Pattern recognition of acorns from different Quercus species based on oil content and fatty acid profile

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RESUMEN
Patrón de reconocimiento de bellotas procedentes de diferentes especies Quercus basado en el contenido de aceite y en el perfil de ácidos grasos.

El objetivo de este estudio fué (i) la caracterización de diferentes especies del género Quercus y (ii) la clasificación de las mismas en base al contenido y composición de ácidos grasos del aceite de sus frutos y/o en sus caracteres morfológicos, via técnicas de patrón de reconocimiento (Análisis de Componentes Principales, ACP, Análisis de Cluster, AC, y Análisis Discriminante, AD). Se han estudiado Quercus rotundifolia Lam., Quercus suber L., y Quercus pyrenaica Willd., pertenecientes a la misma zona del centro de Portugal.

Al emplear el contenido de aceite y sus respectivas composiciones de ácidos grasos para caracterizar a las muestras, el ACP reveló grupos bien separados correspondientes a cada especie, los cuales, a su vez, se confirmaron con el AC y el AD. El "ancho" y "longitud" de las bellotas exhibieron un poder discriminante bajo. Las bellotas de Q. rotundifolia mostraron el contenido más elevado de aceite, seguidas de las de Q. suber y Q. pyrenaica (9.1, 5.2 y 3.8%, respectivamente). Los perfiles de ácidos grasos de los aceites de Q. rotundifolia y Q. suber son similares al del aceite de oliva, mientras que el aceite de las bellotas de Q.pyrenaica es más insaturado.


SUMMARY
Pattern recognition of acorns from different Quercus species based on oil content and fatty acid profile.

The aim of this study was (i) to characterize 3 different species of the Quercus genus and (ii) to discriminate among them on the basis of the content and fatty acid composition of the oil in their fruits and/or on morphological aspects of the fruits via pattern recognition techniques (Principal Component Analysis, Cluster Analysis and Discriminant Analysis). The following different species, grown in the same mixed stand in the center of Portugal, were investigated:

Quercus rotundifolia Lam., Quercus suber L. and Quercus pyrenaica Willd.

Pattern recognition methods were carried out to determine the set of measurements for sample characterization, i.e., to identify the pattern (Miller and Miller, 1993). Therefore, the available data were used simultaneously rather than sequentially and Principal Component Analysis (PCA), Cluster Analysis (CA) and Discriminant Analysis (DA) were performed on multivariate data.

PCA is an attempt to best describe the shape of a multivariate distribution by considering selected
linear combinations of the original variables rather than
the variables themselves (Bollinger, 1975). In addition,
with this technique, the initial m-dimensional space (m
variables) may be reduced to n dimensions (n<m)
without considerable loss of information (Harman,
1976; Hoffman and Young, 1983). The initial system
of m axis is replaced by another system where the
new axis are the principal components (Morrison,
1967; Piggott and Sherman, 1986). The first
component shows the maximum correlation with all
the variables and explains the highest proportion of
the global variance or to the multivariate analysis of
variance if the procedure is identical to the one-way analysis of
variables. If the means for a variable are
significantly different in different groups, then this
variable discriminates between the groups. In fact,
the procedure is identical to the one-way analysis of
variance or to the multivariate analysis of variance if
several variables are used.

2. MATERIALS AND METHODS

2.1. Materials

Ripened fruits were obtained, on the same date,
from adult trees of a mixed stand (Portalegre,
Portugal) with the following Quercus species: Q.
rotundifolia, Q. suber and Q. pyrenaica. Each sample
was collected from one individual tree, i.e., 10
samples of Q. rotundifolia, 11 samples of Q. suber
and 9 samples of Q. pyrenaica, were obtained;
n-hexane p.a. was used for oil extraction.

2.2. Methods

Morphological Characterization of the fruits: The
average length and width (cm) were measured for
100 fruits from each tree picked at random.

Oil extraction: Before oil extraction, the fruits were
dehulled, ground in a household coffee mill (knife
cutter type) and heated at 75°C for 90 minutes. The
oil from prepared material was extracted by
n-hexane p.a in a Soxhlet apparatus for 8 hours
(Ferreira-Dias et al., 2003). Experiments were
conducted in triplicate and the results were
expressed on a dry basis.

Chemical characterization of the oil: The fatty
acids profile of every oil sample was evaluated as
their methyl esters in a gas chromatograph (Carlo
Erba, Vega 2000 GC) equipped with a SUPELCO
capillary column (SP-W 2380, 0.2μm, 60 m x 0.25
mm; fused silica). Both detector and injector (FID)
were heated at 250°C. Temperature was
programmed as follows: 175°C for 25 minutes, a
slope of 5°C/min. from 175°C to 220°C and 220°C for
10 minutes. Hydrogen was the carrier gas at a
column head pressure of 60 kPa.

2.3. Statistical analysis

The experimental results, concerning the average
"length" and "width" of the fruits from each of the 30
trees studied, the average yield in oil and its fatty acid
composition, were put in a matrix form (Matrix A).
Samples were presented in rows and variables in
columns (matrix 30x8). A matrix 30 x 6 was
considered for statistical analysis by removing the
columns corresponding to the "length" and "width" of
the fruits (Matrix B).

Concerning pattern recognition, a Principal
Component Analysis (PCA) was first carried out on
the experimental data (matrices A and B). The
second stage of multivariate data analysis
consisted of a Cluster Analysis (CA) of the data
matrices A and B, in order to confirm the existence
of the groups suggested by the plot of the samples in
the reduced space defined by the significant
principal components.

Finally, a Discriminant Analysis (DA) was used on
data from matrix B, to determine which variables
discriminate between these groups a priori defined
(Morrison, 1967; Burgard and Kuznicki, 1990). The
model of discrimination was built step-by-step and a
forward stepwise analysis was followed. At each
In the next step, it was evaluated which variable would contribute most to the discrimination between groups. This variable would then be included in the model, beginning the next step. The maximum number of discriminant functions will be equal to the number of groups minus one or to the number of variables in the analysis, whichever is smaller. The best combination of variables for discriminant analysis includes variables that represent independent measures of product similarities and differences.

In addition, the Classification Functions can be used to determine to which group each case most likely belongs. The classification matrix shows the number of cases that were correctly classified and those that were misclassified.

PCA, CA and DA were performed by using the software "Statistica™, version 5, from Statsoft, USA.

3. RESULTS AND DISCUSSION

3.1. Sample Characterization by Principal Components Analysis

The average values of the oil content, respective fatty acid profile, average length and width of the fruit samples are shown in Table I.

Table I: Matrix A containing the average values of oil content (% w/w, dry basis), respective fatty acid profile (%), average length and width of Quercus fruit samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Species</th>
<th>Oil</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>C18:3</th>
<th>Fruit Length (cm)</th>
<th>Fruit Width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s1</td>
<td>suber</td>
<td>4.4</td>
<td>14.8</td>
<td>1.0</td>
<td>63.8</td>
<td>18.3</td>
<td>1.7</td>
<td>3.91</td>
<td>1.86</td>
</tr>
<tr>
<td>s2</td>
<td>suber</td>
<td>4.6</td>
<td>15.6</td>
<td>1.0</td>
<td>62.7</td>
<td>18.0</td>
<td>1.7</td>
<td>3.73</td>
<td>1.72</td>
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<tr>
<td>s3</td>
<td>suber</td>
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<td>14.8</td>
<td>0.95</td>
<td>62.6</td>
<td>19.1</td>
<td>1.7</td>
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<td>63.7</td>
<td>18.1</td>
<td>2.0</td>
<td>3.41</td>
<td>1.69</td>
</tr>
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<td>s8</td>
<td>suber</td>
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<td>16.6</td>
<td>1.1</td>
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<td>1.9</td>
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<td>1.69</td>
</tr>
<tr>
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<td>1.1</td>
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<td>17.6</td>
<td>2.1</td>
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<td>1.61</td>
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<td>2.1</td>
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<td>1.2</td>
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<td>3.72</td>
<td>1.86</td>
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<td>2.6</td>
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<td>1.1</td>
<td>3.47</td>
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<td>1.1</td>
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<td>2.0</td>
<td>3.64</td>
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<td>1.5</td>
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<td>3.71</td>
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<td>15.6</td>
<td>1.8</td>
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<td>28.2</td>
<td>1.8</td>
<td>3.63</td>
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<td>1.4</td>
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<td>pyrenaica</td>
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<td>15.1</td>
<td>1.4</td>
<td>48.5</td>
<td>32.2</td>
<td>2.0</td>
<td>3.94</td>
<td>1.96</td>
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<td>pyrenaica</td>
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<td>13.6</td>
<td>1.8</td>
<td>49.4</td>
<td>25.1</td>
<td>1.6</td>
<td>3.47</td>
<td>1.82</td>
</tr>
</tbody>
</table>
samples of Q. rotundifolia, Q. suber and Q. pyrenaica are shown in Table I, displayed in a matrix form (Matrix A). These data were first submitted to a PCA and the eigenvalues and respective variances of the extracted new axis (principal components) are shown in Table II. According to the criterion proposed by Kaiser, only principal components with eigenvalues greater than 1 must be retained for the analysis, since they explain more than the average variability accounted for by one original variable as meaningful (Dagneli, 1977; Burgard and Kuznicki, 1990). Therefore, the initial 8-dimensional space (defined by 8 variables) can be reduced to a plane, $F_1$-$F_2$, defined by the first two principal components since they have eigenvalues greater than one. This plane accounts for about 76% of the variance explained by the original data matrix A.

The correlations between the original variables and the first two principal components, i.e. the loadings of variables are in Fig. 1-A. The first axis is positively correlated with the concentrations of linoleic and linolenic acids (C18:2 and C18:3, respectively) and negatively with oleic acid (C18:1). The amounts of saturated fatty acids (palmitic, C16:0, and stearic acids, C18:0) are better correlated with the second principal component, increasing along it. They are also well correlated with the negative part of the first principal component, which plots both acids very close to the diagonal of the 2nd quadrant. The presence of the variables “Width” and “Length” of the fruits near to the diagonal of the first quadrant indicates that they are equally correlated with both axes. In order to redistribute the weightings of the variables so as to make them

Table II
Principal Component Analysis of matrix A- eigenvalues and variances explained by the principal components extracted from the data matrix A, considering the yield in oil, fatty acid composition and the “length” and “width” of the fruits (see text for details)

<table>
<thead>
<tr>
<th>Principal Component</th>
<th>Eigenvalue</th>
<th>Total Variance (%)</th>
<th>Cumulative Variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.35</td>
<td>54.31</td>
<td>54.31</td>
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<tr>
<td>2</td>
<td>1.73</td>
<td>21.63</td>
<td>75.94</td>
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<td>3</td>
<td>0.78</td>
<td>9.75</td>
<td>85.69</td>
</tr>
<tr>
<td>4</td>
<td>0.47</td>
<td>5.82</td>
<td>91.51</td>
</tr>
<tr>
<td>5</td>
<td>0.38</td>
<td>4.73</td>
<td>96.24</td>
</tr>
<tr>
<td>6</td>
<td>0.20</td>
<td>2.48</td>
<td>98.72</td>
</tr>
<tr>
<td>7</td>
<td>0.08</td>
<td>1.12</td>
<td>99.84</td>
</tr>
<tr>
<td>8</td>
<td>0.01</td>
<td>0.16</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 1
Principal Component Analysis of data from Matrix A- Loadings of morphological characteristics of Quercus fruit samples, oil content and respective fatty acid composition on the first and second principal components (Fig.1-A). Plot of the Quercus fruit samples (from Q. suber (s), Q. rotundifolia (i) and Q. pyrenaica (p)) on the plane defined by first and second principal components (Fig.1-B).
nearer to or farther away from each of the axes, rotation of principal components by Varimax technique was also performed (Burgard and Kuznicki, 1990). However, no improvement on interpreting principal components and/or clustering the samples was achieved.

When the samples were plot on the plane formed by the first and the second principal components, F1F2 (Fig. 1-B), the different samples seem to be grouped according to the species. The *Q. rotundifolia* fruit samples show the highest oil content and their oil is richer in saturated fatty acids (palmitic and stearic acids). Larger and longer fruits are observed for *Q. pyrenaica*. The lowest oil content is obtained for *Q. pyrenaica* acorns. This oil is richer in unsaturated fatty acids (mainly linoleic acid). The *Q. suber* fruits are in an intermediate position in the plane F1F2. This suggests that for *Q. suber*, both oil content and composition are between the values observed for *Q. rotundifolia* and *Q. pyrenaica* fruits.

As an attempt to better separate samples by species, the variables concerning the morphological characterization of the fruits were ignored on Matrix A (Table I.) and a second PCA on these smaller set of data (Matrix B) was performed. Similarly as obtained for Matrix A, the original information can be displayed on the plane F1F2, corresponding to the eigenvalues higher than the unity (Table III). This plane explains about 82% of the initial information contained in the matrix B. The loadings of the initial variables on first and second components (Fig. 2-A) are similar to those observed for Matrix A. With respect to the projection of the samples on the plane F1F2 (Fig. 2-B), a better separation of the clusters according to the species is obtained. Therefore, the length and width of the fruits showed not to be adequate parameters to

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**Table III**

Principal Component Analysis of matrix B- eigenvalues and variances explained by the principal components extracted from the data matrix B, corresponding to the data of matrix A concerning the yield in oil and its fatty acid composition

<table>
<thead>
<tr>
<th>Principal Component</th>
<th>Eigenvalue</th>
<th>Total Variance (%)</th>
<th>Cumulative Variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.73</td>
<td>62.17</td>
<td>62.17</td>
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<td>1.20</td>
<td>20.06</td>
<td>82.23</td>
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<td>0.23</td>
<td>3.82</td>
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<td>0.13</td>
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<td>6</td>
<td>0.02</td>
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**Figure 2**

Principal Component Analysis of data from Matrix B- Loadings of oil content of Quercus fruit samples and respective fatty acid composition on the first and second principal components (Fig.2-A). Plot of the Quercus fruit samples (from *Q. suber* (s), *Q. rotundifolia* (i) and *Q. pyrenaica* (p)) on the plane defined by first and second principal components (fig.2-B).
discriminate between samples from different species of the Quercus genus, as previously suggested by the results from the analysis of variance.

3.2. Sample Characterization by Cluster Analysis

In a second stage, a CA was carried out to investigate the feasibility of the clusters suggested by PCA. The hierarchical tree diagram (dendrogram) for the 30 Quercus samples described by the "oil content" and fatty acid composition (6 variables; Matrix B) is shown in Fig. 3. Similar dendrogram is obtained when data from Matrix A is used (not shown). This confirms the small power of "fruit length" and "fruit width" on discrimination and characterization of Quercus samples. For a linkage distance higher than 5, only two clusters can be defined: a Cluster corresponding to Q. pyrenaica fruits, and another Cluster where the sub-cluster of Q. rotundifolia fruits and the sub-cluster of Q. suber are joined together. For a linkage distance of 4, three clusters, corresponding to the different species, can be identified. Only an outlier from Q. pyrenaica (P9) was observed at this linkage level (Fig. 3).

3.3. Sample Characterization by Discriminant Analysis

After confirming by Cluster Analysis the existence of isolated groups of samples, a Discriminant Analysis was used to determine which variables discriminate between the groups a priori defined, corresponding to the 3 species. Due to the low discriminant power exhibited by the variables "Length" and "Width" of the Quercus fruits on the previous data analysis (PCA and CA) only the oil content of the fruits and their fatty acid composition (Matrix B) were used in Discriminant Analysis. Table IV summarizes the successive steps of the forward stepwise analysis. The highest the F-value of a variable, the highest is the discriminating power of that variable. Therefore, the most important variable to discriminate between Quercus species was oleic acid content followed by stearic acid, oil content and palmitic acid level.

The existence of the 3 entirely distinct Quercus species can be confirmed by the plot of the Quercus samples onto the plane formed by the two Discriminant Functions (canonical roots) found by canonical analysis (Fig. 4).

In addition, the following Classification Functions were established to define every group (species) and can be used to determine to which group each case most likely belongs:

![Figure 3](dendrogram.png)

**Figure 3**
Dendrogram of the Quercus fruit samples (from Q. suber (s), Q. rotundifolia (i) and Q. pyrenaica (p)) based on their oil content and fatty acid composition.

![Figure 4](discriminant.png)

**Figure 4**
Discriminant Analysis - Plot of 30 Quercus samples from 3 different species (•, Q. suber; ●, Q. rotundifolia; ○, Q. pyrenaica) on the plane defined by 2 canonical roots (discriminant functions).

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable to Enter</th>
<th>F to enter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C18:1</td>
<td>201.83***</td>
</tr>
<tr>
<td>2</td>
<td>C18:0</td>
<td>121.47***</td>
</tr>
<tr>
<td>3</td>
<td>Oil</td>
<td>23.89***</td>
</tr>
<tr>
<td>4</td>
<td>C16:0</td>
<td>9.93***</td>
</tr>
<tr>
<td>5</td>
<td>C18:3</td>
<td>1.39 (NS)</td>
</tr>
<tr>
<td>6</td>
<td>C18:2</td>
<td>1.24 (NS)</td>
</tr>
</tbody>
</table>
\[ Q. \text{rotundifolia} = (-4104.60) + 88.88[C18:1] + 53.93[OIL] + 71.58[C16:0] + 49.76[C18:0] + 48.81[C18:3] + 41.76[C18:2] \text{ (eq. 1)} \]
\[ Q. \text{suber} = (-3834.57) + 87.35[C18:1] + 44.04[OIL] + 68.62[C16:0] + 19.15[C18:0] + 59.61[C18:3] + 41.25[C18:2] \text{ (eq. 2)} \]
\[ Q. \text{pyrenaica} = (-3176.34) + 77.41[C18:1] + 34.67[OIL] + 59.36[C16:0] + 41.07[C18:0] + 34.91[C18:3] + 45.42[C18:2] \text{ (eq. 3)} \]

Where \([C16:0], [C18:0], [C18:1], [C18:2]\) and \([C18:3]\) are the concentrations (%) of palmitic, stearic, oleic, linoleic and linolenic acids, respectively; \([OIL]\) represents oil content of the acorns (% w/w), on a dry basis.

In fact, when the observed classifications were compared to the predicted by these classification functions no case was misclassified, i.e. all the cases were correctly classified (Table V).

3.4. Final Remarks

The results concerning oil content of \(Q. \text{suber}\) and \(Q. \text{rotundifolia}\) fruits and respective fatty acid profile are in agreement with those obtained by Ferrão and Ferrão (1988) for the same fruit species harvested from 1964 to 1986 in the Centre and South of Portugal. In fact, data from matrix A (Table I) show that acorns from \(Q. \text{rotundifolia}\) have an average oil content of 9.1% and \(Q. \text{suber}\), of 5.2%. The lowest values were observed for \(Q. \text{pyrenaica}\) fruits (average value of 3.8%).

Both for oils from \(Q. \text{rotundifolia}\) and \(Q. \text{suber}\) fruits, fatty acid profiles are within the variation range exhibited by olive oil fatty acids (Commission of the Codex Alimentarius, 1993). However, \(Q. \text{rotundifolia}\) fruit oil is richer in saturated fatty acids (palmitic and stearic acids) than the oil from \(Q. \text{suber}\) fruits. In addition, a slightly higher linolenic acid content was observed in \(Q. \text{suber}\) fruit oil (1.2-2.1%) when compared to its content in olive oil (maximum of 1.5%). Concerning \(Q. \text{pyrenaica}\) acorns, its oil is rather unsaturated and quite different from olive oil. The highest levels of linoleic (25.1-34.3%) and linolenic acids (1.4-2.3%) and the lowest oleic acid content (43.4-52.2%) were observed in the oil from this fruit.

As a main conclusion, the knowledge of oil content and respective fatty acid profile of \(Q. \text{rotundifolia}\), \(Q. \text{suber}\) and \(Q. \text{pyrenaica}\) fruits allows the identification of acorns from these species via pattern recognition techniques (PCA coupled with CA and DA).

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