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From Plums to Prunes: Influence of Drying Parameters on Polyphenols and Antioxidant Activity

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Prunes, which are industrially obtained by dehydrating fresh plums at 85−90 °C for 18 h, contain higher levels of phenolic compounds than most other fruits. Prune phenolics have shown beneficial effects on human health. Reports are available in the literature on ascorbic acid, phenol composition, and antioxidant activity of fresh plums and prunes, but there is a lack of publications on the influence of drying parameters on the phenolic compounds and antioxidant activity. A study was carried out on two plum cultivars using two sets of air-drying temperatures: (i) air temperature at 85 °C until 50% of prune moisture level and then the temperature was lowered to 70 °C; (ii) air temperature at 60 °C. Whole fresh and dried fruits were assessed for phenolics (catechins, hydroxycinnamic acids, anthocyanins, and flavonols), ascorbic acid, and antioxidant activity (all parameters were calculated on a dry matter basis). Analysis of data shows that chlorogenic and neochlorogenic acid changes were affected by both process parameters and cultivar. Drying destroyed anthocyanins, and there was a significant decrease in flavonols. Ascorbic acid was drastically reduced in relation to process temperature. The most striking result was that drying at 85 °C doubled antioxidant activity in both cultivars, while contradictory results were found for 60 °C processed plums.

KEYWORDS: Chain-breaking activity; drying; hydroxymethylfural; phenols; plums

INTRODUCTION

It is well-known that certain foods help to keep us healthy and prevent chronic disease (*1*). These foods are often classified as functional foods or nutraceuticals. Prunes, obtained by drying plum fruits (*Prunes domestica* L.), fall into this category. The literature reports various biological effects of plum and prune consumption in man and animals, demonstrated in vitro with fruit extracts or single isolated compounds. Besides the well-known action of prunes against constipation, their chemical composition seems to give them other properties, such as the ability to lower the glycemic index in man (*2*−*4*) and rats (*5*), slow osteoporosis in man (*6*) and rats (*7*−*9*), control lipoidoproteinosis in man (*10*) and rats (*11*), and inhibit human low-density lipoprotein (LDL) oxidation in vitro (*12*, *13*). They also have high radical scavenging activity, the strongest of all fruit and vegetable products in the human diet (*14*), while antimutagenic action has been demonstrated in man and in hamsters in in vitro tests (*15*−*17*). Of the various chemical compounds in the prune, the phenolic pool plays a key role in health-promoting action, as it has been demonstrated that chlorogenic and neochlorogenic acids lower the glycemic index in man (*3*) and inhibit LDL oxidation in vitro (*18*), while flavonoids exert an in vitro antithrombotic action in rabbit (*19*). However, despite the many data in the literature on the chemical composition of fresh plums (*20*−*22*) and dried prunes (*18*, *23*), no clear connection between the initial and the final composition of the product has been demonstrated; in other words, the effects of processing are not fully understood although a few papers have been published on the subject (*24*, *25*).

The aim of the present investigation was to demonstrate the effects of hot air-drying at two different temperatures on the main phenolic compounds and assess changes in their antioxidant capacity after the plums become prunes. Optimization of processing procedures could undoubtedly lead to improvements in the functionality and health-promoting capacity of prunes.

MATERIALS AND METHODS

The experiments were conducted on two varieties of plums: Sugar and President.

Plant Material. The fruits were purchased locally at an optimum ripeness stage, and those showing defects were discarded. Fruits were size-graded so that size difference would not affect drying times. They were then submitted to a dewaxing pretreatment by immersing in an alkaline solution (2% NaOH) at 80 °C for 10 (President) and 15 s (Sugar) (water/fruit ratio 10:1). At the end of this procedure, the fruits were immediately cooled in running water, dried, and checked to eliminate any plums that had been damaged and dried.

Dehydration. Fruits were dried in a laboratory pilot dryer, a tangential air-flow cabinet (a modified model of "Scirocco", Società Italiana Essiccatoi, Milan, Italy), equipped with automatic temperature and air moisture control devices. The air flowed tangentially to the fruits, while an air recycling system allowed mixing of the exhaust with fresh air and then reheating and redirecting to the product, to achieve the desired air moisture. The particular construction of the drier

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allowed a continuous air flow on the fruits, avoiding turbulence; thus, it is particularly suited to calculate drying kinetics. The fruits were placed on 56 cm diameter steel food trays (with a 20−28 kg/m^2 load, depending on variety) and loaded into the drier, where they were dried until reaching a predetermined dry matter value of 80% (based on weight loss calculation). The process was carried out at two different temperature settings: in the first case, the air temperature was set at 85 °C until the product reached a moisture value of 50%, and then, the temperature was brought to 70 °C until the end of the process; in the second case, the air temperature was set at 60 °C throughout the process. In both cases, the relative humidity of the air was as high as possible in the first stage (≈40%), the air volume was 1840 m^3 /h, and dehydration was performed by recycling the air (regulated to maintain the prearranged humidity values). The dried fruits (80% dry matter) were packed in coextruded plastic bags (95 μm thick polyethylene/polypropylene) and kept in a freezer at −18 °C before analysis.

Analysis and Quantitative Determinations. Fresh and dried fruits were evaluated differently. Thirty fresh plums were stoned and homogenized using a Waring blender. The following analyses were carried out in triplicate on the puree: water content (%), determined in a vacuum oven for 12 h at 70 °C (*26*); water activity of fruit puree, assessed by an electronic hygrometer (Aw-Win, Rotronic, Huntington, NY) equipped with a Karl-Fast probe calibrated in the range of 0.1−0.95 with solutions of LiCl of known activity; pH, determined on homogenized fruit flesh by a digital pH meter; acidity (expressed as g of malic acid per 100 g of dry matter^{-1}) by titration with 0.1 N NaOH to end point (pH 8.3); total soluble solids by digital refractometer; and ascorbic acid (mg g dry matter^{-1}) by titration with 2,6-dichloroindophenol (*26*). Antioxidant capacity was evaluated using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) (*27*): 25 mL of distilled H_2O was added to 5 g of homogenized puree, placed in the vortex for 1 min, and centrifuged at 6000 rpm at room temperature for 5 min (*31*). The supernatant was filtered through a Whatman filter and then, before the spectrophotometric reading (HP 8453 spectrophotometer, Palo Alto, CA), through a 0.45 μm filter. A 50 μL amount of the solution was made to react for 2.5 h in a cuvette containing 3 mL of a methanol solution of 6×10^{-5} M of DPPH•, at 515 nm wavelength and a temperature of 25 °C, to obtain a decrease in absorbance by the radical DPPH• (the decoloration curve of the radical follows very slow kinetics). A graph of absorbance vs time showed that its decrease followed a fourth order kinetic ($r^2 \geq 0.99$). The antioxidant capacity was expressed with the following $-OD^{-3}$ min^{-1} mg dm^{-1} 10^3 in the equation:

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

where A_0 is the initial optical density, A is the optical density at rising time t, and OD is optical density. The phenols were extracted and analyzed in high-performance liquid chromatography (HPLC) according to the method described by Donovan et al. (*18*) and Nakatani et al. (*29*). HPLC-DAD is a reliable means of analyzing the phenolic compounds in the fruits. The methanol extracts were separated by HPLC, and retention times and UV spectra were recorded. The pure standards of the compounds were available to identify the major peaks. Although studies have been published recently on the determination of phenolics in prunes by HPLC-MS-MS and HPLC-DAD-ESIMS (*21*, *22*), our analytical methods did not permit us to identify the unknown peaks on the basis of similarity with chromatograms in the literature. We used a Hewlett-Packard liquid chromatograph, Series 1090, coupled with an HP 1050 diode array detector. The column was a LiChrosphere C_{18}, 4 mm × 250 mm, 5 μm; 20 μL loop; 0.5 mL/min flow; mobile phase: A = 50 mM $NH_4H_2PO_4$ solution brought to pH 2.6 with H_3PO_4, B = 80% CH_3CN and 20% phase A, C = 200 mM H_3PO_4. The phenols were monitored at four different wavelengths: 280 nm for catechins, 316 nm for hydroxycinnamic acids, 365 nm for flavonols, and 520 nm for anthocyanins. The compounds were quantified by calibration with the following standards: catechin, caffeic acid, *p*-coumaric acid (Sigma Chemical Co., St. Louis, MO), neochlorogenic acid (kindly provided by Dr. Murray Isman, Department of Plant Sciences, University of British Columbia, Vancouver), chlorogenic acid (Fluka Chemical, Buchs, Switzerland), rutin, cyanidin-3-monoglucoside, and cyanidin-3 rutinoside (Extrasynthese B. P. 62-69730, Genay, France). The other flavonols found in the samples, which we did not have standards for, were quantified as rutin equivalents since they presented the same spectral characteristics as rutin. Likewise, the third anthocyanin found in both plum varieties was quantified as a cyanidin-3-rutinoside equivalent, since this anthocyanin was the one found in the highest quantities in plums. All values were expressed as mg/kg of dry matter. The same analyses were performed on the dried samples. We used 12 prunes to determine the phenols and 12 for the other measurements. The prunes were cut into small pieces and minced with a mincer; the minced prunes were analyzed as above, except that 1 g (instead of 5 g) of minced prunes was extracted to assess antioxidant capacity.

Statistical Analysis. All data were submitted to one way analysis of variance (ANOVA) using MSTAT-C software, considering sampling time (fresh and dried) as the "group variable". Means, when required, were separated according to Duncan's multiple range test, significance level $P \leq 0.01$.

Table 1. Physicochemical Parameters in Fruits of Two Plum Cultivars (Fresh and Dried)

cultivar	sample	dry matter (%)	Aw	pH	acidity (g malic acid/100 g dm^{-1})	ascorbic acid (mg g dm^{-1})
Sugar	fresh	18.58c[a]	0.980a	3.38a	6.12a	18.1a
	dried 60 °C	81.24a	0.712c	3.56a	5.79a	8.8b
	dried 85 °C	79.87a	0.759b	3.48a	5.17b	5.1c
President	fresh	18.23b	0.989a	3.29ba	5.99a	22.4a
	dried 60 °C	79.42a	0.799b	3.36a	4.98b	8.8b
	dried 85 °C	78.36a	0.785c	3.3a	4.87b	4.4c

[a] Data followed by different letters within each column and cultivar are significantly different according to Duncan's multiple range test at $P < 0.01$.

RESULTS AND DISCUSSION

Fresh Fruits. Data on the physicochemical parameters of the fresh plums are shown in **Table 1**. It can be seen that the dry matter values indicate that the fruit is suitable for drying, although it is usual to find slightly higher initial values in Italian-grown plums (*30*) and decidedly higher values in those grown in the U.S.A. (*31*). The two plum varieties examined showed fairly similar pH and acidity values, which were slightly lower and higher, respectively, than those reported in the literature (*32*). Ascorbic acid values varied between 18.1 mg/g dry matter for the Sugar variety and 22.4 mg/g dry matter for President, in accord with data in the references (*20*).

Dehydration Kinetics. Under our experimental conditions, the time necessary to reach the estimated value of dry matter varied, as was expected, according to the set of values used. The processing time was 38 and 44 h for President and Sugar respectively, in high-temperature conditions (85 °C), while it was from 60 to 72 h when the dehydration temperature was lower. Considering that the President plums had a much higher mean weight than the Sugar variety, it appears that the former had greater drying efficiency. This behavior can be explained by the greater difficulty of Sugar tissues in letting water pass, rather than by different efficacy of the dewaxing pretreatment.

Physicochemical Changes in the Dried Fruits. The physicochemical changes observed after drying are shown in **Table 1**. A marked decrease in ascorbic acid content was found after the dehydration process. Loss of vitamin C after heat treatment has been widely reported (*33*). As expected, the fruits dried at 85 °C showed significantly higher losses, ranging from a minimum of 61% to a maximum of 80%. With regard to the other chemical parameters, an increase in pulp pH and a decrease in titratable acidity (in dry matter) were generally observed.

Table 2. Absolute and Percent Amounts of Phenolic Compounds in Two Cultivars of Fresh Plum Fruits

	cultivar			
	Sugar		President	
compd	absolute[a]	%	absolute	%
neochlorogenic acid	1952. 53	57.97	3817.77	53.52
chlorogenic acid	584.93	17.37	540.51	7.58
caffeic acid	0.00	0.00	0.00	0.00
p-coumaric acid	0.00	0.00	0.00	0.00
cyanidin 3-glucoside	0.00	0.00	199.20	2.79
cyanidin 3-rutinoside	263.89	7.89	1377.75	19.31
cyanidin 3-rutinoside equivalent	162.73	4.83	593.98	8.33
rutin	150.41	4.47	266.53	3.74
rutin equivalent	253.89	7.54	150.72	2.11
catechin	0.00	0.00	186.76	2.62
percent values per class				
% hydroxycinnamic acids		75.33		61.09
% anthocyanins		12.67		30.43
% flavonols		12.00		5.84

[a] As mg kg dry matter^{-1}.

Water activity values in some cases seemed to indicate a possible increment in the various classes of microorganisms, but because phenol content is high, an increase in microbes is unlikely, as has been confirmed in industrial-scale processing.

Phenolic Composition of Fresh Fruits and Changes after Drying. Table 2 shows the percentages of the single compounds or classes of phenols found in fresh plums. It can be seen, as reported in the literature (*18*, *20*, *22*), that the major phenolic compounds found in plums are the hydroxycinnamic acids, present in 61 and 75% in President and Sugar, respectively. The most common hydroxycinnamic acid is neochlorogenic acid, which has a maximum value of 58% of the total phenols in the Sugar variety. Caffeic and *p*-coumaric acids, the latter reported by Tomas-Barberan et al. (*22*), were not found in the fruits. Other compounds observed were the following: (i) anthocyanins, cyanidin-3 rutinoside, and the other similar anthocyanin, identified by some authors as peonidin 3-rutinoside (*24*), while others believe it to be cyanidin-3-galactoside or cyanidin 3-acetyl-glucoside (*22*); the cyanidin-3 monoglucoside was found only in President plums. This compound was also lacking in another variety, Wickson (*22*). In President, it made up 30% of the total phenol content. (ii) Flavonols: the flavonols were basically rutin (quercetin-3 rutinoside) and other compounds similar to rutin (since the spectra were the same) and therefore quantified as rutin equivalent. In this case also, the results agree with recent data in the literature, which report the presence of quercetin derivatives only (*22*). (iii) Flavan-3-ols (catechin): we found catechin only in the President variety. Our results fall in the interval of values reported by other authors (*18*, *20*, *22*, *29*), although President plums showed very high values of neochlorogenic acid (**Table 2**).

Figures 1−**6** show the changes in phenolic compounds after drying. It is interesting to note the changes in the two hydroxycinnamic acids that were also found in the fresh fruits (**Figures 1** and **2**). No significant changes in the acids took place in either variety when processing was done at the higher temperature whereas the changes were always significant in the prunes obtained at 60 °C. The lower drying temperature must be responsible for the difference. The degradation of the hydroxycinnamic acids under our experimental conditions (high presence of air and, thus, oxygen) could have been influenced by polyphenoloxidase (PPO) enzymatic activity. Reports in the literature show that during the dehydration process PPO activity remains high for long periods when the drying temperature is

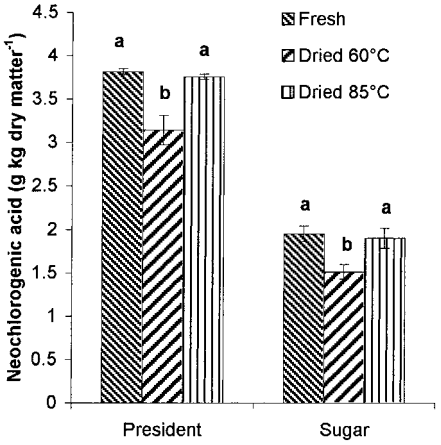

Figure 1. Changes in neochlorogenic acid of plums dried with two different process parameters settings. Data are the mean of six determinations. Vertical bars indicate standard error. Different letters within each bar mean a statistical difference by Duncan's multiple range test at $P \leq 0.01$.

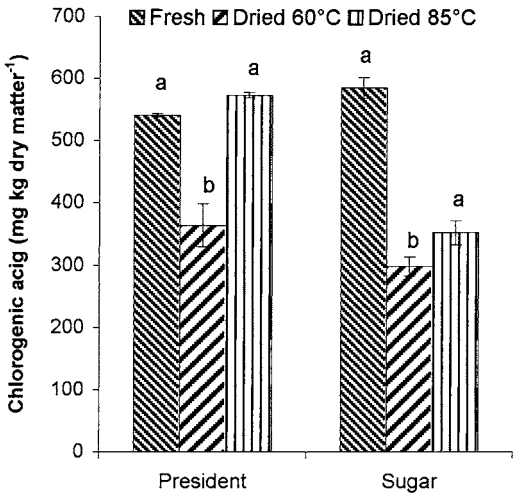

Figure 2. Changes in chlorogenic acid of plums dried with two different process parameters settings. Data are the mean of six determinations. Vertical bars indicate standard error. Different letters within each bar mean a statistical difference by Duncan's multiple range test at $P \leq 0.01$.

around 55 °C, whereas only moderate activity is observed at temperatures higher than 75 °C (*24*). In our case, although PPO activity was not studied, it is likely that degradation kinetics was the same as in the work cited. However, processing time was much longer (double, on average) in fruits dried at a lower temperature; consequently, PPO activity, besides having a higher residual action, went on longer. On the other hand, no significant changes in the two acids were observed in the plums dried at 85 °C, a result in contrast with reports of Raynal et al. (*24*), who found a decrease of 25 and 30% in chlorogenic and neochlorogenic acids, respectively. Besides these acids, present also in the plums, the prunes contained *p*-coumaric acid. This has been reported by other workers (*22*) and can probably be attributed to cinnamic acid hydrolysis during processing (it is one of the steps in the biosynthetic path of shikimic acid). We did not find any caffeic acid, unlike some authors who have reported traces of this compound in the dried fruits (*22*).

The anthocyanins showed a completely different picture (**Figures 3** and **4**). It can be noted that in general there was total destruction of the two glucoside derivatives (present only in President plums) and of the rutinoside and rutinoside equivalent derivatives. This occurred totally in the Sugar variety

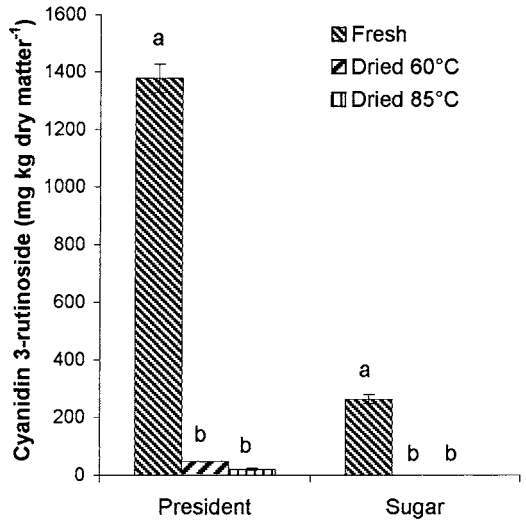

Figure 3. Changes in cyanidin-3 rutinoside of plums dried with two different process parameters settings. Data are the mean of six determinations. Vertical bars indicate standard error. Different letters within each bar mean a statistical difference by Duncan's multiple range test at $P \leq 0.01$.

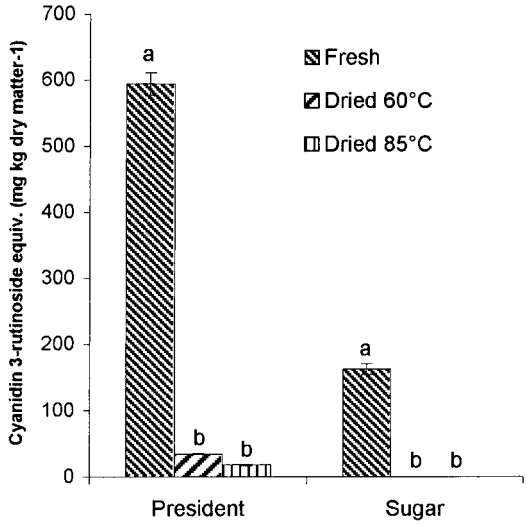

Figure 4. Changes in cyanidin-3 rutinoside equivalent of plums dried with two different process parameters settings. Data are the mean of six determinations. Vertical bars indicate standard error. Different letters within each bar mean a statistical difference by Duncan's multiple range test at $P \leq 0.01$.

while traces of cyanidin 3-rutinoside and cyanidin 3-rutinoside equivalent remained in President prunes at the end of the process. It seems clear, therefore, as reported in the literature, that high temperatures and the high oxygen concentrations involved in the air-drying process lead to rapid degradation of the anthocyanins (*34*). During drying, the balance between the various types of anthocyanins shifts toward either the quinoid base or the colorless chalcones, which are destroyed by different oxidation mechanisms (hypothesized) to produce different types of compounds, some of which are brown and of high molecular weight (*35*). It can easily be explained why traces of the two anthocyanins remained in the President variety: in the fresh fruits, the two compounds are present in much higher concentrations than in the Sugar variety. If we consider, therefore, that anthocyanin degradation seems to follow first order kinetics, as indicated in reports on prunes in the literature (*25*), it is evident that instrumentally detectable quantities were likely to

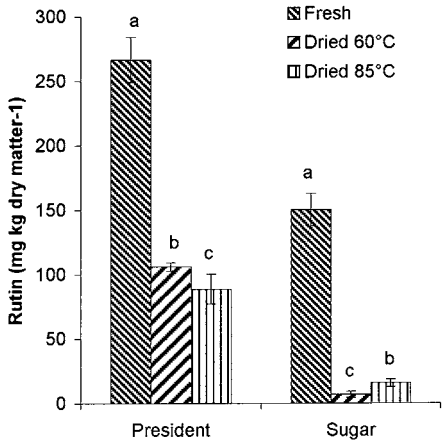

Figure 5. Changes in rutin of plums dried with two different process parameters settings. Data are the mean of six determinations. Vertical bars indicate standard error. Different letters within each bar mean a statistical difference by Duncan's multiple range test at $P \leq 0.01$.

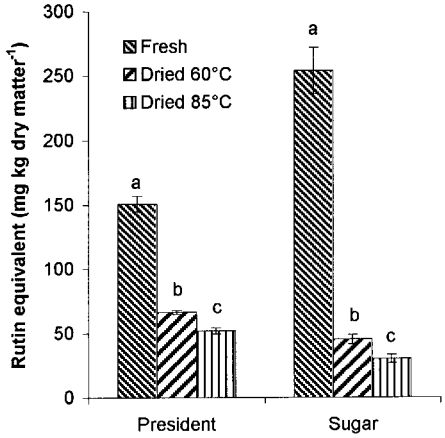

Figure 6. Changes in rutin equivalent of plums dried with two different process parameters settings. Data are the mean of six determinations. Vertical bars indicate standard error. Different letters within each bar mean a statistical difference by Duncan's multiple range test at $P \leq 0.01$.

be found in the President fruits. Catechin, found only in the President variety, was totally destroyed by the high temperature.

The flavonols (**Figures 5** and **6**) showed different behavior from the two categories of compounds previously mentioned. The degrading action of the dehydration process is evident. In all cases except one (**Figure 5**), the decrease in flavonols was significantly more marked in the sample dried at 85 °C. It is clear, therefore, that unlike the case of hydroxycinnamic acids, degradation of the flavonols and anthocyanins is not directly correlated to PPO activity since the latter compounds disappear proportionally to the increase in temperature. The flavonoids are therefore not degraded by the same mechanism as the phenolic acids; that is, they are not direct substrates for oxidases (*36*) since PPO does not act directly on the glycosides. It must be pointed out, however, as demonstrated by Raynal and Moutounet (*25*), that the anthocyanins could have been degraded by joint action of temperature/enzymatic activity as the chlorogenic acid (present in the plums) can act as an intermediary in PPO enzymatic degradation of the anthocyanins (the compounds deprived of glycosides).

Changes in Antioxidant Activity after Processing. The phenols in plums and prunes are responsible at various levels for good antioxidant activity, with their inhibitory action against lipid oxidation, especially in the case of LDLs (with beneficial

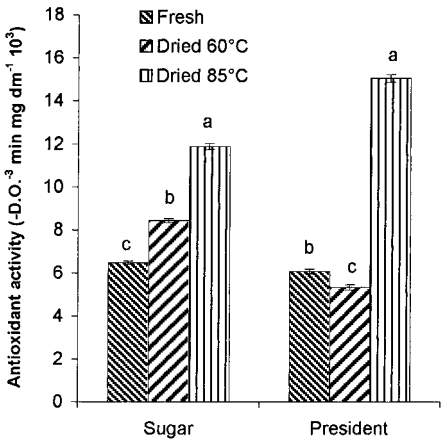

Figure 7. Changes in antioxidant activity of plums dried with two different process parameters settings. Data are the mean of six determinations. Vertical bars indicate standard error. Different letters within each bar mean a statistical difference by Duncan's multiple range test at $P \leq 0.01$.

effects on the prevention of coronary heart disease) and with their chain-breaking activity. Our assays of antioxidant capacity, conducted on a hydrophilic model system, gave particularly interesting results. As can be seen in **Figure 7**, the antioxidant capacity tended to increase significantly with the higher drying temperature in the Sugar variety, while in the President plum sample dried at 60 °C antioxidant capacity was significantly lower than in the fresh fruits. **Figure 8** shows that the total polyphenol value (calculated simply as the sum of the phenols we measured) markedly decreased, particularly in the sample dried at 60 °C. We should therefore expect to find an opposite trend to that observed in antioxidant capacity values. We actually find a value two and a half times higher in the case of President plums dried at 85 °C. This behavior could be the result of two factors: (i) it is known that polyphenols in an intermediate stage of oxidation have greater antioxidant power than initially (*37*) even though this is temporary; and (ii) high temperature stabilization procedures may lead to the formation of new compounds with higher antioxidant activity. This is essentially the case of the Maillard reaction, which creates various products that are the Maillard reaction products (MRPs), with markedly higher antioxidant power, often by a chain-breaking type mechanism (*38*−*40*). One of the intermediary products of the Maillard reaction is hydroxymethylfural (HMF), which has a maximum absorbance around 280−290 and appeared in the chromatograms that we obtained at 280 nm wavelength. **Figure 9** shows a comparison between HMF optical density data and antioxidant capacity. An increase in optical

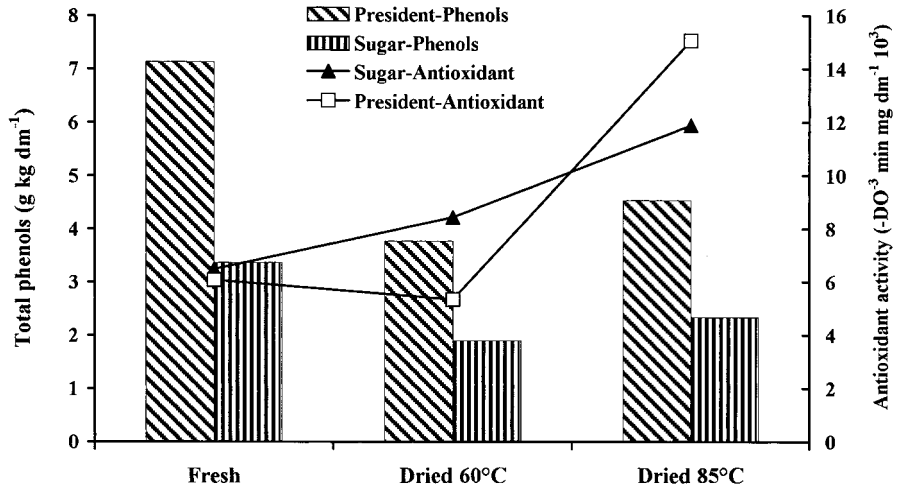

Figure 8. Antioxidant activity and total phenols of plums dried with two different process parameters settings. Data are the mean of six determinations.

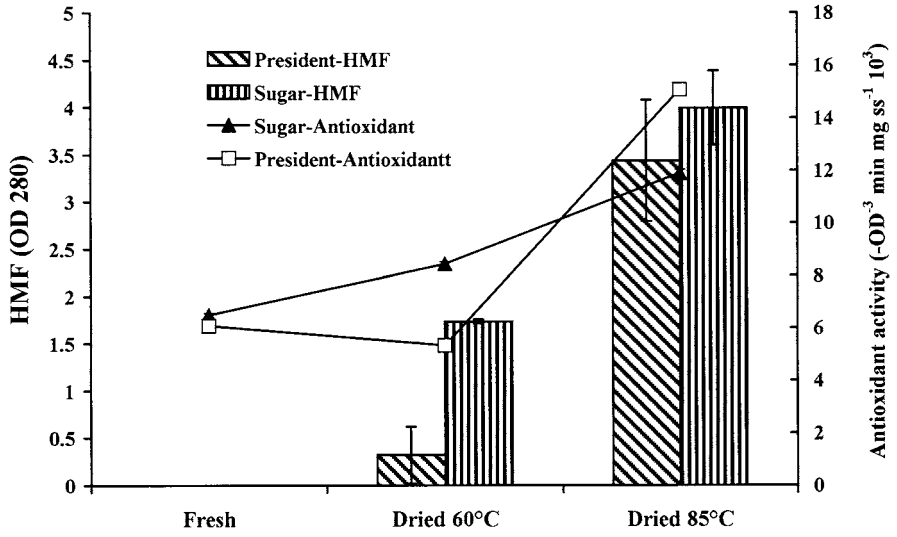

Figure 9. Antioxidant activity and optical density of HMF of plums dried with two different process parameters settings. Data are the mean of six determinations.

density is accompanied by a corresponding increase in antioxidant capacity, except in President plums dried at 60 °C. We can presume, therefore, that the increase in chain-breaking activity values can be attributed to the MRPs. It can also be seen that the higher the MRPs content, the higher the antioxidant capacity value (within each cultivar). The apparent anomaly of the President plums dried at 60 °C remains to be explained. It could be that the increase in antioxidant capacity due to the MRPs did not compensate for the destruction of the phenolics.

The results of the study show that phenol degradation during hot air-drying of the two varieties of plum fruits did not always result in the same effects. Despite the marked reduction in phenols, which are almost in toto responsible for the antioxidant capacity, chain-breaking activity considerably increased in the plums dried at higher temperatures. It is likely that this is caused by MRPs, which are present in important amounts in this type of dried fruit.

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