Comparison in extractives chemical signatures between branch, knot and bark fractions from forestry and agroforestry walnut trees

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Introduction

Walnut agroforestry systems require regular tree pruning, generating a large volume of biomass residues. These agro-forest wastes are today mainly valorized as wood-energy, Ramial Chipped Wood (RCW) or as animal litter (Terrasse et al. 2021). However, walnut is recognized as a rich source of different extractives compounds, which could be recovered as valuable chemicals. In the Framework of the Agrobranch project (Ademe, Graine, 2018-2022), this study aims to improve the knowledge about the composition of the water and ethanol extractives contents of wood, knot and bark fractions from walnut branches, harvested in agroforestry (AF) and forestry control (FC) systems. LC-MS analyses were carried out to identify the chemical composition between all the sample modalities. Additionally, all samples were analyzed by NIR-Spectroscopy with the aim of developing a fast system to assess the branch wood properties from agroforestry and forestry walnut branches, knots and bark wood fractions.

Materials and methods

The experiment was carried out at the Restinclières farm in southern France (43°420 N, 3°510 E and elevation 61 m). The two plots with 25 years old walnuts, an Agroforestry plot (AF, with 140 walnuts) and a Forestry Control plot (FC, with 235 walnuts), are presented in Figure 1. As specified in Figure 1, two hybrid walnut (*Juglans nigra* × *Juglans regia*) trees were sampled from agroforestry (AF) and forestry control (FC) plots.

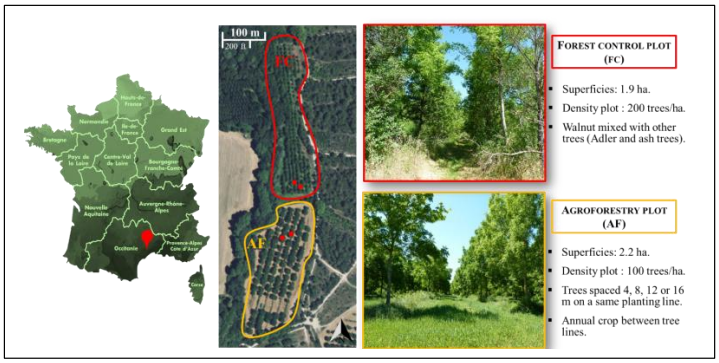

Fig.1: Walnut trees selection in the Agroforestry (AF) and Forestry Control (FC) plots at the Restinclières farm in southern France.

The detailed sampling of Figure 2 is presented for one studied tree. A similar samples repartition was performed for each trunk and branches from AF and FC plots. So, the total number of samples, including branch and trunk wood, knot wood and bark, used for this study is 195. All of these 195 wood samples were ground in powder (0.2 mm and 0.5 mm) before analyses by NIR-Spectroscopy [MicroNIR OnSite-W, VIAVI Solutions Inc.]. Then all samples were extracted with successives sohxlet extraction process using water and ethanol [32221-M; HoneyWell, Germany] solvents. For each extraction step, the extractives rates were determined. Finally, only a representative selection of 14 Ethanol-extracted samples were characterized by LC-MS analyses [Shimadzu LC-20A ultra-HPLC system, Kyoto, Japan].

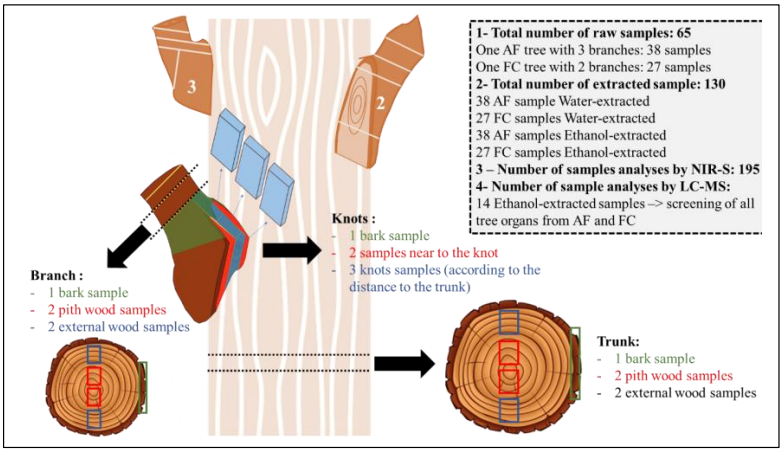

Fig.2: Sampling, processing and selection of wood samples.

Results and Discussions

Extractives contents of AF and FC specimens are not significantly different for branch, knot and bark. However, PLS-DA models developed with NIRS measurements showed that chemical differences exist between AF and FC samples, and these differences in composition (even low) is sufficient to distinguish wood-knots and bark specimens from different forestry systems. Figure 3 highlighted that PLS-DA models based on treated NIR signatures are efficient for classifying walnut wood specimens from forest control (FC) and agroforestry plots (AF).

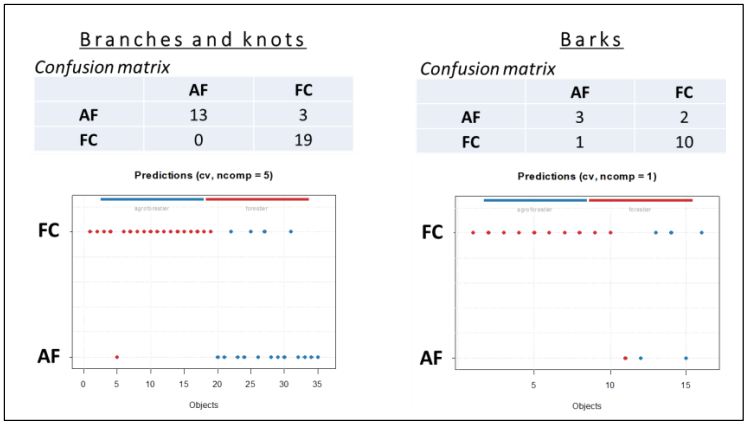

Fig.3: Discriminant analyses (PLS-DA) classifying un-extracted branch, bark and knots samples from AF and FC.

For both forestry systems, branch and knot extractive contents are significantly lower than those of bark specimens. These results are confirmed by the Principal Component Analyses (PCA) highlighting that the chemical composition of branch and knot woods are similar to each other and very different compared to those of bark samples (Figure 4).

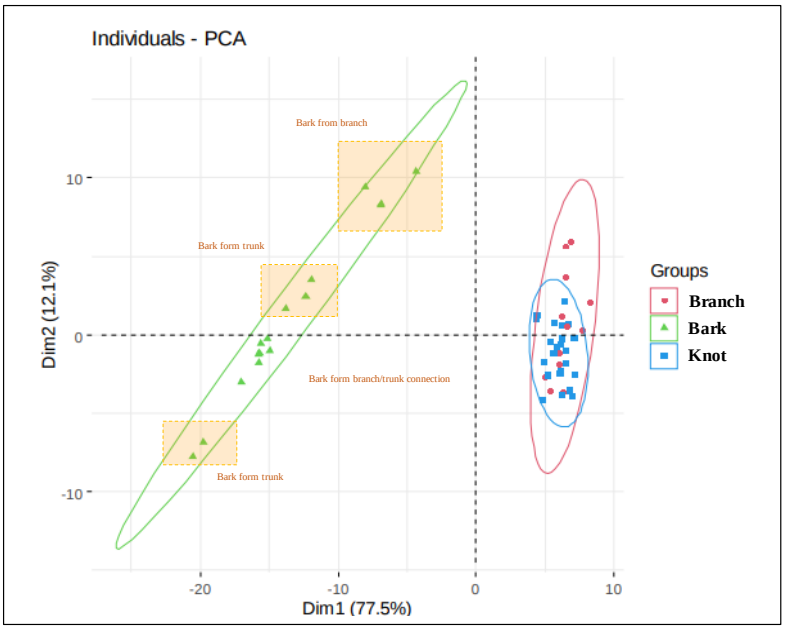

Fig.4: Individuals graph of a PCA led on the branch, knot and bark wood fractions (before extraction) of walnut tree from forestry and agroforestry systems.

LC-MS analyses highlighted that main components of ethanol-extracts of AF and FC branch wood were lignans following by sterols and flavonoids. Ethanol-extracts from knots specimens are mainly composed by lignans following by polyphenols, polyssacharides and flavonoid compounds. Finally, ethanol-extracts from bark specimens are mainly represented by flavonoids components following by polyphenols and sterols. In addition, the chemical composition of ethanol-extracts of bark samples depends on the bark position in the tree: branch, branch-trunk junction and trunk. The ethanol-extractives chemical compositions of branch and knot woods were slightly different for AF and FC samples. Whereas their difference in chemical composition between AF bark and FC bark were most marked, specifically concerning the flavonoids compounds.

Tab. 1 : List of chemical compounds, identified by LC-MS analysis, present in the ethalol-extracts from AF and FC branch, knot and bark fractions.

Chemical compounds	Scheme	Molar Weight ($g.mol^{-1}$)	Presence in ethanol-extracted samples*				Types Of Compounds
			Modality	Branch	Knot	Bark	
Malic acid		134	AF	-	--	-	carboxylic acids
			FC	-	Ø	-	
Gallic acid		170	AF	Ø	+	-	Phenolic acids
			FC	Ø	+	-	
Syringic acid		198	AF	Ø	Ø	++	Phenolic acids
			FC	Ø	Ø	++	
Sakuranetin		286	AF	Ø	Ø	++	Flavonoïds
			FC	Ø	Ø	++	
Quercetin		302	AF	+	+	++	Flavonoïds
			FC	+	+	+	
Rhamnetin		316	AF	-	+	Ø	Flavonoïds
			FC	-	Ø	++	
3,7-diméthylquercetin		330	AF	- -	-	+	Flavonoïds
			FC	-	+	Ø	
Mono-O-galloyl-glucose		332	AF	-	+	-	Phenolic acids glycoconjugate
			FC	-	+	-	
Balanophonin		356	AF	Ø	+	Ø	Lignans
			FC	Ø	+	Ø	
Secoisolariciresinol		362	AF	++	++	Ø	Lignans
			FC	++	++	Ø	
Oleuropein-aglycone		378	AF	Ø	++	Ø	Phenolic acids
			FC	Ø	++	Ø	
Quercitrin		448	AF	Ø	Ø	++	Flavonoïds
			FC	Ø	Ø	++	
Isoquercitrin		464	AF	Ø	Ø	+	Flavonoïds
			FC	Ø	Ø	+	
Campesterol		472	AF	+	Ø	+	Sterols
			FC	+	Ø	++	

* ++ present in high quantity; + present in medium quantity; - present in low quantity; -- present in trace; Ø absent.

Conclusion

This study provides new knowledge on branch woods from agroforestry systems, which are still very under-studied at present. The results obtained make it possible to highlight the evolution and variability of the chemical characteristics of wood branches from agroforestry walnut wood compared to the same species grown in forestry systems.

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