Vibrational Spectroscopy 92 (2017) 111–118

Contents lists available at ScienceDirect

Vibrational Spectroscopy

journal homepage: www.elsevier.com/locate/vibspec

Phenolic compound explorer: A mid-infrared spectroscopy database

Ouissam Abbas[a,*], Géraldine Compère[b], Yvan Larondelle[b], Darly Pompeu[c], Hervé Rogez[d], Vincent Baeten[a]

[a] Valorisation of Agricultural Products Department, Walloon Agricultural Research Centre (CRA-W), Chaussée de Namur 24, B-5030 Gembloux, Belgium
[b] Life Sciences Institute, Université catholique de Louvain, Croix du Sud 2, Bte L7.05.08, 1348 Louvain-la-Neuve, Belgium
[c] Departamento de Tecnologia de Alimentos, Centro de Ciências Naturais e Tecnologia, Universidade do Estado do Pará, Trav. Éneas Pinheiro 2626, 66095-100, Belém-PA, Brazil
[d] Center for Valorisation of Amazonian Bioactive Compounds, Universidade Federal do Pará, Av. Perimetral s/n, 66.095-780, Belém-PA, Brazil

ARTICLE INFO

Article history:
Received 25 January 2017
Received in revised form 30 May 2017
Accepted 31 May 2017
Available online 9 June 2017

Keywords:
MIR
ATR
Spectral profile
Phenolic acids
Flavonoids

ABSTRACT

Analyses of phenolic compounds can be time-consuming and not always very reliable. Mid-infrared (MIR) spectroscopy could be used as a non-destructive tool for food quality analysis and control. The MIR spectra of 36 standard phenolic compounds (13 phenolic acids [hydroxybenzoic and hydroxycinnamic acids], 23 flavonoids [flavonols, flavones, isoflavones, flavanols, and flavanones]) were collected using an Attenuated Total Reflectance (ATR) accessory. Data were collected to build a spectral database in order to identify spectral features of each family of phenolic compounds studied. The phenolic compound spectra showed common spectral bands associated with aromatic six-membered rings and phenol moieties. The bands were numerous between 1640 and 700 cm^{-1}, leading to an overlap with other signals specific to different families. The methoxy group present in some phenolic compounds showed bands between 1470 and 950 cm^{-1}. The phenolic acids were characterized mainly by bands of unsaturated carboxylic acids, esters and alkenes, whereas flavonoids were characterized mainly by bands associated with benzopyrylium, Benzo-γ-pyrone and 2-phenyl-3,4-dihydro-2*H*-chromen-3-ol vibrations. A comparison of flavonoids with phenolic acids showed that the latter could be distinguished by bands of carboxylic acids between 1755 and 1630 cm^{-1}. Flavonoids were characterized by numerous peaks in the 1650–1400 cm^{-1} region and at spectral frequencies inferior to 1200 cm^{-1}. Principal Component Analysis (PCA) was performed on the MIR spectra, resulting in good discrimination of the families of the studied flavonoids and phenolic acids. The spectral regions 1755–1400 cm^{-1} and 1000–870 cm^{-1} were of great importance in distinguishing the studied families of phenolic compounds. The study allows starting to build a MIR spectral database for phenolic compounds as bioactive components of food.

1. Introduction

The consumption of fruits and vegetables has significantly increased in the past 20 years. According to WHO [1], physical activity and healthy eating are essential for good nutrition and a long and healthy life. Plants are an excellent source not only of fibers, vitamins and minerals, but also of phenolic compounds [2]. These organic molecules are known mainly for their antioxidant properties. They help reduce the risk of cardiovascular disease and act as antibacterial, antiviral and anti-inflammatory agents [3,4]. They are also involved in sensory (astringency, bitterness) and nutritional properties [5]. Phenolic compounds play an important role in the agri-food sector. They have also proved to be a problem in some industrial processes because they promote enzymatic browning reactions, formation of haze and by consequence degrade the food quality [6].

Phenolic compounds are secondary plant metabolites with diverse chemical structures [7]. Their chemical structure consists of at least one benzene ring to which is attached one or more hydroxyl groups. Other functional groups, such as ethers and carboxylic acids, could also be connected. Depending on their chemical configuration, phenolic compounds can be grouped into various families (e.g.: phenolic acids, flavonoids, tannins and stilbenes).

The quality and nutritional properties of food and the phenolic compound content of plants are usually determined by conventional methods. Among them, we have colorimetry, electrophoresis and high or ultra-performance liquid chromatography (HPLC or

* Corresponding author.
E-mail address: o.abbas@cra.wallonie.be (O. Abbas).

http://dx.doi.org/10.1016/j.vibspec.2017.05.008
0924-2031/

UPLC) [8–10]. These techniques are targeted to specific or family of compounds, time-consuming, expensive, and requiring significant quantity of solvent. The development of a rapid screening analytical method should make the identification and the evaluation of the phenolic content of plant matrices faster and easier.

Mid-Infrared (MIR) spectroscopy could be a suitable candidate in this regard. It has become a common spectroscopic technique in the food industry. It is used for characterizing molecules, but it could also be applied for evaluating the quality of food matrices (e.g.: determining the composition of milk [11], the quality of oils [12], the authenticity of honey [13]. Literature shows that only a few studies have determined the MIR spectral profile of phenolic compounds. MIR spectroscopy has been used as a new tool for determining *in situ* rosmarinic acid (caffeic acid ester) in *Lavandula officinalis* culture suspensions [14]; for better identification in the culture suspension, the MIR spectrum of a pure commercial rosmarinic acid standard was recorded, interpreted then compared with dried cells of *L. officinalis*. Similarly, Pei et al. used MIR spectroscopy to analyze *Herba epimedii* (traditional Chinese medicine); the correlation value, representing the similarity of two spectra of an herbal sample and the standard phenolic substance, icariin (flavonol glycoside), in the 1280–1200 cm^{-1} region, has been found to be a good indicator for fast and effective quality control and could be used as a screening criterion for the herbs [15]. MIR spectroscopy was also used in the framework of a study on the chelation of isoflavones like biochanin A (*O*-methylated isoflavone) and genistein (isoflavone) with metal [16]. In the same period, the technique was used by Gorinstein et al. to determine the bioactivity of exotic fruits [17]. Spectra of fruits (Avocado, durian and mango) were compared to pure phenolic standards (catechin (flavan-3-ol) and gallic acid (phenolic acid)) to detect the presence of those phenolic compounds in the fruits. Bands of these two compounds were pointed and interpreted. Another study has been realized with the objective to develop a quantitative method for the determination of anthocyanin content in sweet cherries by using MIR spectroscopy, the spectrum of keracyanin (anthocyanin) as chloride salt was recorded and interpreted. A linear relationship between anthocyanin content and the area of the band between 1640 and 1630 cm^{-1} was established (coefficient of correlation 0.99) [18]. Mangolim et al. have characterized, by MIR spectroscopy, curcumin before and after its complexation with ß-cyclodextrin. They studied the stability, the solubility and the food application of the inclusion complex [19].

In recent times, two phenolic acids; *trans*-ferulic acid and gallic acid were analyzed and characterized by MIR spectroscopy in the field of a possible interaction that they can occur in a co-encapsulated complex [20]. More recently, MIR technique associated multivariate analysis was effective in characterizing and distinguishing hydrolysable and condensed tannins [21].

Identification of phenolic compounds in natural products is of a great interest. Several papers attempted to investigate this topic [22–24]. It seems clear that MIR spectroscopy has a good potential for the analysis of phenolic compounds in addition to its usefulness to determine the bioactivity and/or the antioxidant potential of plant materials. It is then important to set a spectral database to easily do identification and characterization. The aim of this study is to perform the analysis of series of pure phenolic standards (powders) in order to identify the main spectral bands and to explore the possibility to establish rules allowing the detection of specific or a family of phenolic compounds by MIR spectroscopy.

2. Material and methods

A total of 61 standards corresponding to 36 phenolic compounds (HPLC grade purity) belonging to phenolic acid and flavonoid families have been analyzed. Table 1 presents the phenolic standards purchased; their name, families (phenolic acids/flavonoids) and the provider company (Vwr via merck, Sigma Aldrich, Extrasynthese, Vwr via alpha aesar, Molekula, Vwr via Molekula, Vwr via Cayman, Vwr, Cayman) as well. Sixteen phenolic compounds were purchased from more than one supplier, giving a total of respectively 32 and 29 samples of phenolic acids and flavonoids.

All the samples have been analyzed in their powder form. MIR spectra were collected with the Attenuated Total Reflectance (ATR) 'Golden Gate' accessory, a single reflection monolithic diamond integrated into a Vertex 70 spectrometer (Bruker Optics, Ettlingen, Germany). This ATR accessory allowed analyzing powders and obtaining spectra of high quality through the use of an integrated press accessory. Around 10 mg of the sample were deposited directly on the crystal and pressed against it to ensure optimum contact with the diamond. Each spectrum was the average spectrum of 64 scans. The spectra ranged from 4000 to 700 cm^{-1} and the data were collected with a resolution of 4 cm^{-1}. Analyses have been done in duplicate after acquiring the spectrum of the background (ambient air).

Before starting the data treatment, all the spectra were cut, keeping only the spectral region between 1800 and 700 cm^{-1}. This range is called the 'fingerprint region' because of its richness in structural information. The region between 4000 and 2500 cm^{-1} contained mainly broad bands associated with the stretching vibrations of hydroxyl functions and bands associated with the aromatic C—H stretching vibrations that were not specific to individual phenolic compounds. The range between 2500 and 1800 cm^{-1} did not contain spectral relevant information, apart from the band corresponding to CO_2. Therefore, the region between 1800 and 700 cm^{-1} was studied in detail in order to characterize phenolic compound families.

OPUS software (version 6.5) was used for MIR spectra recording while data treatment was performed using the UNSCRAMBLER software version X 10.2 from CAMO (Computer Aided Modelling, Trondheim, Norway).

Table 1
List of phenolic compounds studied and suppliers.

Polyphenol family	Compound name
Phenolic acids	Gentisic acid (3,6), *o*-Coumaric acid (2,3), Protocatechuic acid (2,3,4,5), *p*-Hydroxybenzoic acid (2,3), Caffeic acid (2,3,7), Chlorogenic acid (7,3,2), Ellagic acid (2,3,6), Ferulic acid (2,3), Gallic acid (2,3), Sinapic acid (2,3), Syringic acid (2,3,4), Vanillic acid (2,3,4), Salicylic acid (2)
Flavonoids	Catechin (3), Catechin hydrate (7), Cyanidin chloride (3), Cyanidin-3-glucoside (3), Cyanidin 3-rutinoside (3), Epicatechin (3), Epicatechin gallate (3), Epigallocatechin (3), Epigallocatechin gallate (3), Eriodictyol (3), Genistein (3), Quercetin-3- *D* –galactoside (3), Isorhamnetin (3), Kaempferol (2), Luteolin (2,3,9), Luteolin-7-*O*-glucoside (3), Myricetin (2), Delphinidin 3-O-glucoside (3), Quercetin (2,7,9), Quercetin-3-*o*-glucoside (2), Quercetin dihydrate (1,3), Rutin (3), Rutin hydrate (2,8)

(1) Vwr via merck, (2) Sigma Aldrich, (3) Extrasynthese, (4) Vwr via alpha aesar, (5) Molekula, (6) Vwr via Molekula, (7) Vwr via Cayman, (8) Vwr, (9) Cayman.

3. Results and discussion

The development of a spectroscopic method for identifying and characterizing phenolic compounds has involved four main steps: identification of bands; literature search; data compilation; and extraction of characteristic data. As examples, spectra between 1800 and 700 cm^{-1} of one sample from each sub-family of phenolic compounds are illustrated on Fig. 1. Gentisic acid and o-coumaric acid from phenolic acid family and cyanidin chloride, quercetin dihydrate, luteolin, genistein, catechin and eriodyctiol from flavonoid family were selected to exhibit the mid-infrared spectral profile of phenolic compounds.

From this figure, we can observe spectral signals in both phenolic acid and flavonoid families. However, it can be observed that spectra of phenolic acids are better resolved than those of flavonoids. That can be explained by more condensed structures in the latter case implying the occurrence of numerous and more overlapped spectral signals. This is shown in Fig. 2 in which the mean values of the total number of spectral signals of each phenolic compound was reported then values obtained for phenolic acids and flavonoids were compared. Two rectangles were inserted to the figure to easier do comparison and observe higher values for flavonoids.

Phenolic compounds are rich in functional groups which need to be identified and characterized in order to describe the spectral family of each phenolic acid and flavonoid. The bands present in the spectra (calculated, for each compound, on the basis of the average of spectra of samples provided by different vendors) of standards were identified and are presented in Supporting information (Tables S1 and S2) [25]. The structures of phenolic compounds are complex and vibrations of the different functions can be influenced by their environment (other groups forming the phenolic compound). Spectral bands identification and interpretation was done considering a small wavenumber shift equal to the spectral resolution of 4 cm^{-1}.

Phenolic compounds consist of one or more aromatic rings and at least one hydroxyl function directly linked to the ring(s). They present common bands associated with these structures. The aromatic six-membered rings present two or three bands due to ring C=C stretching vibrations in the 1625–1430 cm^{-1} region, the strongest usually being around 1500 cm^{-1} [25]. Depending on the number of substituents on the ring, their positions (*ortho*, *para* or meta), and their electronegativity, bands of aromatic C=C stretching vibrations of variable intensity and position can be observed around 1625–1590 cm^{-1}, 1590–1575 cm^{-1}, 1525–1470 cm^{-1} and 1465–1430 cm^{-1}. Nakanishi and Solomon reported bands around 1640–1630 cm^{-1}, 1604–1585 cm^{-1} and 1580–1562 cm^{-1} corresponding to double bond (C=C) stretching vibrations [26]. An important contribution of OH deformation vibrations can be found in the region 1410–1260 cm^{-1} [27]. The interaction of O—H deformation and C—O stretching vibrations of phenols can present bands around the spectral regions 1390–1330 cm^{-1} and 1260–1180 cm^{-1}. Other bands of phenols appear between 1382 and 1317 cm^{-1}, corresponding to C—O—H deformation [28]. A band around 1339 cm^{-1} was assigned by Schulz et al. to in-plane C—O stretching vibration combined with the ring stretch of phenyl [29,30]. Su et al. reported deformation vibrations of C—H group in-plane in the spectral region 1290–1000 cm^{-1} and OH deformation vibrations appeared in the 1410–1260 cm^{-1} range [31]. The 1290–1000 cm^{-1} region needs to be studied carefully for the presence of several other bands in this range, with C—C (phenyl-carbon) [26] presenting stretching vibrations between 1225 and 1075 cm^{-1} and C—O stretching vibrations [16] between 1150 and 1040 cm^{-1}. The 900–700 cm^{-1} spectral region is associated with out-of-plane deformation vibrations of the C—H group. This region is important because it helps in determining the

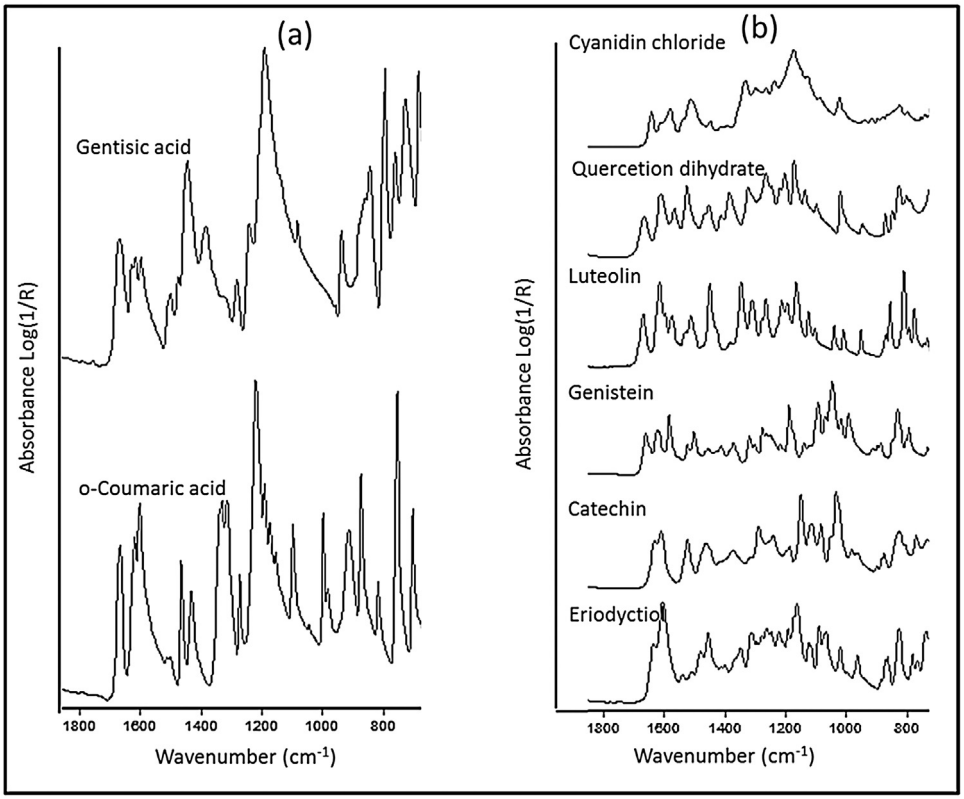

Fig. 1. Examples of MIR spectra of (a) phenolic acids (gentisic acid, o-coumaric acid) and (b) flavonoids (cyanidin chloride, quercetin Dihydrate, luteolin, genistein, catechin, eriodyctiol).

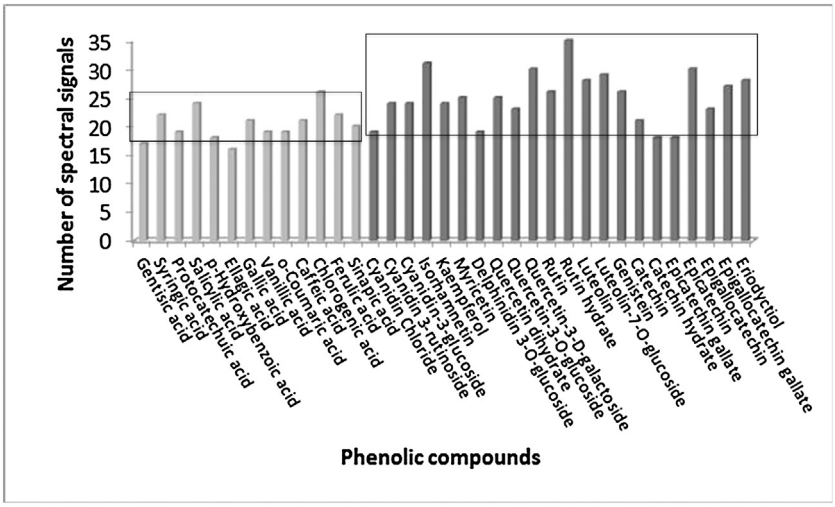

Fig. 2. Comparison of numbers of spectral signals identified for phenolic acids and flavonoids in the range 1800–700 cm^{-1}.

type of aromatic substitution. The presence of bands in specific sub-regions depends on the number of adjacent hydrogen atoms [26]. Broad absorption features appear around 720 cm^{-1} because of the out-of-plane deformation of the hydroxyl group. On another side, methoxy groups can also be present in both some phenolic acids and some flavonoids. They cannot be exploited for the screening of phenolic compound families. Four phenolic acids associated with methoxy groups (syringic, vanilic, ferulic, sinapic acids) show bands around 1470–1435 cm^{-1} and 1200–1185 cm^{-1} assigned to asymmetric and symmetric —C—H deformation vibration and —C—H rocking vibration, respectively. These bands are also observed in the spectrum of flavonol compounds, which contain one methoxy group such isorhamnetin, studied in this paper. Bands due to C—O vibrations of alkyl-aryl ethers were located in the 1310–1210 cm^{-1} and 1120–1020 cm^{-1} regions [25,15]. Methoxy groups and aromatic moieties bands will not be described again in the following determination of the spectral profiles of phenolic compounds families and subfamilies. In the following paragraphs, discussion has been focused on bands helping to do differentiation of phenolic acids and flavonoids and bands distinguishing their respective subfamilies.

3.1. Hydroxybenzoic acids and hydroxycinnamic acid derivatives

Hydroxybenzoic and hydroxycinnamic acids were characterized mainly by the presence of a single aromatic ring (basic structures of C6-C1 and C6-C3, respectively) and a carboxylic function. The frequency of this carboxyl stretching vibration could be affected by its structural environment. The phenolic acids analyzed in our study present several bands, shown in Table S1, Supporting information. Samples are classified according to their sub-class belonging (hydroxybenzoic acids and hydroxycinnamic acids). Spectral bands pointed have been presented in the vicinity of four spectral regions (1755–1630, 1629–1400, 1399–1200, 1199–700 cm^{-1}) arbitrarily chosen on the basis of the major groups that may occur in MIR spectral range. From this table, it can be seen that there were many differences between the phenolic acid vibrational bands. This can be due, among others, to the position and number of substitutions of the aromatic ring by hydroxylic groups or to the presence (or absence) of specific groups as methoxy function (—O—CH_3). In the literature [25,32,33,34], aryl (hydroxybenzoic acids) and/or α,β-unsaturated (hydroxycinnamic acids) carboxylic acid structures (C=O) stretching vibrations show bands in the 1715–1680 cm^{-1} region. But because aryl carboxylic acids with a hydroxyl group in the *ortho*- position[25] absorbs at about 50 cm^{-1} lower than the range defined earlier (1715–1680 cm^{-1}) and the C=C stretching vibration band of α,β-unsaturated acids [25] occurs around 1660–1630 cm^{-1}, several bands are observed between 1715 and 1630 cm^{-1} (1664, 1695, 1658, 1693, (1664, 1643), 1670, 1658, 1641, (1683, 1637), (1685, 1660), and 1660 cm^{-1} for samples studied in this paper as GeA, SyA, PA, SaA, HA, EA, GaA, VA, CoA, CaA, ChA, FA, SiA, respectively) [25,35,36,37]. Some hydroxybenzoic acids (syringic and salicylic acids) present bands around 1730 and 1753 cm^{-1}, respectively, which could be associated with aryl carboxylic acid monomers [20] absorbing in the 1755–1735 cm^{-1} range (the band around 1730 cm^{-1} could be associated with aryl carboxylic acid monomers because of the spectral resolution and the complexity of the phenolic compound structure that might affect the frequencies of individual functional groups). Ellagic acid showed a small band around 1755 cm^{-1} because of its specific structure (dimer of gallic acid). Electronegative groups were directly linked to the alcoholic oxygen atom of the ester group, which tended to increase the frequency of the C=O stretching vibration. Chlorogenic acid, an ester of caffeic acid and quinic acid, presented a band around 1720 cm^{-1}, which could be associated with the vibration of α,β-unsaturated aliphatic ester [25] that occurred between 1740 and 1705 cm^{-1} or the carbonyl C=O stretching of protonated carboxylic acid [38]. Other vibrations of carboxylic acid monomers [25] associated with O—H deformation were observed around 1380–1280 cm^{-1}(see Supporting information, Note S1).

It has to be mentioned that the spectra of some samples of phenolic compounds provided from several companies, such as protocatechic acid, ellagic acid, gallic acid, showed some differences while other compounds (sinapic acid, chlorogenic acid, *p*-hydroxybenzoic acid) provided by various companies showed a small shift in one or two spectral ranges of their samples. In order to understand the source of variations observed, complementary analyses using Ultra Performance Liquid Chromatography (UPLC-DAD) have been undertaken on protocatechic acid, ellagic acid and gallic acid. Chromatograms (not shown) did not present significant differences, but associated UV–vis spectra showed dissimilarities mainly for protocatechic acid (additional little shoulder), ellagic acid (additional bands and shoulders) and gallic acid (different intensities of the second band). These differences could be due to the presence of impurities in some standard phenolic compounds. Impurities ranged from equal or superior to (97%, 90%, unknown%) for protocatechic acid, to (unknown%, 95%, 90%) for ellagic acid and

to (99%, 97%) for gallic acid. Attention needs to be paid to the purity of standard phenolic acids.

3.2. Flavonoids

The spectra of samples from different classes of flavonoids were studied. Flavonoids have a general structure of C15 (C6-C3-C6) and they are composed of thousands of molecules grouped in more than 10 classes that can be distinguished by the central heterocyclic structure and its degree of oxidation [39]. This shows the structural heterogeneity that can exist between flavonoids. Within each class, variations of the basic chemical skeleton (C15) focus on three points: degree of oxidation of the different cycles; level of methoxylation; and level of glycosylation.

The spectral bands were identified and presented in Table S2. Samples are classified according to their sub-class belonging (anthocyanin, anthocyanidins, flavonols, flavones, isoflavones, flavanols, flavanones). Spectral bands pointed have been presented in the field of four ranges (1755–1630, 1629–1400, 1399–1200, 1199–700 cm^{-1}) defined according to the major groups that may occur in each spectral region.

Based on these data a general spectral signature of flavonoids was described. Flavonoids include one or several ether functions. The C—O stretching vibrations of aromatic ethers occurred between 1310 and 1230 cm^{-1}, but caution was needed because ester, carboxylic acid and ether all absorb in the 1300–1000 cm^{-1} region due to C—O stretching vibrations affected by the environment of the C—O function [25,26]. It should also be noted that some flavonoids are linked to some carbohydrates. It has been reported that the spectral region between 1200 and 950 cm^{-1} contains functional groups mainly from carbohydrates [40]; a pyranose structure is presenting bands in the 1200–1030 cm^{-1} region due to C—O stretching vibrations [25]. A band around 1160 cm^{-1} was observed by Huang et al. and affected to C—O—C stretching ring pyranose vibrations [41].

As with phenolic acids, a distinction between classes of flavonoid compounds was also possible. Flavonoids could be characterized by three types of chemical structure, as described here.

3.2.1. Flavylium (benzopyrylium) basis of anthocyanins and anthocyanidins

Because only the pyrylium part of flavylium has been described in the literature [26], the interpretation of some anthocyanin bands was based on the pyrylium nucleus that presented ring in-plane deformation vibration bands in the 1650–1615 cm^{-1}, 1560–1520 cm^{-1}, 1520–1465 cm^{-1} and 1450–1400 cm^{-1} ranges, as well as the 1000–970 cm^{-1} range. The pyrylium nucleus was usually tetrasubstituted, leading to additional vibrations associated with C—H out-of-plane vibrations. According to the literature [31], the position of these substituents is important and relates to bands around 920 cm^{-1} and around 890–870 cm^{-1} if the pyrylium compound is 2,3,4,6-tetrasubstituted and to bands around 900–880 cm^{-1} and around 705 cm^{-1} if the pyrylium compound is 2,3,5,6-tetrasubstituted. The three anthocyanins studied showed a band in the 1650–1615 cm^{-1} range around 1637 cm^{-1} and several bands in the 1520–1465 cm^{-1} (1515, 1508, 1492, 1490 cm^{-1}) and 1450–1400 cm^{-1} (1444, 1440, 1417, 1413 cm^{-1}) regions, in addition to the 1000–970 cm^{-1} range (977 cm^{-1} is present only in cyanidin-3-rutinoside). Bands corresponding to the 2,3,4,6-tetrasubstituted pyrylium compound were present around 869, 877, 885 and 873 cm^{-1}. Bands corresponding to the 2,3,5,6-tetrasubstituted pyrylium compound were present around 885, 877 and 709 cm^{-1}.

3.2.2. Benzo-γ-pyrone structure present in flavonols, flavones and isoflavones

Bands shown in Table 3 between 1670 and 1625 cm^{-1} (mainly a band around 1655 cm^{-1}) were characteristic of ketone C=O (Ar—C=O) stretching vibrations of cyclic conjugate (and planar) systems [26,28,29,38,42,43]. In addition to bands characteristic of benzene, the literature [31] shows that the specific bands of γ-pyrone are due to a combination of C=O and C=C stretching vibrations localized around 1570–1540 cm^{-1}, 1535–1525 cm^{-1}, 1465–1445 cm^{-1} and 1420–1400 cm^{-1}. For rutin (flavonol) Solimani identified bands around 1263 cm^{-1} (not observed in our study) associated with C—C—O stretching vibration and around 1167 cm^{-1} (also present in our study) for C—O—C antisymmetric stretching vibration [28]. Corredor et al. showed luteolin presenting a band around 1655 cm^{-1} associated to C=O stretching vibrations. They have also explained that the absence or presence of one or more hydroxyl groups on the B ring of flavone compounds (chrysin, apigenin, luteolin) affected the intensity of a band around 1000 cm^{-1}, which could be helpful in discriminating the different structures of flavones [44]. In the present study, flavonols, flavones and isoflavones showed several bands in the ranges described above associated with γ-pyrone vibrations between 1570 and 1540 cm^{-1} (1573, 1571, 1568, 1566, 1564, 1561, 1556 and 1554 cm^{-1}, apart from quercetin-3-*o*-glucoside and genistin), between 1535 and 1525 cm^{-1} (1529 and 1523 cm^{-1} present only in the two studied flavones), between 1465 and 1445 cm^{-1} (1465, 1463, 1461, 1456, 1454, 1452, 1448, 1446 1443 and 1442 cm^{-1}, apart from luteolin-7-*o*-glucoside) and between 1420 and 1400 cm^{-1} (1407, 1406 and 1402 cm^{-1}). In this study, bands in the 1420–1400 cm^{-1} region were visible only in two flavonols (quercetin dihydrate and rutin hydrate (1407, 1402 cm^{-1}) and in the only studied isoflavone (genistein (1406 cm^{-1})). However, bands in this region were not reported previously in the literature [16,45]. This region cannot be considered to characterize genistein compound.

3.2.3. 2-Phenyl-3,4-dihydro-2H-chromen-3-ol present in flavanols and flavanone

Unlike anthocyanins, anthocyanidins, flavonols, flavones and isoflavone flavonoid classes, flavanols and flavanones did not show bands above 1625 cm^{-1}. The 2-phenyl-3,4-dihydro-2*H*-chromen-3-ol structure of flavanols included a central six-membered ring comprising only one substitution in the 3-hydroxyl OH (free or complexed with other groups). This central ring consisted of cyclic ethers that showed bands around 1110–1090 cm^{-1} and 820–805 cm^{-1} associated with asymmetric and symmetric C—O—C stretching vibrations, respectively [25]. In our study, only few bands were visible in these ranges.

Two bands around 1110 cm^{-1} (1109, 1112 cm^{-1}) (apart from epigallocatechin gallate and epicatechin) and 820 cm^{-1} (824, 823, 821, 817 cm^{-1}) were observed in most of the flavanols and flavanones studied. Epigallocatechin, epigallocatechin gallate and epicatechin showed bands around 1099, 1097 and 1091 cm^{-1}, respectively. Epicatechin also showed a band around 806 cm^{-1}. The complexity of structures could cause a shift in the symmetric C—O—C stretching vibrations.

As for phenolic acids, the spectra of some flavonoid samples like luteolin and quercetin provided from three companies each. Ultra-Performance Liquid Chromatography (UPLC-DAD) analyses permitted to observe differences in the UV–vis spectra associated to the chromatographic characterizing peak. They present additional bands in the case of luteolin and different intensities of the second band in the case of quercetin. These differences could be due to the purity equal to (98%, 99%) for luteolin, and to (90%, 95%, 98%) for quercetin.

3.3. Discrimination of families of phenolic compounds

Phenolic compounds have a large infrared spectral signature. Fig. 3 has the ambition to clarify and summarize the interpretation of the spectral bands of the phenolic compounds studied. All phenolic compounds had common bands corresponding to aromatic rings and phenol functions (regions highlighted in grey in the figure) and some had a methoxy group (regions highlighted in grey in the figure). It is possible, however, to identify some bands that characterized different families of phenolic compounds. It is worth noting that there was an overlap between spectral regions of the different families of phenolic compounds.

From this description, we sought to identify the general spectral signature of different phenolic compound families, but we had to bear in mind the existence of some shifts in spectral bands due to the presence of several hydroxyl groups in different positions and, in some cases, of glucoside molecules. It should be noted that in addition to specific bands for each family of phenolic compounds, there were several bands associated with the vibrations of the aromatic rings and phenolic functions common to all compounds.

The spectral profile of phenolic compound families was then identified. The second step involved applying PCA to the spectral data in order to investigate if it was possible to discriminate the different families of phenolic compounds.

The spectra (1800–700 cm^{-1}) of samples from the same standard were averaged in order to obtain only one spectrum per phenolic compound then corrected using a Standard Normal Variate SNV pretreatment to remove scatter effects from the spectra. The PCA was then applied to the corrected spectra. The results (not shown) showed the repartition of samples in the space of the two first components. Ellagic acid was separated from its own group and mixed with flavonoids, probably because of its condensed structure, unlike other phenolic acids. Luteolin was also separated from samples of its class, probably because it belonged to the flavone class, whereas most of the flavonoids studied are flavanols or flavonols. In order to study the discriminant power of more specific spectral regions, PCA has been applied on spectral ranges associated with the main functional groups of phenolic acids (carboxylic acids, ester and alkenes) and the main functional groups of flavonoids (ϒ-pyrone and pyrylium) were combined (1755–1400 cm^{-1} and 1000–870 cm^{-1}). The spectral ranges have been defined on the basis of the general spectral signature of the families of phenolic compounds, presented in Fig. 3. The results presented in Fig. 4 show two clusters in the PCA space separated along the first component (that explains 23% of the total variance), indicating good discrimination of phenolic acids and flavonoids.

The examination of loading associated with this component presented on Fig. 5 allowed us to identify the spectral bands, explaining the separation observed. The positive part of the loading describing phenolic acids was composed of bands around 1724 cm^{-1} (α,β-unsaturated aliphatic ester and carbonyl C=O stretching of protonated carboxylic acid), 1680 and 1637 cm^{-1} (aryl and/or α,β-unsaturated carboxylic), 1581, 1540, 1515 and 1454 cm^{-1} (C=C aromatic ring stretching) and 972 cm^{-1} (alkene

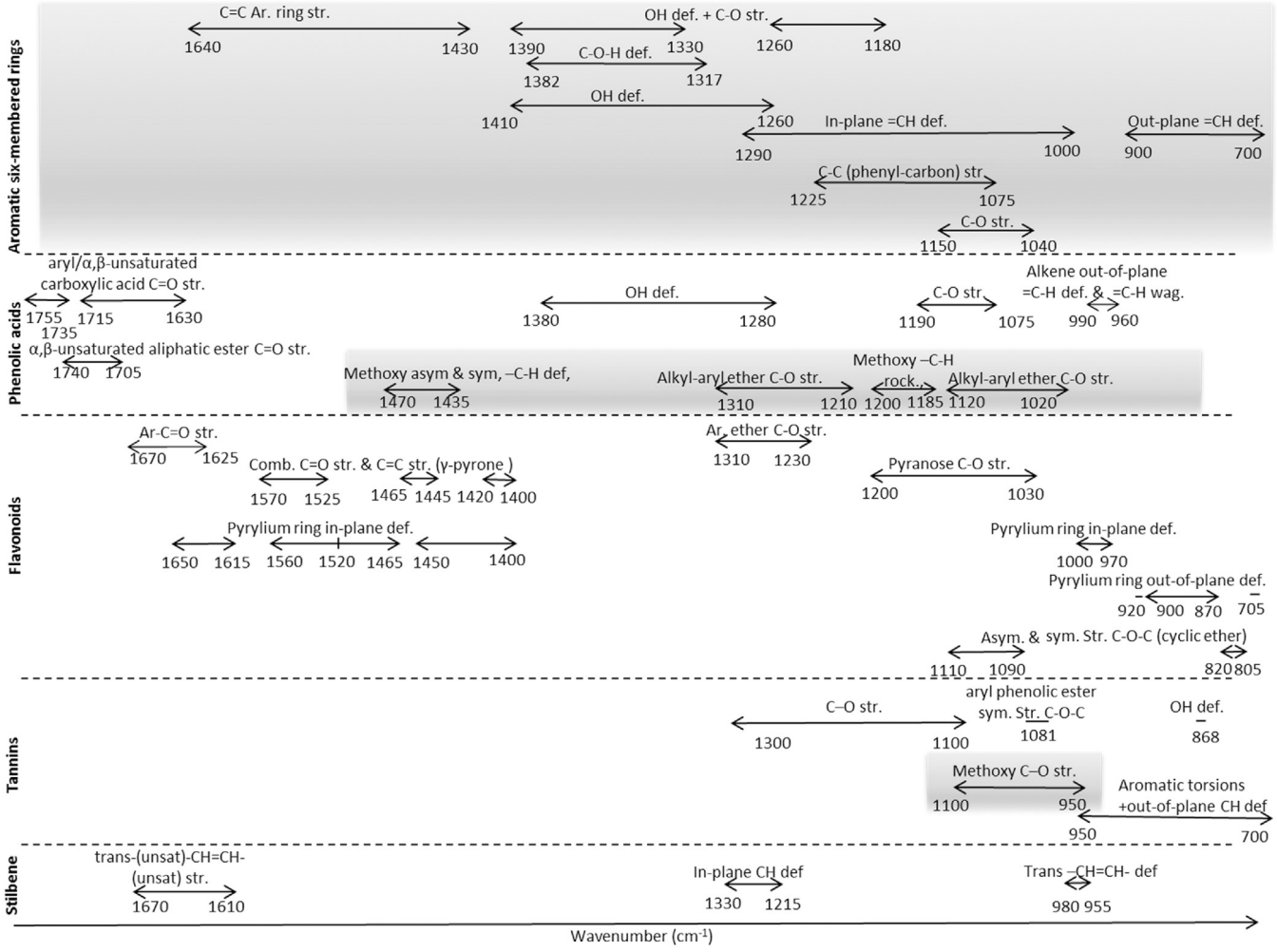

Fig. 3. Characteristic MIR spectral regions of major families of phenolic compounds.

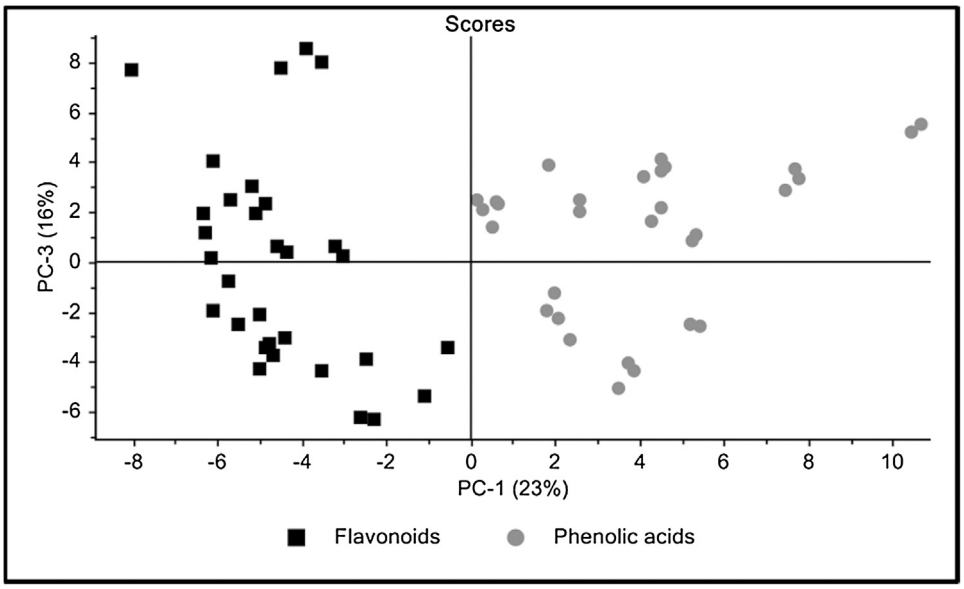

Fig. 4. PCA applied on SNV corrected MIR spectra of major families of phenolic compounds (phenolic acids, flavonoids).

=C—H wagging vibrations), in addition to 908 and 881 cm^{-1}(out-of-plane C—H deformation vibration of the aromatic ring). The negative part of the loading describing flavonoids was composed of bands around 1604 cm^{-1} (C=C aromatic ring stretching), 1556 and 1498 cm^{-1} (pyrylium ring in plane deformation vibration) and 962 cm^{-1} (out-of-plane C—H deformation vibration of the aromatic ring) or (pyrylium ring in plane deformation vibration) taking account of a small shift).

The exclusion of the spectral region associated mainly with the vibrational groups of phenolic moieties (between 1400 and 1000 cm^{-1} and bands between 870 and 700 cm^{-1}) helped considerably in the discrimination of the phenolic compound families studied. The spectral bands included in 1755–1400 cm^{-1} and 1000–870 cm^{-1} are important in the separation.

The PC3 is not discriminative (see Supporting information, Fig. S1). In fact, the examination of the loading associated with the third principal components shows that all bands appear on the positive part, indicating that all compounds are composed of the structures associated to the bands revealed by this loading.

The band around 1662 cm^{-1} can be associated to aromatic —C=O stretching vibrations which can be found both in phenolic acids and flavonoid compounds. Bands pointed at 1608, 1590, 1570, 1525, 1504, 1444 and 1434 cm^{-1} correspond to C=C aromatic ring stretching vibrations. The little bands around pointed around 989 and 974 cm^{-1} can be associated with alkene out-of-plane =C—H deformation and =C—H wagging vibrations that can be found in phenolic acids or may be affected to pyrylium ring out-of-plane deformation vibrations that can be encountered in flavonoids. These observations indicate that the spectral profile behind the third component is only describing phenolic acids and flavonoids common structures.

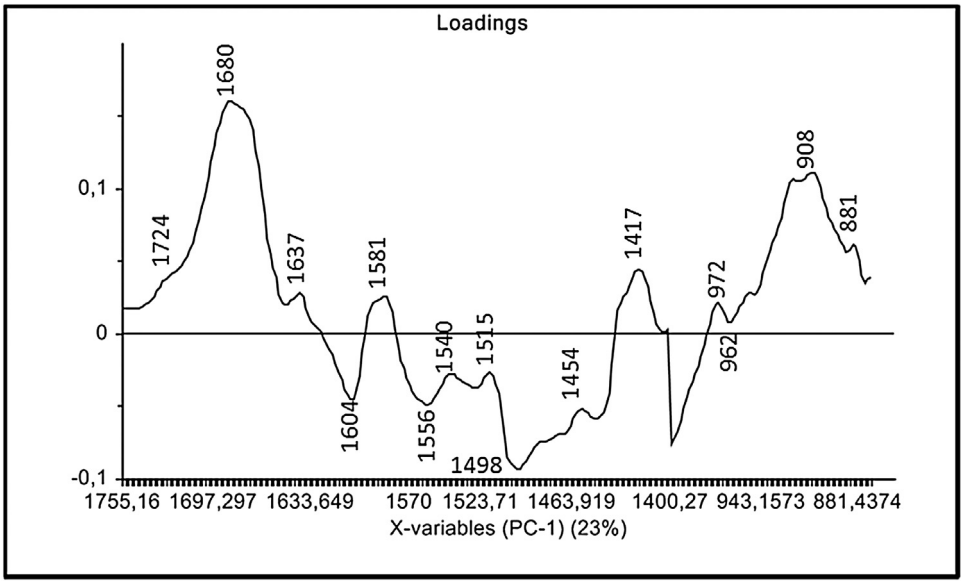

Fig. 5. Loading associated to the first principal component (PC1).

4. Conclusion

The study, we assessed the potential of MIR spectroscopy as a technique for identifying the profile of phenolic compounds that could be present in plant matrices. The results show that MIR spectroscopy is a good tool for characterizing chemical compounds such as phenolic compounds. Taking into account the great number of absorption bands, it was not easy to identify spectral regions specific to each family of phenolic compounds. A general description was given, however, for each family of phenolic compounds.

The application of the PCA spectral 1755–1400 cm^{-1} and 1000–870 cm^{-1} ranges allowed us to distinguish the studied families of phenolic compounds. The number of samples in different classes was not balanced, however, hence the need to work on a larger number of samples in order to better characterize the different classes and to validate the potential of PCA for discriminating phenolic compound classes and families.

More work is needed on using the combination of MIR spectroscopy and statistics to automate the identification of phenolic compound families and to devise a method for recognizing them in natural food matrices. The overall objective is to develop a rapid and simple method for assessing the quality of plants in terms of their richness in one or more phenolic compounds.

Acknowledgments

We would like to thank two technicians in the Walloon Agricultural Research Centre (CRAW), Christophe Jasselette (supervised by Dr. Jean-Michel Romnée and Dr. Georges Sinnaeve) and Quentin Arnould, who participated to the chromatographic and MIR measurements, respectively.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at **http://dx.doi.org/10.1016/j.vibspec.2017.05.008**.

References

[1] www.who.int/dietphysicalactivity/publications/trs916/summary/fr/index.html.
[2] A.L.K. Faller, E. Fialho, J. Food Compos. Anal. 23 (2010) 561–568.
[3] M. Aidilla, The Function of Dietary Phenolic Acids in Cardiovascular Health, (2013) .
[4] A. Rodriguez-Mateos, R. Del Pino-García, T.W. George, A. Vidal-Diez, C. Heiss, J. O.E. Spencer, Mol. Nutr. Food Res. 58 (2014) 1952–1961.
[5] Y. Peng, F. Liu, Y. Peng, J. Ye, Food Chem. 92 (2005) 169–175.
[6] P. Sarni-Manchado, V. Cheynier, Les polyphénols en agroalimentaire, Lavoisier collection sciences et techniques agroalimentaire, Ed TEC&DOC, 2006.
[7] F. Shahidi, M. Naczk, Phenolics in Food and Nutraceuticals, CRC Press, Boca Raton, FL, 2004.
[8] M. Ceymann, E. Arrigani, H. Scharer, A.B. Nising, R.F. Hurrell, J. Food Compos. Anal. 26 (1–2) (2012) 128–135.
[9] A. Raal, A. Orav, T. Pussa, C. Valner, B. Malmiste, E. Arak, Food Chem. 131 (2012) 632–638.
[10] S. Khanizadeh, R. Tsao, D. Rekika, R. Yang, M.T. Charles, H.P.V. Rupasinghe, J. Food Compos. Anal. 21 (2008) 396–401.
[11] M. De Marchi, V. Bonfatti, A. Cecchinato, G. Di Martino, P. Carnier, Ital. J. Anim. Sci. 8 (2) (2009) 399–401.
[12] O. Abbas, P. Dardenne, V. Baeten, Near-infrared, mid-infrared, and Raman spectroscopy, in: Y. Pico (Ed.), Chemical Analysis of Food: Techniques and Applications, Elsevier Science, Burlington, 2012, doi:http://dx.doi.org/10.1016/B978-0-12-384862-8.00003-0 (chapter 3, pp. 59).
[13] L. Svečnjak, D. Bubalo, G. Baranović, H. Novosel, Eur. Food Res. Technol. 240 (6) (2015) 1101–1115.
[14] K. Stehfest, M. Boese, G. Kerns, A. Piry, C. Wilhelm, J. Plant Physiol. 161 (2003) 151–156.
[15] L.K. Pei, S.Q. Sun, B.L. Guo, W.H. Huang, P.G. Xiao, Spectrochim. Acta Mol. Biomol. Spectrosc. 70 (2) (2008) 258–264.
[16] S. Dowling, F. Regan, H. Hughes, J. Inorg. Biochem. 104 (2010) 1091–1098.
[17] S. Gorinstein, R. Haruenkit, S. Poovarodom, S. Vearasilp, P. Ruamsuke, J. Namiesnik, H. Leontowicz, M. Suhaj, G.P. Sheng, Phytochem. Anal. 21 (2010) 355–362.
[18] C.S. Pappas, C. Takidelli, E. Tsantili, P.A. Tarantilis, M.G. Polissiou, J. Food Compos. Anal. 24 (2011) 17–21.
[19] C.S. Mangolim, C. Moriwaki, A.C. Nogueira, F. Sato, M.L. Baesso, A.M. Neto, G. Matioli, Food Chem. 153 (2014) 361–370.
[20] G. Olga, C. Styliani, R.G. Ioannis, Food Chem. 15 (2015) 33–40.
[21] F.D.S. Grasel, M.F. Ferrão, C. RodolfoWolf, Spectrochim. Acta Mol. Biomol. Spectrosc. 153 (2016) 94–101.
[22] K.S. Singh, M.S. Majik, S. Tilvi, Compr. Anal. Chem. 65 (2014) 115–148.
[23] Y.S. Park, M.H. Im, K.-S. Ham, S.-G. Kang, Y.-K. Park, J. Namiesnik, H. Leontowicz, M. Leontowicz, S. Trakhtenberg, S. Gorinstein, Food Sci. Technol. 63 (1) (2015) 346–352.
[24] C.W. Huck, Phytochem. Lett. 11 (2015) 384–393.
[25] G. Socrates, Infrared Characteristic Group Frequencies, J. Wiley & Sons, Chichester, 1997.
[26] K. Nakanishi, P.H. Solomon, Infrared Absorption Spectroscopy, 2nd edn., Holden-Day, INC, San Francisco, 1977.
[27] M. Hesse, H. Meier, B. Zeeh, Infrarot- und Ramannischen spektren, Spektroskopische Methoden in der Organischen Chemie, Thieme, Stuttgart, Germany, 1991.
[28] R. Solimani, Biochim. Biophys. Acta 1336 (1997) 281–294.
[29] X. Lu, C.F. Ross, J.R. Powers, B.A. Rasco, J. Agric. Food Chem. 59 (2011) 6376–6382.
[30] H. Schulz, M. Baranska, Vib. Spectrosc. 43 (2007) 13–25.
[31] S. Xin-Fang, Z. Hong, S. Ju-Xiang, W. Hai-Ying, J. Mol. Struct. 847 (2007) 59–67.
[32] R. Świsłocka, E. Regulska, M. Samsonowicz, W. Lewandowski, J. Mol. Struct. 1044 (2013) 181–187.
[33] R. Świsłocka, Spectrochim. Acta Part A 100 (2013) 21–30.
[34] G. Olga, C. Styliani, R.G. Ioannis, Food Chem. 185 (2015) 33–40.
[35] R. Świsłocka, E. Regulska, M. Samsonowicz, W. Lewandowski, J. Mol. Struct. 1044 (2013) 181–187.
[36] R. Świsłocka, Spectrochim. Acta Part A 100 (2013) 21–30.
[37] G. Olga, C. Styliani, R.G. Ioannis, Food Chem. 185 (2015) 33–40.
[38] J. He, L.E. Rodriguez-Saona, M.M. Giusti, J. Agric. Food Chem. 55 (2007) 4443–4452.
[39] P. Sarni-Manchado, V. Cheynier, Les polyphénols en agroalimentaire, Collection Science & Techniques Agroalimentaires, Editions TEC&DOC, 2006.
[40] X. Lu, J. Wang, H.M. Al-Qadiri, C.F. Ross, J.R. Powers, Juming Tang, B.A. Rasco, Food Chem. 129 (2011) 637–644.
[41] A. Huang, Q. Zhou, J. Liu, B. Fei, S. Sun, J. Mol. Struct. 883–884 (2008) 160–166.
[42] A.M. Mendoza-Wilson, H. Santacruz-Ortega, R.R. Balandrán-Quintana, Spectrochim. Acta Mol. Biomol. Spectrosc. 81 (2011) 481–488.
[43] J. Pusz, B. Nitka, A. Zielinska, I. Wawer, Michrochem. J. 65 (2000) 245.
[44] C. Corredor, T. Teslova, M.V. Cañamares, Z. Chen, J. Zhang, J.R. Lombardi, M. Leona, Vib. Spectrosc. 49 (2009) 190–195.
[45] R. Sekine, E.G. Robertson, D. McNaughton, Vib. Spectrosc. 57 (2011) 306–314.