JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Effect of Drying Conditions and Storage Period on Polyphenolic Content, Antioxidant Capacity, and Ascorbic Acid of Prunes

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In this study the main chemical parameters, ascorbic acid and polyphenol content, and antioxidant activity of two varieties of prunes, dried by high-temperature (85 + 70 °C) and low-temperature (60 °C) procedures, were monitored during storage. Ascorbic acid content was higher in the prunes dried at 60 °C but significantly decreased in both varieties during storage. The different classes of polyphenols analyzed (cinnamates, anthocyanins, flavonols) showed different stabilities during storage. Neochlorogenic acid decreased only in the President variety, whereas chlorogenic acid increased in both varieties; anthocyanins, present only in the President prunes, disappeared in the first months of storage, and the flavonol content fell significantly in both cultivars during the year of the study. Drying temperature significantly affected the polyphenol content, with different effects according to the class of polyphenols. Antioxidant activity showed a significant increase at the end of the storage period and in the President variety was higher in the sample dried at the higher temperature.

**KEYWORDS: Storage; antioxidant activity; phenols; prunes**

## INTRODUCTION

Studies conducted over the past 20 years on different foods, particularly fruits and vegetables, have shown how these products can promote an excellent state of health in humans, lower the risk of several chronic diseases such as heart disease, cancer, and diabetes, and even slow the aging process. These effects are mainly associated with biologically active components that are naturally present in the product, the most important of which are the phenolic compounds, the carotenoids, vitamins C and E, and fibers. Prunes have a prominent place among the many so-called "functional" foods, and the effect of these fruits on human health has been known for a long time (*1*). We now have a fairly complete understanding of the chemical composition of prunes and their biological effect on human health (*1*−*10*). Their action is attributable in particular to their high polyphenol content and antioxidant capacity (*2*−*8*), due mostly to chlorogenic and neochlorogenic acid (*3*, *9*) and the flavonoids (*5*, *10*). The phenolic compounds in prunes act by inhibiting oxidation of the low-density lipoprotein (LDL) in vitro. They play a very important role in the absorption and metabolism of glucose and counteract the effects of hypertension. They also lower the blood cholesterol level, and it appears that they have an inhibitory effect on the development of tumors by neutralizing free radicals (*1*, *3*), which form by oxidative stress. Few studies are available in the literature on the effect of drying on these compounds (*11*−*13*) or on the changes that occur during storage (*14*, *15*), particularly with regard to the cinnamate content (*16*). Research is scarce on the effects of processing and cooking on the cinnamates in foods (*16*). Recent studies are trying to evaluate whether any compounds that can improve the health properties of the food are formed during processing of the product. The objective of the present study was to investigate the effect of two different drying procedures and storage time on polyphenol content (cinnamates, flavonols, anthocyanins), ascorbic acid (AA), and the antioxidant capacity of two varieties of prunes.

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## MATERIALS AND METHODS

**Sampling.** The study was carried out on fruits of the President and Sugar varieties of prunes, bought in a local market at an optimum stage of ripening, pretreated and dried in ways described in a previous work (*13*). Two different drying procedures were used; the first one is the standard high temperature used to obtain prunes (SHT) (85 °C up to 50% humidity, then lowered to 70 °C until the end of the process), and the other one is a low-temperature procedure (LT) (60 °C). Air flow and humidity were kept the same in both processes. The dried fruits were packed and wrapped in a polypropylene pouch and stored at 20 °C. Relative humidity (RH) inside the packages was 50%.

**Physicochemical Analyses.** The following parameters were measured in the fruits immediately after drying and after 4, 8, and 12 months of storage, after homogenization of the flesh of 12 prunes: *pH*, by pH-meter (model 710/A, Orion); *titratable acidity* (grams of malic acid per 100 g of dry matter), by titration with an 0.1 N NaOH solution up to a pH of 8.3; *total soluble solids (TSS)* (in degrees Brix), by digital

10.1021/jf049889j CCC: $27.50 
Published on Web 07/02/2004

**Table 1.** Influence of Technology and Storage Period on Chemical Parameters, Ascorbic Acid Content, and Antioxidant Activity in President Prunes[a]

| source of variation | $a_w$ | acidity (g of malic acid 100 g of $dm^{-1}$) | ascorbic acid (mg g of $dm^{-1}$) | antioxidant activity ($-DO^{-3}$ min mg of $dm^{-1}$ $10^3$) |
|---|---|---|---|---|
| technology | | | | |
| SHT | 0.765a | 5.16a | 3.04b | 15.68a |
| LT | 0.748b | 5.25a | 6.34a | 7.22b |
| significance[b] | ** | ns | ** | ** |
| storage period | | | | |
| 0 months | 0.792a | 4.93c | 6.62a | 9.98bc |
| 4 months | 0.771ab | 5.01c | 4.60b | 7.55c |
| 8 months | 0.749b | 5.23b | 4.38b | 10.54b |
| 12 months | 0.715c | 5.66a | 3.17c | 17.73a |
| significance | ** | ** | ** | ** |

[a] Data followed by different letters within each column and source of variation differ significantly according to Duncan's multiple-range test at $P \leq 0.001$. [b] **, *, significant for $P \leq 0.001$ or 0.01, respectively; ns, not significant.

**Table 2.** Influence of Technology and Storage Period on Chemical Parameters, Ascorbic Acid Content, and Antioxidant Activity in Sugar Prunes[a]

| source of variation | $a_w$ | acidity (g of malic acid 100 g of $dm^{-1}$) | ascorbic acid (mg g of $dm^{-1}$) | antioxidant activity ($-DO^{-3}$ min mg of $dm^{-1}$ $10^3$) |
|---|---|---|---|---|
| technology | | | | |
| SHT | 0.696a | 5.57b | 4.18b | 10.75a |
| LT | 0.671b | 7.20a | 4.65a | 11.37a |
| significance[b] | ** | ** | * | ns |
| storage period | | | | |
| 0 months | 0.735a | 5.47c | 6.98a | 10.16b |
| 4 months | 0.689b | 6.15b | 3.48b | 8.40b |
| 8 months | 0.677b | 6.87a | 3.84b | 10.17b |
| 12 months | 0.634c | 7.06a | 3.35b | 15.53a |
| significance | ** | ** | ** | ** |

[a] Data followed by different letters within each column and source of variation differ significantly according to Duncan's multiple range test at $P \leq 0.001$. [b] **, *, significant for $P \leq 0.001$ or 0.01, respectively; ns, not significant.

refractometer (model PR-101, Atago); *dry matter (dm)* (%), using a vacuum oven for 12 h at 70 °C; *water activity* ($a_w$), determined by an electric hygrometer (Rotronic Aw-Win equipped with a Karl-Fast probe, PBI International, Switzerland), calibrated with solutions of known activity; *AA* (milligrams per gram of dry matter), by titration with a 2,6-dichlorophenolindophenol solution (*17*). All of the analyses were carried out in triplicate.

**Antioxidant Activity.** Antioxidant activity was evaluated using the radical DPPH according to the Brand-Williams method (*18*), as described in ref *13* and expressed as $-DO^{-3}$ $min^{-1}$ mg $dm^{-1}$, the value of which was obtained by the equation

$$\frac{1}{A^3} - \frac{1}{A_0^3} = -3kt$$

where $A_0$ is the initial optical density and $A$ is the optical density at rising time $t$. Prunes are extracted as reported in refs *19* and *20* with some modifications: 25 mL of distilled $H_2O$ was added to 1 g of cut and minced prunes; the sample was homogenized in an Ultra-Turrax (IKA-Labortechnik) for 30 s in ice and then centrifuged at 4000 rcf at 20 °C for 5 min. The supernatant was filtered through a 0.45 mm filter before the spectrophotometric reading.

**Phenolics Analysis.** The polyphenols were extracted in duplicate and analyzed in HPLC-DAD according to the methods described in refs *3* and *21*. A Hewlett-Packard series 1090 liquid chromatograph coupled with a diode array detector was used. Operating conditions were as reported in a previous work (*13*). The identified compounds, belonging to classes of cinnamates, flavonols, and anthocyanins, were measured as described in ref *13*, calibrated against the pure standards of the polyphenols examined. All values were expressed as milligrams per kilogram of dry matter and were obtained by the average of four measurements. The absorption value of the hydroxymethylfurfural (HMF) was also read, at a wavelength of 280 nm.

**Statistical Analysis.** The data of each variety were analyzed by a two-factor randomized complete block design (2 × 4), the two factors being the different temperatures used (85 and 60 °C) and the sampling period (0, 4, 8, and 12 months). When necessary, the means were separated by Duncan's multiple-range test at a significance level of $P \leq 0.001$ and 0.01. A linear correlation analysis was also performed for the two temperatures, between the values of antioxidant capacity and HMF content, at a significance level of $P \leq 0.001$ and 0.01.

## RESULTS AND DISCUSSION

**Chemical Changes during Storage. Tables 1** and **2** show the values of $a_w$, acidity, AA, and antioxidant capacity of the samples dried with the two procedures, besides the changes that occurred during storage in the President and Sugar varieties, respectively. In both cultivars a lower $a_w$ value was observed in the prunes LT dried as well as a significant decrease ($P \leq 0.001$) during storage, probably due to the fact that the prune water activity reached equilibrium with the relative humidity (RH) inside the packages. Acidity increased significantly during storage, with a higher value in the Sugar prunes LT dried than in those SHT processed, whereas no difference by drying temperature was found in the President variety. Ascorbic acid content was significantly affected in the two cultivars by both the drying temperature and the storage time. The plums SHT dried had a lower AA content than those LT dried. This confirms the negative effect of high temperatures and storage on AA (*22*, *23*).

**Changes in Antioxidant Activity during Storage.** Antioxidant capacity was higher at 12 months of storage in both cultivars, with a value of 1.5 times the initial value in Sugar prunes and twice the initial value in the President variety (**Tables 1** and **2**). On the other hand, the temperature had a significant effect only in the President cultivar. The increase observed between the values at 8 and 12 months of storage may be explained by the formation of new compounds with antioxidant activity, such as Maillard reaction products, which continue to

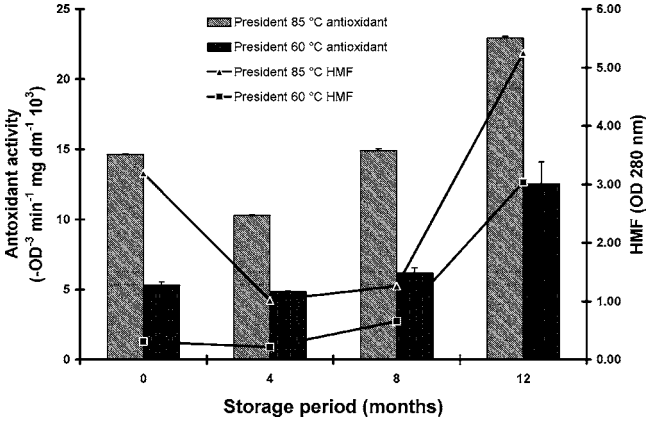


**Figure 1.** Changes in antioxidant activity and HMF optical density during storage of President prunes. Data are the mean of six determinations.

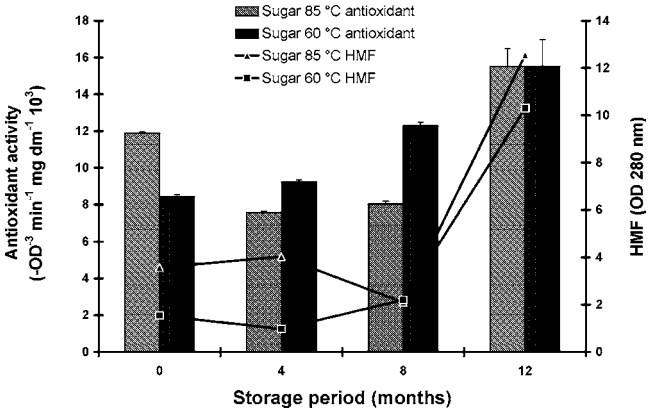


**Figure 2.** Changes in antioxidant activity and HMF optical density during storage of Sugar prunes. Data are the mean of six determinations.

**Table 3.** Correlation Coefficients between Antioxidant Activity and HMF Optical Density in Prunes

| cultivar | temperature | $r^b$ | cultivar | temperature | $r^b$ |
|---|---|---|---|---|---|
| President | SHT | 0.899** | Sugar | SHT | 0.853* |
| | LT | 0.933** | | LT | 0.878* |
| | SHT + LT | 0.898** | | SHT + LT | 0.816** |

[b] **, *, significant for $P \leq 0.001$ and 0.01, respectively.

be formed even over long storage times (*24*, *25*). It seems that the increased antioxidant activity of a food during storage can be attributed to the formation of brown compounds with a high molecular weight, which appear in the later stages of the Maillard reaction (*25*). The literature reports how even the decrease in ascorbic acid (as occurred in our case during storage) as well as the decrease in polyphenols (*24*, *25*) is correlated to the capacity of the acid to act as a reagent in the formation of Maillard reaction products. In our work, the formation of new products by Maillard reaction was confirmed also by the HMF content, an intermediary product of nonenzymatic browning. The optical density of this molecule (**Figures 1** and **2**) was markedly higher in the 12th month of storage, together with higher antioxidant capacity. These data are supported by the coefficients obtained by correlation analysis performed between the values of antioxidant capacity and HMF content, for both varieties of prunes and drying procedures (**Table 3**). The coefficients were highly significant ($P \leq 0.001$ for President and $P \leq 0.01$ for Sugar) and show how the rise in HMF content also increased the antioxidant activity of the food. They remained significant also when the two drying procedures (SHT and LT) were considered together and the two varieties separately.

**Variations in Phenolic Content during Storage. Tables 4** and **5** report the effects of drying procedure and storage time on the polyphenol content of the two varieties of prunes.

In both the President and Sugar cultivars higher amounts of neochlorogenic and chlorogenic acids were found in the fruits SHT dried than in those LT dried. In the LT sample the lower content is likely to be due to greater residual enzymatic activity of polyphenol oxidase (PPO), which is probably present in this sample owing to the lower drying temperature used (*11*). In fact, it is reported in the literature that PPO activity is high at 55 °C and lasts, although at a much lower level, up to temperatures of >75 °C (*11*). Neochlorogenic acid decreased significantly over the period of a year in the President variety, whereas chlorogenic acid increased in both cultivars in the 12th month of storage. It is known that PPO acts prevalently on neochlorogenic acid, which in fact showed greater degradation than chlorogenic acid. Because most neochlorogenic acid is present in the flesh, oxidation takes place mainly in this substrate (*11*). The greater stability and even the increase in chlorogenic acid at 12 months of storage could be linked to coupled oxidation reactions in which chlorogenic acid is involved in the degradation of the anthocyanins, with a reaction mechanism that leads to the partial regeneration of the acid (*12*, *26*). Coupled oxidation reactions, again with phenol regeneration, can appear in the presence of ascorbic acid, which reacts with the quinones formed during oxidation, giving rise to phenol regeneration, a reaction that continues until all of the acid present has been used up (*26*). A recent work reported an increase in the chlorogenic acid content during storage of a product at different temperatures (*23*).

With regard to the *p*-coumaric and caffeic acids, which in dried prunes are present in tiny amounts compared with the total polyphenols, probably as a result of hydrolysis of the cinnamates during processing of the plums into prunes, a highly significant difference can be seen according to the two drying procedures. In the President variety, higher amounts of both acids were present in the prunes SHT dried than in those LT dried, whereas the opposite occurred in the Sugar prunes. On the other hand, during storage a sharp fall in *p*-coumaric acid content was observed in the President fruits, whereas caffeic acid appeared only in the sample taken at 4 months, probably due to the greater degradation of the cinnamates in this sample, before it disappeared until the end of the trial. The fall in *p*-coumaric and caffeic acid content over a period of time is probably due to enzymatic oxidation. In the Sugar variety, the *p*-coumaric acid content remained fairly constant and caffeic acid appeared also in these prune samples at 4 months of storage and then disappeared in the 12th month. The anthocyanins, which were present only in the President variety, differed significantly according to the drying procedures: prunes dried at the lower temperature showed a higher anthocyanin value (cyanidin 3-rutinoside and cyanidin 3-rutinoside equivalent). Rapid degradation of the anthocyanins after subjection to high temperatures and high oxygen concentrations, as occurs during drying, has been described (*27*). It has also been reported that the phenolic acids are also involved in the degradation of the anthocyanins, with mechanisms of coupled oxidation (*12*). Furthermore, the anthocyanins, like the flavonols, are not direct substrates of PPO but are degraded by the mechanisms described above (*28*), which are able to produce also a partial regeneration of the acid involved. These compounds usually disappear more

**Table 4.** Influence of Technology and Storage Period on Phenolic Content (Expressed as Milligrams per Kilogram of Dry Matter) in President Prunes[a]

| source of variation | neochlorogenic acid | chlorogenic acid | *p*-coumaric acid | caffeic acid | cyanidin 3-rutinoside | rutin | cyanidin 3-rutinoside equivalent | rutin equivalent |
|---|---|---|---|---|---|---|---|---|
| technology | | | | | | | | |
| SHT | 3045.53a | 562.44a | 17.30a | 3.51a | 9.57b | 89.36a | 8.51b | 40.76a |
| LT | 2083.20b | 343.92b | 2.75b | 0.00b | 15.49a | 72.04b | 12.32a | 41.54a |
| significance[b] | * | * | * | * | * | * | * | ns |
| storage period | | | | | | | | |
| 0 months | 3452.26a | 468.35b | 18.10a | 0.00b | 33.99a | 99.42a | 26.21a | 59.16a |
| 4 months | 2441.82b | 412.76c | 5.69c | 7.02a | 16.14b | 100.75a | 15.44b | 50.55b |
| 8 months | 2198.54c | 394.67c | 7.78b | 0.00b | 0.00c | 78.89b | 0.00c | 40.57c |
| 12 months | 2164.84c | 536.94a | 8.54b | 0.00b | 0.00c | 43.74c | 0.00c | 14.32d |
| significance | * | * | * | * | * | * | * | * |

[a] Data followed by different letters within each column and source of variation differ significantly according to Duncan's multiple-range test at $P \leq 0.001$. [b] *, significant for $P \leq 0.001$; ns, not significant.

**Table 5.** Influence of Technology and Storage Period on Phenolic Content (Expressed as Milligrams per Kilogram of Dry Matter) in Sugar Prunes[a]

| source of variation | neochlorogenic acid | chlorogenic acid | *p*-coumaric acid | caffeic acid | cyanidin 3-rutinoside | rutin | cyanidin 3-rutinoside equivalent | rutin equivalent |
|---|---|---|---|---|---|---|---|---|
| technology | | | | | | | | |
| SHT | 1885.51a | 434.70a | 31.00b | 7.60b | 0.00a | 12.26a | 0.00a | 20.35b |
| LT | 1614.56b | 413.15b | 42.89a | 10.87a | 0.00a | 6.81b | 0.00a | 42.37a |
| significance[b] | * | * | * | * | ns | * | ns | * |
| storage period | | | | | | | | |
| 0 months | 1652.26b | 317.67d | 33.13bc | 0.00c | 0.00a | 9.81b | 0.00a | 36.36ab |
| 4 months | 2138.05a | 485.06b | 46.06a | 20.24a | 0.00a | 16.67a | 0.00a | 39.99a |
| 8 months | 1560.65b | 368.77c | 31.56c | 16.69b | 0.00a | 9.15b | 0.00a | 33.10b |
| 12 months | 1649.18b | 524.20a | 37.02b | 0.00c | 0.00a | 2.53c | 0.00a | 16.00c |
| significance | * | * | * | * | ns | * | ns | * |

[a] Data followed by different letters within each column and source of variation differ significantly according to Duncan's multiple-range test at $P \leq 0.001$. [b] *, significant for $P \leq 0.001$; ns, not significant.

rapidly as the temperature rises (*12*). The total disappearance of the anthocyanins occurred in the eighth month of storage. The flavonols are not direct substrates of PPO either, because the enzyme is unable to act directly on the glucosides (*12*); however, they are degraded by coupled oxidation reactions (*28*). Moreover, the behavior of the flavonols during storage is not very clear because flavonol content can increase, decrease, or remain unaffected by storage (*29*, *30*). This may depend on the class of flavonoids measured in the food (*29*). Rutin showed a significantly higher value in the sample SHT dried. The other flavonols, quantitatively determined as rutin equivalents, showed a significant difference between the two temperatures only in the Sugar variety. During storage, degradation of rutin was first seen in the third sampling (8 months), whereas the other flavonols showed significant variations at different times.

The Sugar variety had a higher polyphenol content in the fourth month of storage than at the beginning of the trial, but the same behavior was not found in the President fruits. At present, the reason for the variations is unknown but could be due to the variety. This needs to be investigated further.

Our work was aimed at contributing to an understanding of the changes that occur in the polyphenols, particularly the cinnamates, which are rarely studied, during storage of a food such as the prune that has such a high nutritional value and health-promoting properties. The results of our study show that during storage a decrease in polyphenol content occurs (apart from chlorogenic acid), although the antioxidant capacity of the food increases, probably due to the formation of Maillard reaction products. This is an aspect not often investigated and could be the starting point for further studies to identify new antioxidant molecules that may be formed during storage of a product, followed by an assessment of their activity and protective action against cell damage, first in vitro and then in vivo.

## ACKNOWLEDGMENT

We thank Dr. Murray Isman of the Department of Plant Sciences, University of British Columbia, Vancouver, for kindly supplying the standard for neochlorogenic acid.

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**Received for review January 21, 2004. Revised manuscript received May 24, 2004. Accepted June 2, 2004.**

JF049889J