Pinus sylvestris wood resin acids from trees with different provenances and contrasting resin content compared by GC-MS and PCA analysis

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Abstract

Pines trees produce resin in its wood in varying quantities, depending on species and environmental conditions. When injured or attacked by any means, pine trees can produce high quantities of resin. In *Pinus sylvestris* the wood resin is concentrated in the heartwood and, in normal conditions, in relatively low quantities, less than 5%. In the present work we analysed the resin acid content and composition of the wood resin of *P. sylvestris* trees from 4 provenances in north and central Portugal. The resin acids content found were very high, varying from 4 to 31% of the wood mass. The resin acid samples were pooled according to their resin acid content and provenance and analysed by GC-MS. The resin composition was dominated by dehydroabietic acid and its hydroxylated forms (80-90%), with much smaller quantities of abietic, pimaric and isopimaric acid. The GC-MS resin acid composition was analysed by PCA to detect eventual associations with resin acid content or tree provenance. The sample of lowest resin acid content, 4%, was discriminated mainly due to its exceptionally high dehydroabietic acid content; within the "16% resin acid" content group two provenances were separated due their very different phenolic acid content.

Introduction

The wood of pines (*Pinus* sp.) have different resin contents depending on the species, intra-species genetic variability, varying environmental conditions associated with provenance or forestry management. The total resin content in normal conditions does not exceed 5%, and typically has a value around 1% of the wood mass. However, when the tree suffer external aggressions, either physical or biotic, or other forms of injury, the wood in the affected areas can produce much higher amounts of resin. This

is exploited in industrial resin tapping, where wounds are cut down to the wood, and strong acids applied in the exposed surface, to enhance resin production by the trees.

Pine resins are dominantly composed by two main terpenoid components: a volatile fraction made mostly of monoterpenes (corresponding to the industrial "turpentine") and a mixture of diterpenic acids, also named resin acids (corresponding to the "rosin"). In the present work we analysed the resin acids from the wood of Scots pine (*Pinus sylvestris*) trees, obtained as the dichloromethane-soluble extract, For this work trees were sampled in northern Portugal, located in the westernmost point of the distribution area of *P. sylvestris*, which covers most of central and north Eurasia.

Most of the studies regarding the resin acids content and composition in *P. sylvestris*, wood, and their variability, (in the available literature), were made in tree populations in the Scandinavian countries (Manninen et al., 2002; Ekeberg el al., 2006; Hovelstad et al., 2006; Arshadi et al., 2013). Within-tree variability was found in the wood radial direction, showing that resin acids were mostly present in the heartwood: the resin acid content of the wood of *P.sylvestris* in northern Sweden averaged 1.5% in the heartwood, and less than 0.3% in the sapwood, with a mean of 1% for the total wood (Arshadi et al., 2013); in Central Norway the values for resin acid content in sapwood and heartwood were 0.3%-1.1% and 1.4-4.4%, respectively (Hovelstad et al., 2006). An interesting point in the Portuguese *P.sylvestris* samples analysed here is that they have abnormally high resin contents when compared to the ones from the Scandinavian countries just mentioned.

According to the reviewed literature, the resin acid composition in *P.sylvestris* wood is dominated by the abietane-type diterpenic acids, namely abietic, neoabietic, levopimaric, palustric and dehydroabietic acids, which together represent 80% or more of the diterpenic acids mixture. The remaining is made of pimarane-type resin acids, namely pimaric and isopimaric acids. Abietic acid can be the more important component, with percentages of more than 40% of the resin acids mixtures (Manninen et al., 2002), but other results show levopimaric and palustric acids with high percentages (Ekeberg el al., 2006) or dehydroabietic acid as the dominant one (Ekeberg el al., 2006). The resin acid composition is affected by the trees provenance (Manninen et al., 2002; Arshadi et al., 2013), site altitude, part of the tree sampled and even the wood particle size from where the resin acids were extracted (Ekeberg el al., 2006).

Most of these analyses, normally made by GC-MS, face however a question: the abietane-type resin acids abietic, neoabietic, levopimaric and palustric easily isomerize among them – they only differ by the position of the conjugated two double bonds within the ring system of their otherwise identical structure (see Figure 1 in the NMR part of the Report). Also, all of the later can be dehydrated to generate dehydroabietic acid (DHA). Further, DHA can be oxidized by heat or even in ambient conditions, giving rise to a number of hydroxylated and keto DHA derivatives. So, in practise, the resin acid composition is highly dependent on the story of the manipulation of the resin acid extracts, experimental set up and analytical conditions.

Here we present the GC-MS analysis of *P. sylvestris* resin acid extracts from the wood of trees located in 4 sites in northern Portugal (which we call 4 provenances). The wood of these trees had contrasting resin acid content, with values in most cases much higher than the ones found in Scandinavian trees (mentioned above). For GC-MS analysis some of the resin acid extracts were pooled in groups of identical resin acid content and/or provenance (as described in Material and Methods). The composition of the analysed extracts, was dominated by the dehydrated and oxidized forms of the resin acids, namely DHA and its derivatives, showing the effects of the experimental conditions used. The resin acid composition of the different group samples was compared by a PCA analysis to detect eventual associations related to resin acid content or tree provenance, and the respective results are discussed.

Material and Methods
Wood material. Wood samples were taken from *Pinus sylvestris* (Scots pine) trees located in four provenances in northern Portugal: Serra do Gerês (8 trees sampled), Serra da Peneda (3), Vinhais (3) e Serra da Estrela (5). The wood samples were taken from the living trees with an increment (Pressler) borer at 1.30 height. The wood bores included all the radial growth, from the pith to the cambial zone. The wood samples were air dried and ground in a Retsch SM 2000 knife mill to pass a 6 mm sieve, and further ground in a Retsch ZM 100 ball mill to pass a 0.5 mm reverse sieve.

Resin extraction. Aliquots of the wood ground materials (1.5 g) were Soxhlet-extracted with dichloromethane (6 hours) in ANKOM filter bags (ANKOM Technology, New York, USA). The resin content was assessed by the weight loss after the dichloromethane extraction and drying the extracted material.

GC-MS analysis. For GC-MS analysis the wood resin fractions, in form of the dichlorometane extracts, were grouped in 8 classes with similar resin content, and identical provenance, as follows:

4% Class – Serra da Peneda – 1 sample (Tree reference ***30***, 4.3% Extract)

7% Class – Serra do Gerês – 3 samples (***41***, 5.9%; ***44***, 7.7%, ***54***, 7.0%)

11% Class – Serra do Gerês – 3 samples (***46***, 10.8%; ***47***, 12.6%, ***51***, 11.0%)

16% Class – Serra da Estrela – 2 samples (***66***, 17.2%; ***75***, 16.2%)

16% Class – Vinhais – 3 samples (***2***, 17.3%; ***9***, 15.8%, ***11***, 17.0%)

20% Class – Serra da Estrela – 3 samples (***67***, 21.0%; ***72***, 20.7%, ***77***, 19.4%)

28% Class – Serra do Gerês – 1 sample (***45***, 28.1%)

31% Class – Serra do Gerês – 1 sample (***49***, 31.3%)

Aliquots from the described mixed dried extracts (1 mg) were derivatized with 20 uL of Pyridine and 20 uL of BSTFA, and heated in an oven at 60ºc for 30 min before analysis. GC-MS were carried out in an Agilent Technologies 78990A/5975C MSD, with the following GC conditions: injector 320ºC; Initial temperature 80ºC (5 min), 5ºC/min up to 110ºC, 20ºC/min up to 250ºC, and 8ºC/min up to 320ºC (15 min).

PCA analysis.

PCA was performed using the UnscramblerTM X Vsn. 10.4.1 (CAMO). Prior PCA the percentage of each peak from the pyrogram (the area of a peak divided by the sum of the area of all used peaks multiplied by 100% was mean centred and standardized (weighted by the standard deviation of each variable/peak).

Results and Discussion

The results of the GC-MS analysis of the 8 group samples from the wood resin of *P. sylvestris* trees grown in Portugal are shown in Table 1. The respective GC-Ms chromatograms are shown in Figure 1.

Table 1. Composition of *Pinus sylvestris* wood resin from 4 provenances in northern Portugal and 8 resin-content groups, analysed by GC-MS (compounds as TMS derivatives). Pen – Serra da Peneda; Ger – Serra do Gerês, Est – Serra da Estrela; Vin – Vinhais. % values – resin content (dichloromethane extract); DHA - Dehydroabietic acid
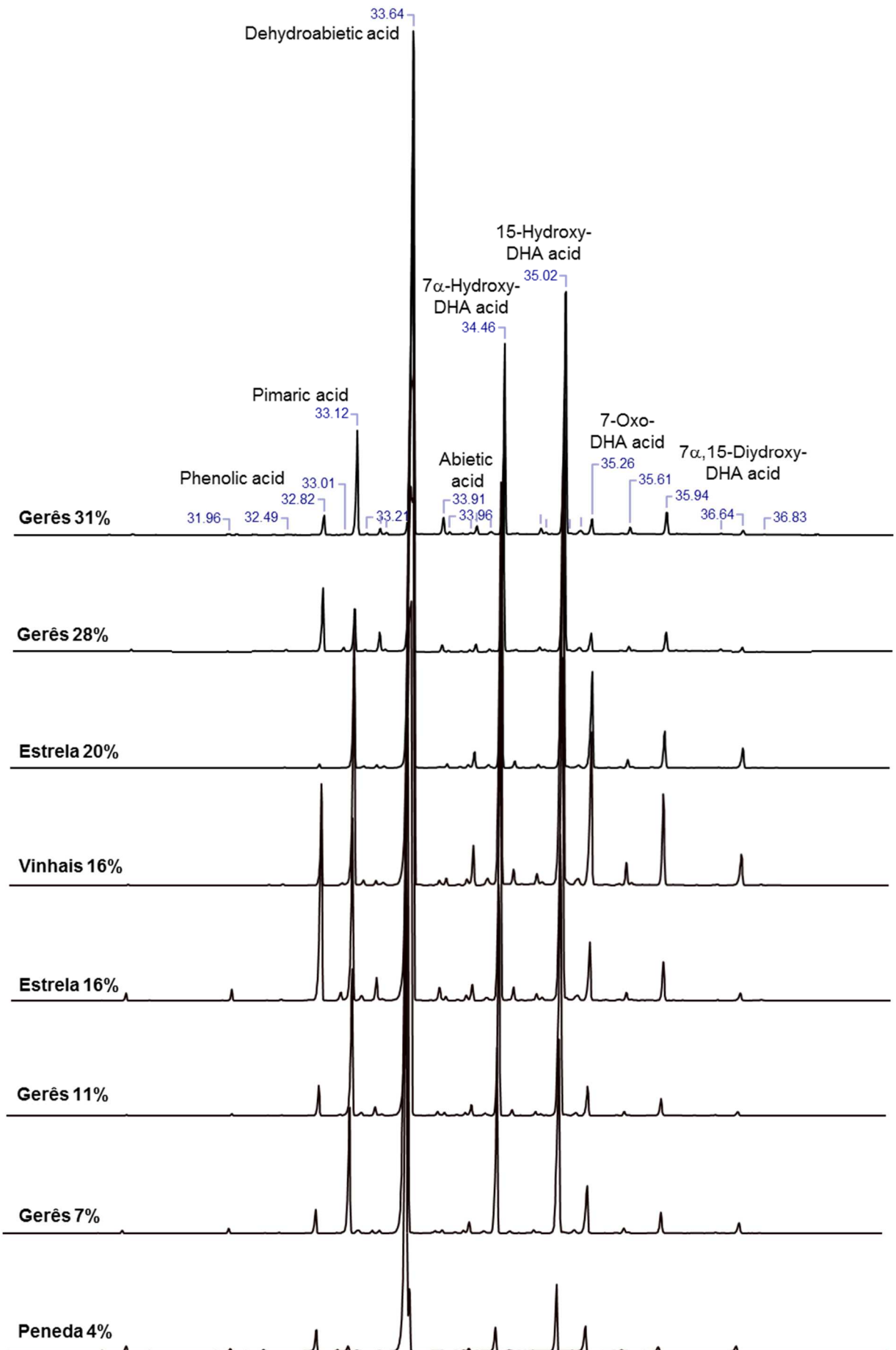
	Pen	Ger	Ger	Est	Vin	Est	Ger	Ger
	4%	7%	11%	16%	16%	20%	28	31%

Phenolic acid[1]	3.5	1.4	1.3	8.1	1.4	0.2	4.2	1.3
Pimaric acid	0.8	7.3	6.8	5.7	7.4	4.6	2.9	7.1
Isopimaric acid	0.1	0.2	0.4	0.7	0.1	0.2	1.2	0.5
Dehydroabietic acid	70.2	59.3	60.2	49.7	46.3	56.6	54.2	50.3
Abietic acid	0.0	0.2	0.2	0.5	0.2	0.2	0.5	1.3
7β-Hydroxy-DHA	0.4	0.7	0.5	0.5	1.0	0.8	0.5	0.6
7α-Hydroxy-DHA	3.0	9.7	10.4	12.3	13.4	9.9	11.6	12.3
?-Hydroxy-DHA	0.0	0.2	0.2	0.2	0.3	0.2	0.3	0.4
15-Hydroxy-DHA	7.7	13.4	15.6	16.3	18.9	17.2	19.5	20.3
7-Oxo-DHA	3.5	3.2	1.5	1.9	4.8	5.3	1.2	1.2
7β,15-Dihydroxy-DHA	0.2	0.3	0.2	0.2	0.6	0.4	0.3	0.5
7α,15-Dihydroxy-DHA	0.6	1.2	0.8	1.3	2.5	1.8	1.4	1.8
15-Hydroxy-7-oxo-DHA	0.8	0.7	0.3	0.3	1.0	1.2	0.3	0.4
NID[2]	9.3	2.1	1.6	2.3	2.1	1.6	1.9	2.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] Mass spectrum compatible with 3,4,5-Methoxyphenylacetic acid, but not positively identified

[2] Non-identified compounds

Dehydroabietic acid — 33.64

15-Hydroxy-
DHA acid — 35.02

7α-Hydroxy-
DHA acid — 34.46

Pimaric acid — 33.12

Abietic
acid — 33.91

7-Oxo-
DHA acid — 35.26
35.61

7α,15-Diydroxy-
DHA acid
36.64   36.83

Phenolic acid
33.01
32.82

Gerês 31%   31.96   32.49   33.21   33.96   35.94

Gerês 28%

Estrela 20%

Vinhais 16%
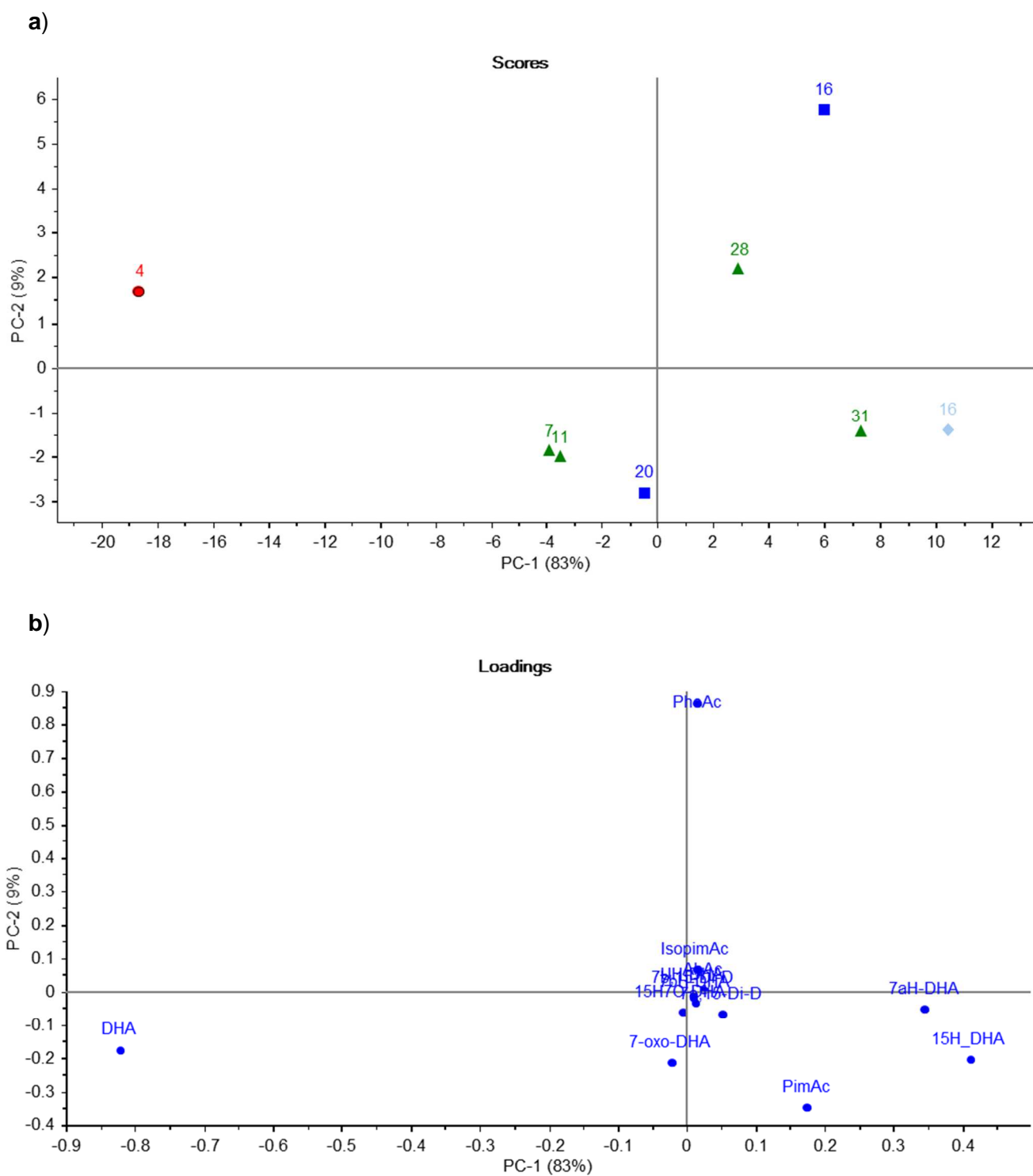
Estrela 16%

Gerês 11%

Gerês 7%

Peneda 4%

Figure 1. GC-MS TIC chromatograms of *Pinus sylvestris* wood resin acids from 4 provenances in northern Portugal and 8 resin-content groups (compounds as TMS derivatives)

In the analysed Portuguese *P. sylvestris* trees the observed variability in the total resin acid content is very high, from 4% (Serra da Peneda), up to 31% (Serra do Gerês). Only the lowest value is in consonance with the ones known for the resin acid content of the wood *of P. sylvestris* in other regions, namely in Scandinavia (see Introduction); all the others can be considered high, or even abnormally high in comparison. The reason(s) behind these very high percentages of resin acids in these Portuguese samples were not elucidated. However we can speculate that genetics can have a role, since the Portuguese trees sampled are in the limit of the distribution area of this pine, very far away from its core region, in central and northern Eurasia. Also climate may have a role: all the Portuguese sites sampled are mountainous locations, with hot summers and wet and cold winters; this climatic context can be significantly different from the one found in the other regions where this pine is common, typically with much less summer heat, and very cold winters. Also, some undisclosed systematic stress or biotic aggression can be behind the very high resin content observed in the Portuguese *P. sylvestris* trees.

The resin acid composition of the resin extracts analysed is also atypical: the Portuguese *P. sylvestris* resin acids mixtures were dominated by dehydroabietic acid (DHA) and their oxidized forms; other abietanane-type resin acids like abietic acid and the pimarane-type pimaric and isopimaric acid were found in small relative quantities (Table 1). This means that the analysed dichloromethane extracts, as the resin acid mixtures were obtained, were in contact with the air and at the same time heated, leading to the dehydration/oxidation observed. This could have happened during the intense drying of the extracts (100ºC, 24 hrs).

The PCA analysis (Figure 2) shows some discrimination of the lowest resin content observed, 4% in the sample from Serra da Peneda. The very high percentage of dehydroabietic acid in this ample explains this separation. Also, comparatively higher percentages of the one phenolic acid found, not a resin acid, lead to the separation observed of the 16% resin content group (two trees) from Serra da Estrela, significantly apart in the Scores graph from the other 16% group from Vinhais (3 trees), eventually showing a provenance-based effect in the resin acid composition.

a)

b)

Figure 2. PCA of the *Pinus sylvestris* resin acid composition as analysed by GC-MS: a) Location of the group samples along the two first Principal Components (Scores), *numbers* show resin acid content; *colours* show provenances (*dark blue* Estrela, *red* Peneda, *green* Gerês, *light blue* Vinhais); b) Resin acids explaining the observed variance along the two first Principal Components (Loadings)

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