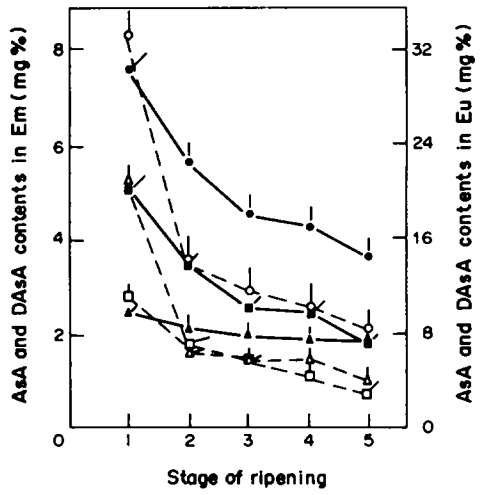

Fig. 7. Changes in L-ascorbic acid (AsA) and dehydroascorbic acid (DAsA) contents in the ripening fruit pulp of *E. multiflora* var. *gingantea* (*Em*) and *E. umbellata* (*Eu*). ●—● : total AsA and DAsA content in *Em*; ■—■ : AsA content in *Em*; ▲—▲ : DAsA content in *Em*, O—O : total AsA and DAsA content in *Eu*; □—□ : AsA content in *Eu*; △—△ : DasA content in *Eu*.

the oleaster fruits was identified by comparison with authentic samples on TLC [8, 9]. The determination of L-ascorbic acid and dehydroascorbic acid by the 2,4-dinitrophenylhydrazine method is reported to be applicable to most fresh vegetables and fruits [10]. In addition, the values of L-ascorbic acid content obtained by this method was confirmed to be in accordance with the values obtained by the 2,6-dichlorophenolindophenol method [11] to within *ca* 6.3% for the same sample of the oleaster fruits. In view of this result the 2,4-dinitrophenylhydrazine method was used for the determination of both L-ascrobic acid and dehydroascorbic acid in the fruits. The highest contents of L-ascorbic acid and dehydroascorbic acid were observed in the pulp of the youngest fruits of *E. multiflora* var. *gingantea*, after which they gradually decreased to one-third (1.79 mg%) and three-quarters (1.86 mg%) with ripening, respectively (Fig. 7). The L-ascorbic acid and dehydroascorbic acid contents were also highest in the pulp of the youngest fruit of *E. umbellata*, after which they decreased remarkably at the early stage of ripening and then gradually diminished to one-third (4.03 mg%) and one-fifth (4.16 mg%), respectively. Such a decrease in the content of L-ascorbic acid is unusual, because an increase in the content of L-ascorbic acid with ripening of the fruits is usual for fruits of other wild plants, such as *Rubus Sieboldi* and *R. parvifolius* [12], *Ribes nigrum* [13] and *Rubus chamaemorus* [14] and for those of cultivated plants, such as peaches [15], pears [16] and papaya [17].

The distinct increase in sweetness of the pulp of the oleaster fruits at the final stage of ripening resulted from the remarkable increase in its content of glucose and fructose, in addition to the decrease in its acidity. The distinct decrease in astringency of the pulp resulted from the reduction in the content of its polyphenols (composed predominantly of associated tannins). The level of the sugar and polyphenols in the stone remained constant during ripening of the fruits. This seems to indicate that ripening of the pulp takes place after ripening of the stone. The content of L-ascorbic acid and dehydroascorbic acid in the pulp decreased as the taste of these fruits became better. The fall in the L-ascorbic acid content is unusual and is paralleled by the fall in the polyphenols.

EXPERIMENTAL

Plant material. The fruits of *Elaeagnus multiflora* Thunb. var. *gingantea* Araki and *E. umbellata* Thunb., were collected in the suburbs of Hiroshima city and in Miyajima Natural Botanical Garden, Hiroshima University, in June and October, respectively. On the basis of the visual assessment of their colour, the fruits of the oleasters were classified into five stages of ripening, which were defined by values of L (lightness) and a_L and b_L (chromaticness) in the ULCS (Hunter) system [18, 18a, 18b], using a photoelectric reflectometer (Tokyo Denshoku, Model TR-1000) as follows: for *E. multiflora*, stage 1, greenish yellow (L 34.5, a_L −0.2, b_L 9.6); stage 2, yellow (L 32.8, a_L −9.8, b_L 12.5); stage 3, yellowish orange (L 28.8, a_L 6.0, b_L 9.1); stage 4, orange or weak reddish orange (L 25.3, a_L 9.8, b_L 5.9); stage 5, strong reddish orange or red (L 24.1, a_L 14.8, b_L 3.9). For the fruits of *E. umbellata* at the various stages of ripening, the visual assessment and the above-described values were as follows: stage 1, greenish yellow (L 41.4, a_L −2.4, b_L 12.7); stage 2, yellow (L 42.1, a_L −6.0, b_L 13.9); stage 3, moderate orange (L 40.5, a_L 6.3, b_L 11.0); stage 4, reddish orange (L 38.5, a_L 13.6, b_L 8.0); stage 5, red (L 35.1, a_L 17.6, b_L 3.5).

Triplicate batches of the fruits (10–30 fruits) of each oleaster were analysed for sugars, organic acids, polyphenols and L-ascorbic and dehydroascorbic acids. The data obtained were statistically treated and are shown as the mean ± s.d., which is indicated by the vertical or oblique line in Figs 1–7.

Sugars. The fruits were extracted by refluxing with 80% EtOH for 5 min. The EtOH soln obtained was immediately cooled and centrifuged at 3000 rpm for 10 min to remove the residual fruit material, which was re-extracted with 80% EtOH by grinding with a small amount of sea sand, followed by centrifugation of the soln. The combined supernatant was concd *in vacuo* below 40° to a small vol. and subjected to the analyses described below. TLC { (silica gel 60 (70–230 mesh, Merck) and silica-gel impregnated with 0.03 M H_3BO_3, EtOH–EtOAc–H_2O (7: 30: 3) [19] and *n*-

BuOH–Me_2CO–H_2O (4: 5: 1) [20]. Spots visualized by spraying with an anthrone reagent, a naphthoresorcinol reagent [21] and a H_2SO_4–HNO_3 (19: 1) mixture} of the 80% EtOH extract showed the presence of glucose, fructose and sucrose in the pulp and the stone. Determination of aldohexoses, reducing sugars and total soluble sugars were performed by the *o*-aminodiphenyl acetic acid method [22], method of ref. [23] and the PhOH–H_2SO_4 method [24], respectively. The contents of glucose, fructose and sucrose were calculated using the following equations: glucose % = aldohexoses %; fructose % = reducing sugars % − aldohexoses %; sucrose % = total soluble sugars % − reducing sugars %.

Organic acids. The pulp was ground with H_2O in the presence of a small amount of sea sand. The aq. soln obtained was centrifuged at 3000 rpm for 10 min and the supernatant was titrated with 0.02 M NaOH. The content of organic acids in the pulp is shown in Fig. 4 as the titrable acidity, that is, ml of 1 M NaOH/100 g fresh pulps.

Polyphenols. The fruits were extracted by the same method as that used to extract the soluble sugars. Polyphenols were determined by Folin–Denis' method [25]. Associated tannins were determined by the method reported in ref. [7] as described below. Following Löwenthal's method, gelatin was added to the 80% EtOH soln to give a precipitate. After removal of the precipitate, the EtOH soln was subjected to determination of polyphenols by Folin–Denis' method. The polyphenol contents obtained were multiplied by 1.7 to give the amount of non-tannin material [7]. The associated tannin content was calculated as the difference in the content between the polyphenols and the non-tannin materials.

L-Ascorbic acid and dehydroascorbic acid. The material was ground with 5% metaphosphoric acid in the presence of a small amount of sea sand and centrifuged at 3000 rpm for 10 min. The supernatant, after addition of 80% EtOH, was left at 2° for 2 days, followed by concentration to a small vol. under reduced pressure. The concentrate was subjected to TLC analysis (co-TLC) using *n*-BuOH–AcOH–H_2O (4: 1: 5) as solvent and 2,6-dichlorophenolindophenol soln as a colour-producing reagent [8].

For identification of dehydroascorbic acid, the material was ground with 8 vols 5% metaphosphoric acid in the presence of a small amount of sea sand, followed by addition of 11 vols of H_2O. The 2% metaphosphoric acid soln thus obtained was centrifuged at 3000 rpm for 10 min. Following the method reported in ref. [9], the 2,4-dinitrophenylhydrazone derivative obtained from the supernatant was extracted with EtOAc. The EtOAc extract, after concentration under reduced pressure, was subjected to TLC analysis (co-TLC) using toluene–Me_2CO–5% HOAc(2: 1: 1) as solvent.

Following the method reported in ref. [10], L-ascorbic acid and dehydroascorbic acid were determined by the 2,4-dinitrophenylhydrazine method, in which 2,6-dichlorophenolindophenol was used as the oxidant, for the above-described 2% metaphosphoric acid extract of the material. The reliability of the 2,4-dinitrophenylhydrazine method was defined by duplicate analysis of the 2% metaphosphoric acid extract of *E. umbellata* fruits using the 2,6-dichlorophenolindophenol method [11]. The levels of L-ascorbic acid detected by the 2,4-dinitrophenylhydrazine method were found to be slightly higher (3.10–11.5 vs 2.94–10.45 mg% at the 1st to 5th stage of ripening) than those detected by the 2,6-dichlorophenolindophenol method.

REFERENCES

1. Sitnikova, A. S. (1962) *Tr. Inst. Botan. Akad. Nauk Kaz. SSR.* **14**, 170.
2. Dovlatyan, A. L. (1977) *Izu. S-kh. Nauk* **20**, 37.
3. Tahara, T. and Sakuda, Y. (1977) *Kochi Joshi Daigaku Kiyo, Shizen Kagaku Hen* **25**, 11.
4. Tagahara, K., Kato, A., Hashimoto, Y. and Suzuta, Y. (1984) *Shoyakugaku Zasshi* **38**, 131.
5. Geiger-Vifian, A. and Muller, B. (1945) *Ber. Schweiz. Botan. Ges.* **55**, 320.
6. Hayashi, K. and Noguchi, T. (1953) *Misc. Repts. Res. Inst. Nat. Resources* **30**, 1.
7. Nakabayashi, T. (1968) *Nippon Shokuhin Kogyo Gakkaishi* **15**, 73.
8. Mapson, L. W. and Partridge, S. M. (1949) *Nature* **164**, 479.
9. Fujita, A., Hirose, F. and Uchida, Y. (1969) *Vitamins (Japan)* **40**, 17.
10. Shokuhin Kogyo Gakkai (1982) *Shokuhin Bunseki-ho*, p. 464. Korin, Tokyo.
11. Fujita, A. (1955) *Vitamin Teiryo-ho* p 543. Nankodo, Tokyo.
12. Sakamura, F. (1982) *Kaseigaku Zasshi* **33**, 14.
13. Buthkus, V., Baranauskaite, A., Butkiene, Z. and Peseckiene, A. (1965) *Lietuvos TSR Moskslu Akad. Darbai, Ser. C.* **1**, 31.
14. Nordnes, T. and Werenskiold, B. Q. (1952) *Food Res.* **17**, 117.
15. Naito, K. Ishimaru, K. and Daijo, M. (1942) *Rikagaku Kenkyusho Iho* **21**, 481.
16. Naito, K. Ishimaru, K. and Daijo, H. (1942) *Rikagaku Kenkyusho Iho* **21**, 641.
17. Selvaraj, Y. and Pal, D. K. (1982) *J. Food Sci. Technol.* **19**, 257.
18. Shikisai Kagaku Kyokai (1980) in *Handbook of Colour Science* p. 124. Nankodo, Tokyo.
18. (a) Hunter, R. S. (1948) *J. Opt. Am.* **38**, 1094.
18. (b) Hunter, R. S. (1958) *J. Opt. Am.* **48**, 985.
19. Sakamura, F. (1972) *Oshimo Joshi Tanki Daigaku Kenkyu-shuho* **9**, 49.
20. Prey, V., Berbalk, H. and Kausz, M. (1961) *Mikrochim. Acta.* 968.
21. Pastuska, G. (1961) *Z. Anal. Chem.* **179**, 427.
22. Timell, T. E. Glaudemans, C. P. J. and Currie, A. L. (1956) *Anal. Chem.* **28**, 1916.
23. Somogyi, M. (1952) *J. Biol. Chem.* **195**, 19.
24. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Anal. Chem.* **28**, 350.
25. Swain, T. and Hillis, W. E. (1959) *J. Sci. Food Agr.* **10**, 63.