Multivariate Analysis of Superficial Scald Susceptibility on Granny Smith Apples Dipped with Different Postharvest Treatments

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Multivariate methods were used to present the results of different postharvest treatments applied to control superficial scald disorder in Granny Smith apples. In a two-season study, the treatments applied by dips were diphenylamine (DPA), Semperfresh (a sucrose ester coating) formulated alone or with either ascorbyl palmitate or *n*-propyl gallate, and $CaCl_2$. DPA correlated negatively with scald, whereas the rest of the treatments showed no clear relationship with the disorder. By means of partial least squares, the incidence of superficial scald after cold storage was shown to correlate with quality parameters (firmness and acidity), measured at the end of storage. In contrast, ethylene production did not appear as a relevant factor in the incidence of scald.

Keywords: *Apple; Granny Smith; principal component analysis; diphenylamine; superficial scald; quality*

INTRODUCTION

Superficial scald is a physiological disorder that appears during marketing, after cold storage of apples. Granny Smith apples are particulary prone to scald and, in some areas, the incidence of scald on this cultivar reaches 100% after several months of storage (Barlow, 1988). Until now, this disorder has been well controlled by diphenylamine (DPA) (Dodd et al., 1993; Chellew and Little, 1995), but it is likely that this product will be withdrawn from the market. Thus, a need has arisen for alternative, non-DPA, strategies for controlling scald.

It is currently common practice to use controlled atmosphere (CA) storage, especially with low oxygen concentrations, to prevent scald. This practice also improves the quality parameters of the fruit (Visai et al., 1997). However, CA is an expensive technique, and sometimes its use may not be cost-effective (Bauchot et al., 1995). Some success in controlling scald has already been claimed for a number of other treatments approved for foodstuffs, these being relatively easy to apply without major modifications to existing storage and dipping equipment. Sucrose ester-based coatings, such as Semperfresh, were found to reduce superficial scald when applied singly (Meheriuk and Lau, 1988; Van Schaik and Slaats, 1990) or in combination with food-compatible antioxidants (Little and Barrand, 1989). These products could also modify apple quality attributes.

Another aspect of superficial scald prevention is to investigate the biochemical origin of the disorder. Scald formation is thought to result from the oxidation of α-farnesene, producing hydroperoxides and peroxides, commonly known as conjugated triene hydroperoxides (CTH) (Huelin and Coggiola; 1970; Anet, 1972). Scald has also been related to other biochemical processes, such as ethylene production (Du and Bramlage, 1994).

The aim of this work was to evaluate the susceptibility of Granny Smith apples to superficial scald development in relation to their postharvest treatment. The evolution of some biochemical and/or quality attributes during cold storage was also considered. To obtain an overview of almost all the results, the data were analyzed by principal component methods (Martens and Naes, 1989; Esbensen et al., 1994). For a deeper insight, the correlation between scald and ethylene production and quality parameters was analyzed by means of partial least squares (PLS).

MATERIALS AND METHODS

Plant Material. Granny Smith apples from a commercial orchard in Lleida, Spain, were harvested in two consecutive years (seasons I and II). The level of maturity of the apples was determined within 4−5 h of harvest (T0). Measurements at harvest included quality attributes and ethylene production.

Treatments and Storage Procedure. The apples were treated immediately after harvest (F1) or after 6 weeks into cold storage (F6). After harvest, 10-apple replicates were left undipped as controls (T1) or dipped for 20 s in DPA emulsions at 2500 ppm (T2), in Semperfresh at 1% (T3), in ascorbyl palmitate at 1875 ppm in 1% Semperfresh (T4), and in *n*-propyl gallate at 1875 ppm (season I) or at 4867 ppm (season II) in 1% Semperfresh (T5) or in $CaCl_2$ at 1% (T6). All of the emulsions were prepared with tap water 18 h before use. The emulsions were supplied by Surface Systems International Ltd., East Challow, U.K. After dipping, the treated and some untreated apples were stored at 0.5 °C for 2, 4, or 6 months under normal atmosphere. In season I, 6 weeks into cold storage, the untreated apples were removed to room temperature and were then dipped with DPA, Semperfresh, ascorbyl palmitate plus Semperfresh, *n*-propyl gallate plus Semperfresh, and $CaCl_2$. In season II, 6 weeks into cold storage, the untreated apples were removed to room temperature and were then dipped with only Semperfresh. Some replicates were kept as control samples in the same conditions but without treatment. Fruit samples were evaluated after 2, 4, and 6 months of storage (S2, S4, and S6, respectively) and after 1, 4, and 10 days at room temperature after cold storage (01, 04, and 10, respectively).

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Scald Incidence. This was evaluated visually using three replications of 10 fruits each. Only those fruits exhibiting >0.5 cm^2 of browning were considered to be scald-damaged. Data were expressed as a percentage of damaged fruit.

Determination of Ethylene Production. Five apples were enclosed in a 10-L jar continuously aerated with humidified air, as one replicate, with two replicates used per treatment. Ethylene production rates were measured by analyzing the ethylene concentration of a 1-mL gas sample from each jar using a gas chromatograph with a flame ionization detector.

Evaluation of Quality Attributes. The fruits were evaluated for weight, flesh firmness, titrable acidity, and soluble solids content. Weight was measured on 0.1 g sensitive scales. Flesh firmness was measured on two opposite peeled sides using an Effegi tester fitted with an 11-mm diameter plunger. Titrable acidity was assessed by titrating with 0.1 N NaOH to pH 8.1 and expressing the result as malic acid. The soluble solids content was measured with an Attago digital refractometer. All measurements were made with three replicates of 10 fruits each.

Chemometrics. For analysis of the data, principal component (PC) methods were used. Principal component analysis (PCA) permits a simultaneous study of all variables and decomposes the data to detect "hidden phenomena". PLS was used to analyze the correlation between scald incidence and the variables studied. We used Unscrambler ver. 6.11b software [by Camo, A. S., Ed., 1996).

The data set included seven category variables to identify the treatments (T0−T6), two category variables for the moment of treatment application (F1 or F6), three category variables for the length of storage (S2, S4, and S6), and three category variables for the days at room temperature after cold storage (01, 04, and 10). There were also variables related to the assessment: incidence of scald (scald), ethylene production (ethylene), weight, firmness (two measurements and the mean expressed as fir1, fir2 and firm), acidity, and soluble solids content (SSC). Category variables were codified using a discrete variable for each category, which reaches the value +1 for the samples of such category and −1 in the opposite case. Samples were labeled using the codes for the treatment, storage time, and moment of treatment application as described above. The resulting data matrix contains 141 samples and 23 variables. As variables were measured in different units, there were large differences between them with respect to the mean values, variance, and standard deviation. As a result, it was necessary to scale the data prior to performing PC models. The factor used was the inverse of the standard deviation (SDev). In addition, the original variables have been centered by subtracting the mean. Thus, PCA and PLS models were made with scaled and centered data to avoid dependence of the results on the measurement units.

RESULTS AND DISCUSSION

PCA Models. As up to 23 variables are being using to characterize the samples, graphic display of the results involves multidimensional spaces. A good method of retaining the main information using a low number of variables is through the axis defined by PCs, which indicates the directions of maximum variance of the samples. The score plot of a full-data PCA model showed two clearly differentiated groups: one corresponding to harvest samples and the other including all samples after cold storage. As this model is not of great value for obtaining an overview of the treated samples after cold storage, the model was recalculated by removing the harvest data. The first three PCs in the resulting model describe 42% of the total variance in the data. Although this value is not very high, it is higher than the explained variance that could be obtained using only three of the variables studied. Moreover, the objects are well spread over the whole PC space, so the model could be considered useful for our aims. The scores PC1 versus PC2 are plotted in Figure 1 using different labels for the samples. Figure 1a shows a group of objects labeled S2 situated near the bottom and to the right. This implies that all samples kept in cold storage for 2 months are similar, independent of the treatment applied. S4 samples are situated in the middle of the plot, and the S6 group is situated to the left, but near S4. It seems that S2 forms a separate group, whereas the difference between S4 and S6 is not so notable. Consequently, storage time characterizes the samples more than other variables and is related to the meaning of PC1. Figure 1b shows that, within each group, the samples treated just after harvest (F1) are in the upper half of the plot, whereas those treated 6 weeks into storage (F6) are found in the lower half. PC2 describes the moment of treatment application. When the samples are identified by the labels of treatment or days at ambient temperature after cold storage, there is no clear grouping inside each cluster (data not shown). Accordingly, no relationship appears between these variables and the meaning of the first two PCs.

A loading plot PC1 versus PC2 (Figure 2) shows the influence of the variables in the PCs and the main relationships among them (variables close together in the loading plot are positively correlated). Acidity and firmness form a group of variables with a strong influence on PC1, which is also influenced by the storage time, as mentioned above. This indicates that acidity and firmness will be affected by storage time, as is well-known. Likewise, SSC and ethylene production appear to correlate to each other and with respect to PC2, which is also influenced by the moment of application of the treatment. However, as the variance explained by these two PCs is not very high, models to explain ethylene production were produced (data not shown) which indicated that the correlation between the moment of application of the treatments and ethylene production is not relevant, ethylene production being mainly affected by storage time. Maximum production was after 4 months of cold storage (S4). Two months into cold storage (variable S2) positive correlation with firmness and acidity was shown, and it correlated negatively with scald incidence (opposite loading with respect to PC1). In contrast, 6 months into cold storage (S6) is clearly correlated with scald and negatively correlated with acidity and firmness. Thus, a long storage period increases the incidence of scald (Soria and Recasens, 1997) and the loss of firmness and acidity in the fruit, which is corroborated by the literature (Mahajan, 1994).

PLS Model for Superficial Scald. The information concerning the success of any treatment in preventing scald is easily obtained by looking at a model designed to describe the variable scald. Thus, PLS was used to model scald using post-cold-storage samples (T1−T6). This model attempts to explain the maximum variance of the scald variable in the first factors or PCs. Figures 3 and 4 represent the score and loading plots for PC1 versus PC2. In Figure 3, PC1 is related with storage time (as in the PCA model), and PC2 is most closely related to the treatments. However, it is remarkable that T2 samples (corresponding to DPA treatment) form a more separate group at the bottom of each cluster corresponding to each withdrawal from storage. In contrast, the rest of the treatments are mixed, spreading the rest of the cluster to a greater or lesser extent (see

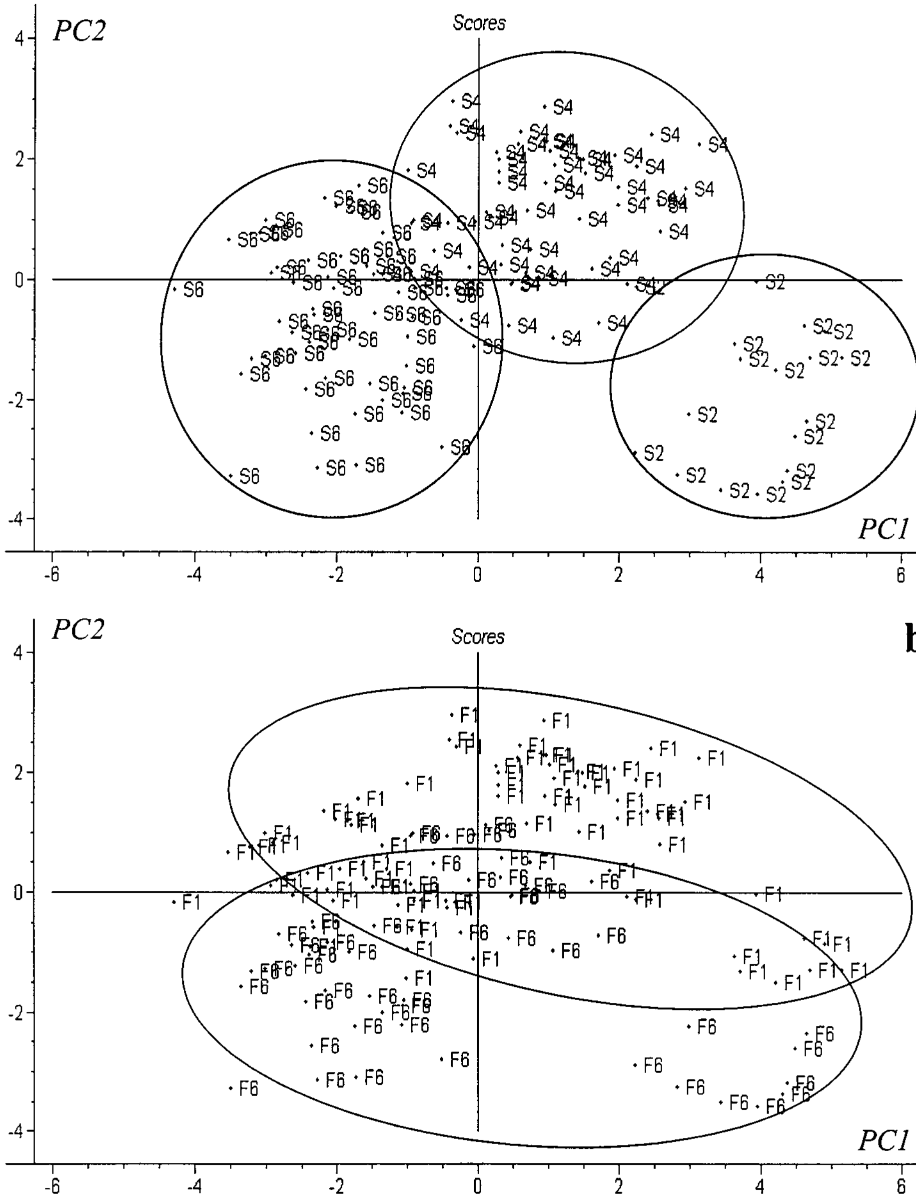

Figure 1. Score plot of PC1 vs PC2 from a PCA model made using all data except that corresponding to harvest; 135 samples from seasons I and II are included. Samples are labeled using coding as defined under Treatments and Storage Procedure, displaying only the storage length label in panel a (S2, S4, and S6) and the moment of treatment application label in panel b (F1 and F6).

Figure 3). These two factors (PC1 and PC2) explain up to 54% of the total variance of scald.

The loading plot (Figure 4) shows that the treatment with DPA (T2) is negatively correlated with the scald variable with respect to both PC1 and PC2. The rest of the treatments have small loading values in PC1, except *n*-propyl gallate (T5), which is situated near scald. We can deduce from this that DPA is the most effective of the compounds tested, but probably none of the other compounds reduced scald incidence, and the application of *n*-propyl gallate could even aggravate the incidence of the disorder. The moment of treatment application appears to have very minor importance for scald development (see Figure 4).

The regression coefficients quantify, for a given number of PCs, the importance of each variable and summarize the results observed in the loading plots. Figure 5 plots the regression coefficients containing samples from seasons I and II separately, because the incidence of the scald was higher during the latter

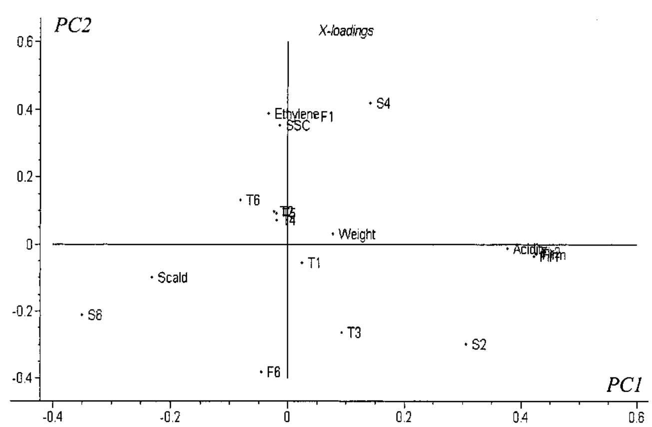

Figure 2. Loading plot of PC1 vs PC2 from a PCA model constructed using all data except that corresponding to harvest; 135 samples from seasons I and II are included. Variables are labeled using coding defined under Treatments and Storage Procedure and Chemometrics.

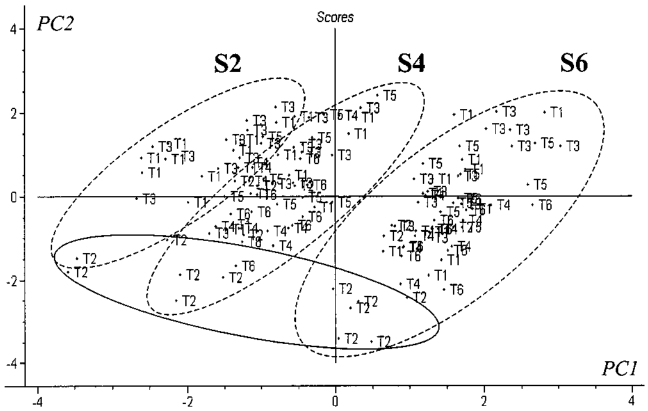

Figure 3. Score plot of PC1 vs PC2 from a PLS model of variable scald made using all data except that corresponding to harvest; 135 samples from seasons I and II are included. Samples are labeled using coding as defined under Treatments and Storage Procedure with the treatment label (T1−T6). Samples corresponding to the same storage time are circled in discontinuous line and labeled S2, S4, or S6.

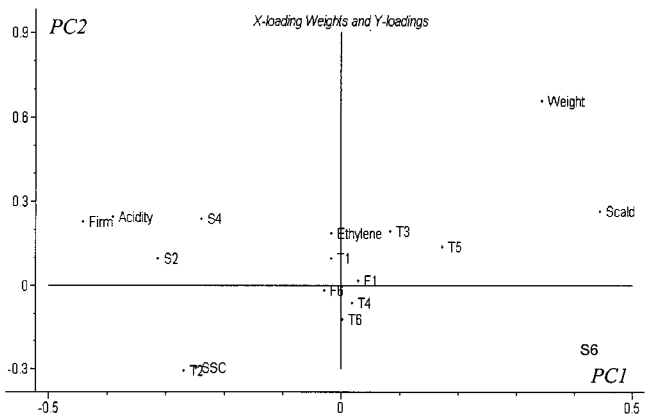

Figure 4. Loading plot of PC1 vs PC2 from a PLS model of variable scald made using all data except that corresponding to harvest; 135 samples from seasons I and II are included. Variables are labeled using coding defined under Treatments and Storage Procedure and Chemometrics.

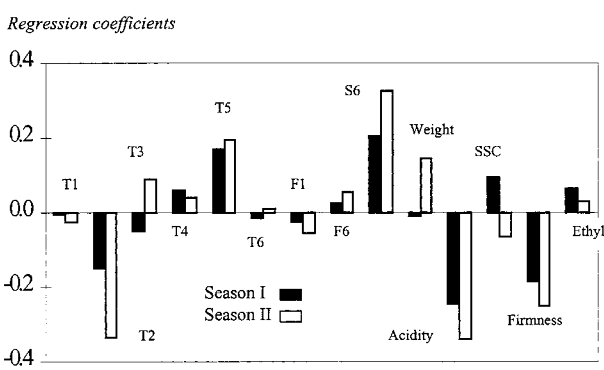

Figure 5. Regression coefficients between scald and the rest of the variables. Samples from seasons I (black) and II (white) are represented separately. Models were constructed using only the data from the withdrawal after 6 months of storage. Variables are labeled using coding defined under Treatments and Storage Procedure and Chemometrics.

season. We have represented the data for only the last withdrawal from storage (6 months), when scald was most obvious (high regression coefficient value in Figure 5). However, the behavior of the variables with respect to scald for withdrawal after 2 or 4 months of cold storage (data not shown) was similar to the behavior obtained after 6 months.

Figure 5 reaffirms the results observed on the PLS loading plot (Figure 4) with respect to the effectiveness of the different treatments at controlling scald. DPA (T2) presents the highest negative correlation with scald, so it is the most effective treatment for controlling the disorder. Moreover, this control is also greater when the incidence is higher (season II). The antioxidant *n*-propyl gallate applied with Semperfresh shows the highest positive correlation. Hence, this treatment could be harmful on cultivars susceptible to scald, such as Granny Smith apples. The application of Semperfresh alone (T3) made a slight impact in season I, when the development of scald was lower. The behavior of $CaCl_2$ (T6) is similar to that of Semperfresh. Although the regression coefficients are low, Figures 4 and 5 suggest that the application of the treatment just after harvest (F1) is negatively correlated with scald, whereas the application after 6 weeks into cold storage has a positive correlation.

Ethylene production does not appear as a relevant factor for explaining the incidence of scald (Figure 4), unlike Du and Bramlage's findings (1994). The poor correlation between these two variables could explain the results obtained by other studies which report that ethylene scrubbing in storage is not always effective in reducing scald (Lau, 1990; Graell and Recasens, 1992).

Quality parameters show a clearer correlation with scald (Figures 4 and 5). Weight is positively correlated with this variable (for PC1 and PC2; see Figure 4), but its regression coefficient value depends on the season. The greater the incidence of scald, the higher the positive coefficient obtained. According to Bramlage (1993), larger fruit tend to scald more than smaller fruit. This could be explained by considering fruit calcium content, which is negatively correlated with scald (Bramlage et al., 1985), and declines as size increases. The SSC presents a negative correlation with scald for both PC1 and PC2 in the loading plot (Figure 4). However, the regression coefficient varies depending on the season, likewise with the weight. Firmness and acidity are strongly correlated with each other and negatively correlated with scald (opposite coordinate with respect to PC1 in Figure 4). Their regression coefficients with respect to scald show a high negative value in both seasons I and II (Figure 5).

The irregular results obtained with weight and SSC variables prevent the establishment of a clear relationship between these and scald development, and they cannot be considered as factors in scald susceptibility. In contrast, firmness and acidity during cold storage are very closely related to scald development: the higher the level of these two quality parameters in the fruit, the lower scald development. Thus, it seems interesting to evaluate whether the maintenance of these quality parameters provides any protection against scald development or whether there is simply a correlation between these and scald, but without a causative relationship. It is widely recognized that early maturity at harvest predisposes fruit to superficial scald development during storage (Lidster et al., 1987; Dilley, 1993; Truter and Combrink, 1993; Lau and Yastremski, 1993). Early mature fruit is characterized by a high firmness and acidity. This contrasts with our results, as after withdrawal from cold storage, firmer and more acid fruit is less prone to scald.

The different roles played by these quality parameters in scald development, depending on whether they are measured at harvest or on withdrawal from storage, lead us to conclude that there is no causal relationship between quality parameters and scald development. Thus, these parameters cannot be considered predetermining factors for scald. It is probable that the biochemical reactions involved in the parameters which lead to maturity and/or the ripening process do not affect the biochemical changes that lead to scald development.

The retention of firmness and acidity may provide resistance to scald, but there could be another factor that predetermines the disorder. It is possible that the evolution of some components (such as phenolic fatty acid esters) in the peel, where biochemical reactions leading to scald occur, has implications for resistance to the disorder. At present, research into the origin of scald is turning this way (Whitaker, 1998). Therefore, we can mention the effect of the harvest date, rather than the effect of maturity as determined by quality parameters, on the susceptibility of fruit to scald.

CONCLUSION

The most important factors that differentiate the fruit are cold storage treatment and the length of this storage. This latter factor mainly determines acidity and firmness of the fruit and also appears to be positively correlated with scald development.

With respect to the effectiveness of the postharvest treatments tested for controlling scald, the best results were obtained with DPA. The rest of the treatments do not show a clear relationship with scald development.

Both firmness and acidity quality parameters measured on withdrawal from cold storage show a negative correlation with scald incidence. Despite this correlation, we cannot prove the existence of a causal relationship.

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