

## Supplementary information

# Rapid characterisation of hypanthium and seed in wild and cultivated rosehip: application of Raman microscopy combined with multivariate analysis

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**Figure S1.** Raw **average** Raman spectra of the individual hypanthium samples 1H-10H

**Figure S2.** Raw **average** Raman spectra of the individual seed samples 1S-10S

**Table S1.** Vibrational bands and their assignments in average spectra (Figures 2 and 3) collected from tissue sections of studied rosehip hypanthium and seed samples, and literature data.

### References

Hyanthium

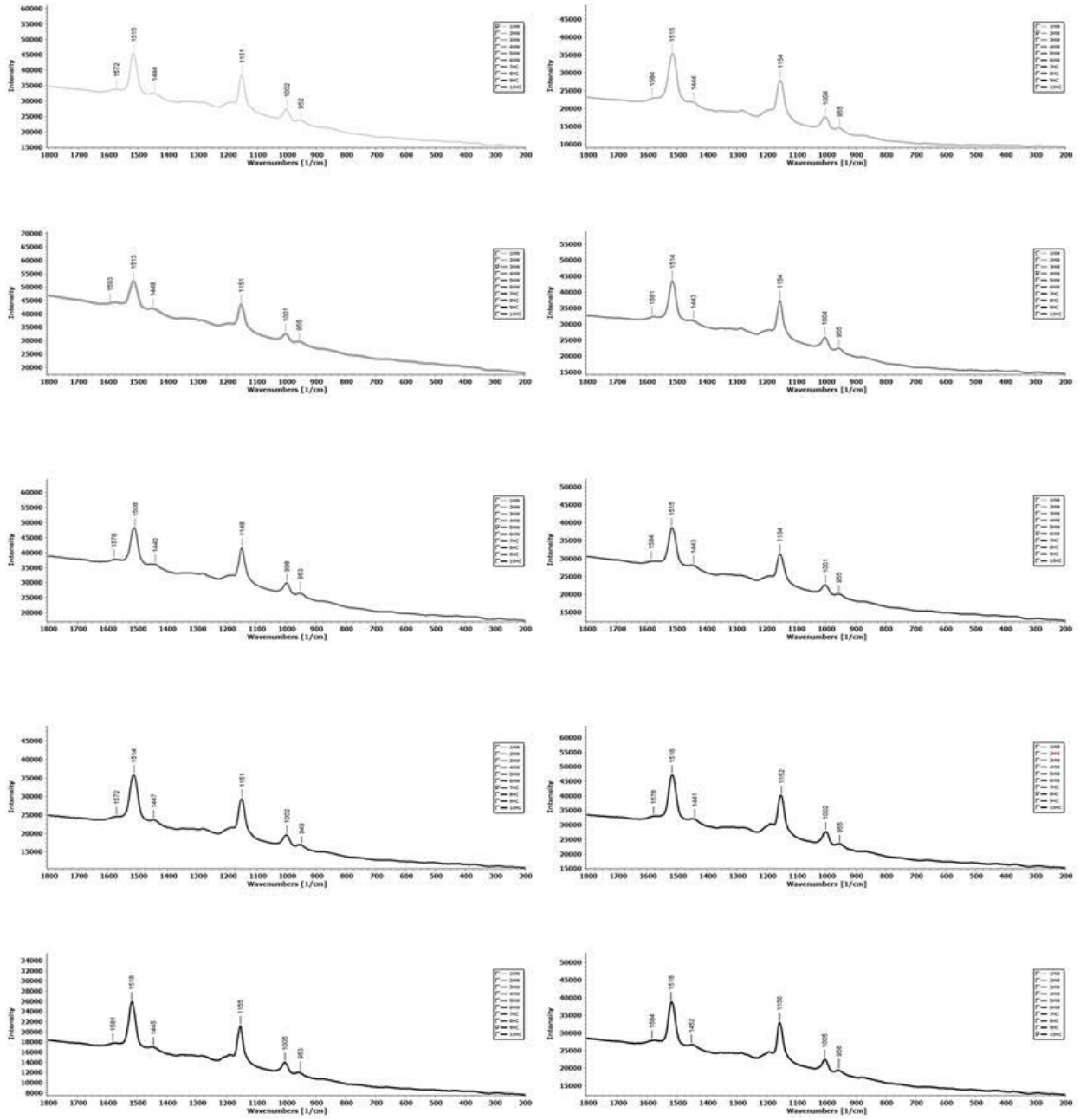


Figure S1. Raw average Raman spectra of the individual hyanthium samples 1H-10H



**Table S1.** Vibrational bands and their assignments in average spectra (Figures 2 and 4) collected from tissue sections of studied rosehip hypanthium and seed samples and literature data.

Hypantium	Seed	Literature data	Vibrational mode	Chemical moiety	Reference
Wave number (cm <sup>-1</sup> )					
	328	336 (rosehip)	$\delta(\text{C-C-C})$	Glucosidic ring	[25]
		360 (black spruce milled-wood)		Lignin	[64]
	372	372 (orange peel)		Polygalacturonic (pectic) acid	[42]
		380 (pure compound)	$\delta(\text{C-C-C})$ ring	Celulose	[46]
		378 (rosehip seed)	$\delta(\text{C-C-C})$	Glucosidic ring	[25]
	413	415 (pure compound)	$\delta(\text{C2-C1-O1})$ bending	$\alpha$ -Glucose	[35]
	460	441 (orange peel)	T(C-O-C) def	Polygalacturonic (pectic) acid	[42]
		479 (pure compound)	C-C-O and C-O-C	Glycosidic ring skeletal deformations	[46]
		480	$\nu(\text{C-O-C})$ Glycosidic	Pectin	[45]
	483	480	C-C-O and C-C-C Deformations; Related to glycosidic ring skeletal deformations	Carbohydrates	[26]
			$\delta(\text{C-C-C}) + \tau(\text{C-O})$ scissoring of C-C and out-of-plane bending of C-O		
		486 (orange peel)		Polygalacturonic (pectic) acid	[42]
		520	$\nu(\text{C-O-C})$ Glycosidic	Cellulose	[65, 45]
		522	$\delta_{\text{ip}}(\text{COC})$	Glucosidic ring	[25]
	523	527 ( fructose)	CCO, CCC and OCO deformations	Fructopyranose	[48]
		537 (orange peel)		Polygalacturonic (pectic) acid	[42]
	610	620	Phenylalanine (skeletal)	Protein	[35]
	625-7	631, 634	Ring deformation	Fructose (pure compound)	[35]
		686 (orange peel)	Low frequency vibrations of pyranoid ring	Polygalacturonic (pectic) acid	[42]
	698,721	709	Skeletal vibration	Fructose	[35]
		710 (orange peel)	$\square\gamma(\text{C-OH})_{\text{ring}}$	Polygalacturonic (pectic) acid	[42]
		747	$\gamma(\text{C-O-H})$ of COOH	Pectin	[42, 45]
		789	$\nu(\text{CO})_{\text{ring}}$ , $\beta(\text{COC})$ , $\beta(\text{CCO})$ , $\beta(\text{OCO})$		[48]
	781				

	800	v(C-C)	Fructose, sucrose	[35]
	840	vs(C-C)	Linoleic acid	[35]
	843	$v_s(\text{CO})_{\text{ring}}$ , v(CC), v(CO)	L(+)- Arabinose	[48]
843	849–853	(C6–C5–O5–C1–O1)	Pectin	[66, 45]
	856 (tomato)	(COC) skeletal mode of $\alpha$ -anomers	Pectin	[46]
	890 (sea weed)	v(CC)stretching	$\beta$ -Galactose	[48]
894	899 (rosehip seed)		Glucosidic ring	[25]
	917	v(C-O-C) in plane, symmetric	Cellulose, phenylpropanoids	[65]
	917		Carbohydrates	[45]
931	922, 928	$\rho(\text{CH}_3)$	Methyl and acetyl ester groups respectively, in pectins	[42]
955	953 (orange peel)	$\delta(\text{CCH})$ , $\delta(\text{COH})$	Polygalacturonic (pectic) acid	[42]
	967	964	Aliphatic groups	[45]
		971 (tomato)	Cellulose	[46]
	1000		Carotenoids	[45]
1003	1004 -1005 (rosehip carrot, pepper)	v(C-C)	Carotene	[25]
	1008	(C–CH <sub>3</sub> ) rocking	$\beta$ -Carotene (pure compound)	[37]
	1010	1000-1005	In-plane CH <sub>3</sub> rocking of polyene aromatic ring of phenylalanine	[45]
	1037	1030 (orange peel)	Carotenoids	[67]
		1037 (black spruce)	Protein	[45]
			Polygalacturonic (pectic) acid	[42]
			Carbohydrates	[68]
		1078	Linoleic acid (pure compound)	[35]
		1086		[25]
1092	1090 (tomato)	$v(\text{C-O-C})$ glycosidic, asymmetric	Cellulose	[46]
	1095 (rosehip)	□		[25]
	1090 (carrot)		Glycosidic bonds in cellulose	[69]
	1098 (tomato)	v(C-O-C)		[46]
	1092 (cucumber)			[44]
	1115–1119	Sym v(C-O-C), C-O-H bending	Cellulose	[65,45]
	1120 (tomato)	v(C-O-C) glycosidic, symmetric	Cellulose	[46]
	1119	1121 (tomato)		[46]
		1122 (carrot)		[69]
		1124 (rosehip)	Glycosidic bonds in cellulose	[25]
1153	1145	v(COC)glycosidic bond, ring	Polygalacturonic (pectic) acid	[42]

	1048		Celulose	[45]	
	1155		Carotenoids	[45]	
	1156 (cucumber)			[44]	
	1157 (pumpkin)	$\nu(\text{C-C})$	Carotene	[37]	
	1156	$\nu(\text{C-C})$	$\beta$ -Carotene (pure compound)	[35]	
	1157 (rosehip)	$\nu_s(\text{COC})$		[25]	
	1155	C-C Stretching; $\nu(\text{C-O-C})$ , $\nu(\text{C-C})$ in glycosidic linkages, asymmetric ring breathing	Carotenoids, carbohydrates	[67] [48]	
	1224	1218	$\delta(\text{C-C-H})$	Aliphatics, xylan	[68, 70]
		1254 (orange peel)	$\delta(\text{CH})$	Polygalacturonic (pectic) acid	[42]
	1264	1265, 1270 (rosehip)	$=\text{C-H}$	<i>Cis</i> - Lipids	[25]
		1265	$-\text{C}=\text{C}-$	Unsaturated fatty acids	[45, 49]
1281		1286	$\text{CH}_2/\text{CH}_3$ vibrations	Aliphatic groups	[45]
	1300	1301	$\delta(\text{C-C-H}) + \delta(\text{O-C-H}) + \delta(\text{C-O-H})$	Carbohydrates	[26]
		1327	$\delta\text{CH}_2$ bending	Aliphatics, cellulose, phenylpropanoids	[45, 65]
	1330	1330 (orange peel)	$\delta(\text{CH})$	Polygalacturonic (pectic) acid	[42]
		1332		$\alpha$ -anomer of glucose	[35]
		1375		$\alpha$ -anomer of glucose	[35]
	1374	1378	$\delta_s(\text{CH}_3)$	Methyl ester groups in pectins	[42]
		1440	$\nu(\text{C-C})$	Linoleic acid	[35]
1443		1443	$\text{CH}_2/\text{CH}_3$ vibrations	Aliphatic groups	[45]
		1444 (rosehip)	$\delta(\text{CH}_2)$	Lipids and glucosidic signal	[25]
		1440	$\text{CH}_2$ Scissoring vibration	Carotenoids	[35]
		1451, 1456 (rosehip)	$\delta(\text{COH})$	Glucosidic signal	[25]
	1453	1455 (apple/pear pulp)	$\delta_{as}(\text{CH}_3)$	Fructose	[47]
		1458, 1440	$\delta(\text{CH}_2) + \delta(\text{CH}_3)$	Methyl and acetyl ester groups in pectins, respectively	[42]
		1513	$\nu \square \square \text{C C} \square$ Stretching	$\beta$ -Carotene	[35]
1514		1520 (rosehip)	$\nu(\text{C}=\text{C})$	Carotene	[25]
		1524 (pumpkin)			[37]
		1526	$-\text{C}=\text{C}-$ (in plane)	Carotenoids	[45, 50]
1576		1576 (citrus fruit)	$\nu(\text{C}=\text{C})$	center of polyene chain	[71]

1596	1601 (rosehip)	v(C=C)	Phenolic compounds	[25]
	1598 (cucumber)			[44]
	1606-1632	v(C-C) Aromatic ring + $\sigma$ (CH)	Phenylpropanoids	[72, 73]
	1606		Phenylpropanoids (including lignin)	[45]
1654	1658 (rosehip)			[25]
	1656 (rapeseed)			[74]
	1656-1658 (coconut, babassu, peanut, grape)	v(C=C)	Unsaturated fatty acids assigned to <i>cis</i> isomer and lignin	[63]
	1658 (cucumber)			[44]
	1654-1660	-C=C-	Unsaturated fatty acids	[49]
	1654-1660	C=O Stretching, amide I	Proteins	[50]
1694	1600-1700 (soy and whey protein isolates)		Amide I band (predominantly $\beta$ -sheet)	[35]
	1654-1660		Amide I band – proteins	[40]
	1660		Proteins	[45]
	1680-1715	C=O	in nucleic acids	[40]
	1682		Carboxylic acids	[45]

## References

- [64] Agarwal, U. P. Analysis of cellulose and lignocellulose materials by Raman spectroscopy: A review of the current status. *Molecules*, **24**, 1659, <https://doi.org/10.3390/molecules24091659> (2019).
- [65] Edwards, H. G., Farwell, D. W. & Webster, D. FT Raman microscopy of untreated natural plant fibres. *Spectrochim. Acta A* **53**, 2383–2392, [https://doi.org/10.1016/S1386-1425\(97\)00178-9](https://doi.org/10.1016/S1386-1425(97)00178-9) (1997).
- [66] Engelsen, S. B. & N rregaard, L. Comparative vibrational spectroscopy for determination of quality parameters in amidated pectins as evaluated by chemometrics. *Carbohydr. Polym.* **30**, 9–24, [https://doi.org/10.1016/S0144-8617\(96\)00068-9](https://doi.org/10.1016/S0144-8617(96)00068-9) (1996).
- [67] Schulz, H., Baranska, M. & Baranski, R. Potential of NIR-FT-Raman spectroscopy in natural carotenoid analysis. *Biopolymers*. **77**, 212–221, <https://doi.org/10.1002/bip.20215> (2005).
- [68] Agarwal, U. P. 1064 nm FT-Raman spectroscopy for investigations of plant cell walls and other biomass materials. *Front. Plant. Sci.* **5**, 1–12, <https://doi.org/10.3389/fpls.2014.00490> (2014).
- [69] Baranski, R., Baranska, M. & Schulz, H. Changes in carotenoid content and distribution in living plant tissue can be observed and mapped *in situ* using NIR-FT-Raman spectroscopy. *Planta*. **222**, 448–457, <https://doi.org/10.1007/s00425-005-1566-9> (2005).
- [70] Yu, M.M.L., Schulze, H.G., Jetter, R., Blades, M.W. & Turner R.F.B. Raman microspectroscopic analysis of triterpenoids found in plant cuticles. *Appl. Spectrosc.* **61**, 32–37 [10.1366/000370207779701352](https://doi.org/10.1366/000370207779701352) (2007).
- [71] Nekvapil, F., Brezestean, I., Barchewitz, D., Glamuzina, B., Chiş, V., & Pinzaru, S. C. Citrus fruits freshness assessment using Raman spectroscopy. *Food chemistry*, **242**, 560-567. <https://doi.org/10.1016/j.foodchem.2017.09.105> (2018).
- [72] Kang, L., Wang, K., Li, X. & Zou, B. High pressure structural investigation of benzoic acid: Raman spectroscopy and x-ray diffraction. *J. Phys. Chem. C.* **120**, 14758–14766, <https://doi.org/10.1021/acs.jpcc.6b05001> (2016).
- [73] Agarwal, U. P. Raman imaging to investigate ultrastructure and composition of plant cell walls: Distribution of lignin and cellulose in black spruce wood (*picea mariana*). *Planta* **224**, 1141–1153, <https://doi.org/10.1007/s00425-006-0295-z> (2006).
- [74] Reitzenstein, S. *et al.* Nondestructive analysis of single rapeseeds by means of Raman spectroscopy, *J. Raman Spectrosc.* **38**, 301-308, <https://doi.org/10.1002/jrs.1643> (2007).