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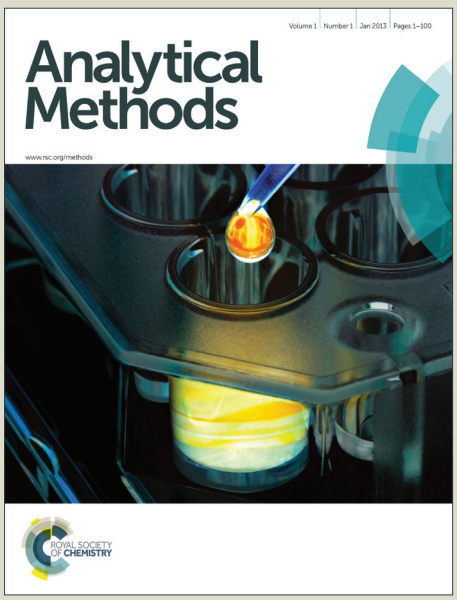

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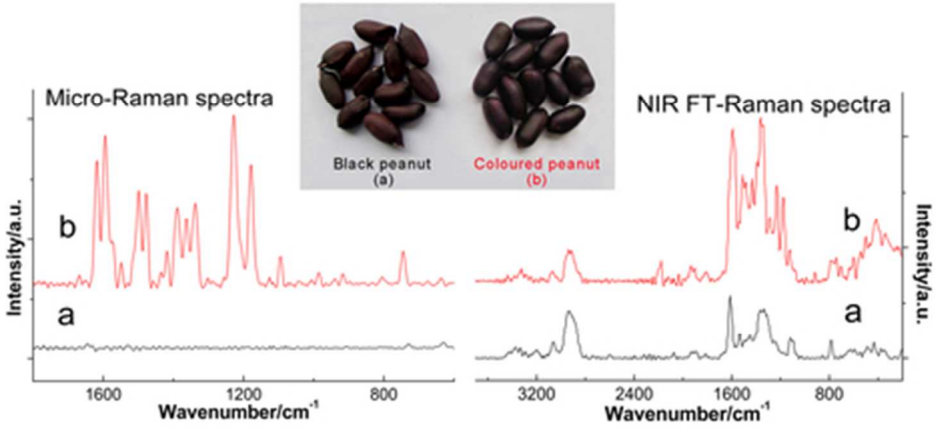

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Rapid identification of black peanut seeds by confocal micro-Raman and near-infrared FT-Raman spectroscopy

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Confocal micro-Raman spectroscopy and near-infrared (NIR) FT-Raman spectroscopy were used to analyse and identify cotyledons and skins of black, white, red and ordinary pink peanut seeds. The former method was equipped with a 785 nm semiconductor laser and a 514 nm Ar^+ laser; the latter method was equipped with a 1064 nm Nd:YVO_4 laser. These methods were also utilised to distinguish black peanut seeds from cheap and imitated coloured black peanut seeds. Significant Raman vibration peaks were detected and assigned by 785 nm Raman and 1064 nm NIR FT-Raman spectroscopy in black peanut cotyledons. Three bands in the 1064 nm NIR FT-Raman spectra of peanut skins could have critical functions in distinguishing black peanut from the other three peanut varieties. The two rapid, simple spectral analysis approaches were also demonstrated to distinguish black peanut seeds from coloured black peanut seeds, which is important to ensure food quality and safety.

1. Introduction

Peanut (*Arachis hypogaea* L.), a globally popular edible food grown extensively as a source of oil and protein, has many biologically active polyphenols such as the stilbene *trans*-resveratrol[1] and flavonoids (e.g., proanthocyanidins).[2,3] These polyphenols have adipogenesis inhibitory,[4] antioxidant and anti-inflammatory[5,6] properties. The bioactive polyphenols and flavonoids from peanuts have been used to prevent and mitigate many chronic diseases, such as cancer and cardiovascular disease.[7,8] A multitude of efficacy and health benefits have been recently discovered in peanut skins (testa, seed coats).[3,9] The composition of peanut skin varies with cultivar and growing conditions. A typical proximate composition is 19.7% fat, 18.6% protein, 2.2% ash, 18.1% fibre and 41.4% other components.[10] Peanut skin colours vary from white to deep red, even black. Most pigments in plants, especially red and purple, belong to the flavonoid class of anthocyanins, with other flavonoid compounds acting as co-pigments.

Black peanut, a purple-dark Chinese specialty peanut type, contains large quantities of mineral substances, such as calcium, potassium, selenium, iron and zinc, as well as vitamins, essential amino acids and other nutrients. In addition, black peanut is rich in anthocyanins and has various nutritional benefits, such as antioxidant, anti-radiation, anticancer, anti-aging, anti-inflammatory, promoting brain cells development, enhancing memory, improving physical ability and bolstering immune system functions.[4,11] Thus, the price of black peanut seeds is relatively higher than that of other types of peanut seeds. Moreover, black peanut seeds can be faked by staining peanut seeds. Selling coloured black peanut seeds dyed with azo pigments will greatly damage human health due to the accumulated toxic effects. Therefore, discriminating between true and fake black peanut seeds is important to ensure the quality of health-promoting products.

Currently, many conventional methods such as gas chromatography–mass spectrometry (GC–MC) for peanut kernels[12] and peanut oil,[13] high performance liquid chromatography (HPLC)[14] and LC–MS^n [15] for peanut skins and proteomics analysis of peanut seeds by two-dimensional gel electrophoresis[16] have been applied. These methods are sensitive and reliable, but they need complex sample preparation and are also time-consuming. Furthermore, determining the contents of protein and amino acids in peanuts using near-infrared reflectance spectroscopy (NIRS),[17] quantitative determination of saturated and unsaturated fatty acids in edible oils by IR,[18] 2D-NIR for discriminating edible vegetable oils[19] and direct monitoring of lipid oxidation in edible oils by 1064 nm Nd:YAG NIR FT-Raman spectroscopy[20] have been recently investigated. These methods propose the fundamental research of peanuts or edible oils and provide important contributions to monitor food quality and safety.

Raman spectrum is a non-elastic light scattering spectroscopy with high sensitivity, non-destructive and rapid detection, without complicated sample pre-treatment. The spectral resolution of a confocal micro-Raman spectrometer is better than 1 cm^{-1}, whereas NIR FT-Raman spectroscopy demonstrates better reduction of fluorescent background. Inspired by these methods, we applied confocal micro-Raman and NIR FT-Raman spectroscopy to analyse

and assign the Raman scattering spectral bands of cotyledons and skins of black peanuts and the other three peanut varieties. Moreover, we employed these methods to authenticate black peanut seeds from coloured black peanut seeds. Our study will be beneficial to the subsequent research and development of correlative health products, especially peanut varieties. The spectral approaches establish rapid screening methods for detecting the quality of black peanut seeds.

2. Materials and methods

2.1. Materials and reagents

Four colours of peanut seed cultivars, namely, black peanut, white peanut, red peanut and ordinary peanut were donated by Guangdong Academy of Agricultural Sciences (China). Composite synthetic pigment brilliant black (mainly including lemon yellow, sunset yellow, amaranth red and indigo) and carmine were purchased from Guangzhou Jingsai fine Chemical Co. Ltd. (Guangzhou, China). Purified water was prepared by an Elga water purification system (ELGA, London, UK).

2.2. Pretreatment of peanut seeds

The fully matured dry peanut seeds were stored at 4 ℃ until use. For the convenience of measurement, the skins were removed by hand. Tweezers and a knife were utilised to cut a flat. Each cotyledon was divided into two parts by hand. The cotyledons were measured flat side and were not from the same small lot.

For coloured black peanut seeds, brilliant black (50 g) and carmine (4 g) pigments were dissolved in water (1 L) for 1 h by a magnetic stirrer to fully mix the dye. Ordinary peanut seeds were poured to the dye liquor, shook for approximately 3 min, filtered and then naturally dried.

2.3. Raman spectral collection and data processing

The micro-Raman spectra were obtained by applying the InVia+Plus confocal micro-Raman spectrometer of Renishaw Inc. in the range of 3200 cm^{-1} to 200 cm^{-1}, with 20× Leica microscope objective times (NA = 0.35) and a spectral resolution of 1 cm^{-1}. The excitation radiations were 785 nm generated by a semiconductor laser and 514 nm generated by an Ar^{+} laser, with the confocal mode, CCD detector and laser beam spot diameter of 2.5 μm.

In this research, the 785 nm laser power at the surface of the samples was approximately 45.75 mW, with 15 s exposure time and three accumulations for peanut cotyledons, whereas it was approximately 0.92 mW, with 1 s exposure time and three accumulations for peanut skins and coloured black peanut skins. The 514 nm laser power at the surface of the samples was approximately 1.5 mW, with 3 s exposure time and three accumulations for peanut cotyledons, whereas it was approximately 0.15 mW, with 1 s exposure time and three accumulations for peanut skins and coloured black peanut skins.

The NIR FT-Raman spectra were recorded by using the 960 FT-Raman spectrometer of Thermo Fisher Nicolet within the scope of 3700 cm^{-1} to 100 cm^{-1}, accumulating 208 scans and a spectral resolution of 8 cm^{-1}. The excitation source was a 1064 nm Nd:YVO_4 laser working at 0.3 W, equipped with an InGaAs detector, a CaF_2 beam splitter and a mirror velocity of 0.3165.

Sample spectra were obtained at least three times to evaluate the reproducibility. Each specimen sampling position was studied randomly in the absence of beyond the scope, thereby guaranteeing the integrity of the spectral results presented. The Raman spectra of all samples were collected at room temperature. As for all spectral pre-treatments and analyses, baseline correction was performed by using the baseline Wavelet library of R version 2.8.1 software (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org/). Smoothing and normalisation were conducted through Originpro8.0 software.

3. Results and discussion

3.1. Black peanut seeds and other three peanut cultivars

Peanut skins have colours ranging from white to deep red, even black. Higher redness indicates that these cultivars are highly pigmented and possibly contain more abundant nutrients, especially in peanut skins. The black peanut has attracted considerable attention as a novel, healthy, delicious food. The black peanut seeds have relatively deeper colour and larger particles than the other three varieties (Fig. 1). Moreover, each black peanut seed has a relatively sharp feature at one end. Therefore, the identification of black peanut seeds and other peanut cultivars can be based on the stated differences. In this research, we used Raman technology for the exploration, interpretation and identification of peanut seeds.

Fig. 1 Shapes of mature seeds of black peanut, white peanut, red peanut and ordinary peanut.

3.2 Peanut cotyledon

3.2.1. Raman spectra of cotyledons of the four peanut cultivars in 785 nm Raman spectroscopy. The Raman spectra of the cotyledons of the four peanut cultivars range from 3200 cm^{-1} to 200 cm^{-1}, as measured by a 785 nm semiconductor laser (Fig. 2). The Raman spectra of the peanut cotyledons proclaim the presence of unsaturated fatty acids composed of *cis*-isomers and reveal that peanut cotyledon possesses protein. The Raman characteristic peaks of the cotyledons of the four peanut cultivars are strikingly similar. This result indicates that the main components of the different peanut cotyledons are similar. Consequently, no significantly different Raman vibration peaks were identified.

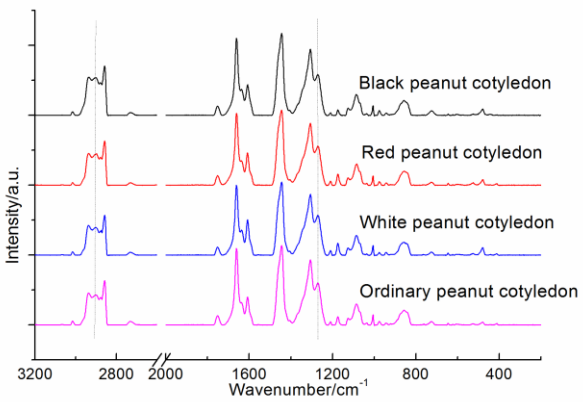

Fig. 2 Raman spectra of cotyledons of black peanut, red peanut, white peanut and ordinary peanut in a 785 nm semiconductor laser.

3.2.2. Raman spectral comparison of black peanut cotyledons by the 785 nm laser and 1064 nm laser. The Raman spectral bands of black peanut cotyledons by the 785 nm semiconductor laser and the 1064 nm Nd:YVO_4 laser are shown in Fig. 3, and their tentative assignments are listed in Table 1. The Raman spectra of black peanut cotyledons showed characteristic peaks with low fluorescence backgrounds in both 785 and 1064 nm laser excitation. The spectra obtained differ in Raman bands, relative intensity ratio and signal-to-noise ratio. The 785 nm radiation reduces the undesirable fluorescence background from naturally occurring substances and balances sensitivity with fluorescence suppression.[21] Signal-to-noise ratio is affected by the size of the excitation light power up to a certain extent. NIR FT-Raman laser power at the surface of the samples is poor, and NIR-excited Raman spectra are intrinsically weaker than the corresponding spectra obtained with visible excitation.[22] In addition, the spectral resolution significantly affects the spectral quality of NIR FT-Raman spectra. The confocal micro-Raman with a spectral resolution of 1 cm^{-1} possesses advantages over the 1064 nm NIR FT-Raman with a spectral resolution of 8 cm^{-1}. Therefore, black peanut cotyledon has higher signal-to-noise ratio and relative intensity ratio under the 785 nm laser than under the 1064 nm laser.

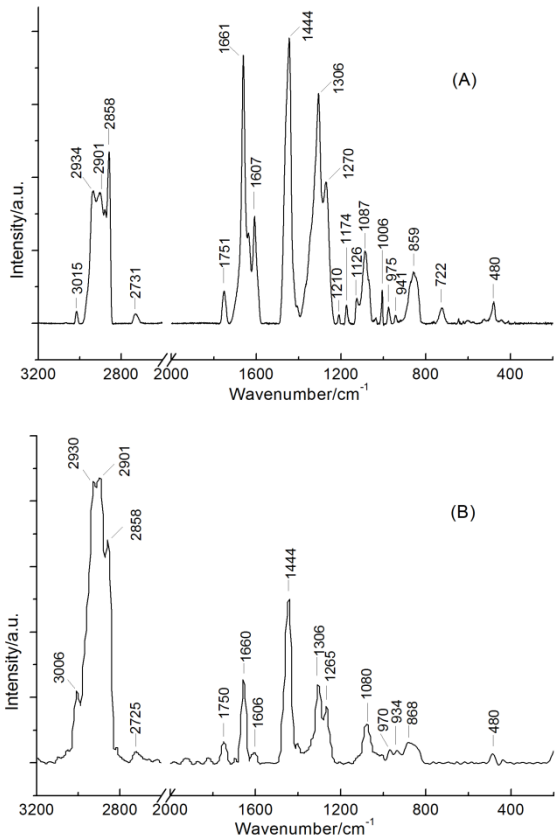

Fig. 3 Raman spectra of black peanut cotyledons in (A) a 785 nm semiconductor laser and (B) a 1064 nm Nd:YVO_4 laser.

No sufficiently complete interpretation of the Raman spectra of peanuts exists. Therefore, the following discussion concentrates upon tentative assignments of the wavenumbers of Raman spectra. We determined the assignments basing on the correlations with proposed assignments in several related substances, such as edible oils[18,20,23] containing high proportion of unsaturated fatty acids, amino acids[24,25], and the vibrations of the chemical functional groups.[26]

Most of the bands in the 785 nm Raman spectrum and 1064 nm NIR FT-Raman spectrum for black peanut cotyledons are similar. However, dissimilarities such as a few small offsets and different intensities could still be present and easily detectable. In general, spectral features within the scope of 3100 cm^{-1} to 2800 cm^{-1} are characteristic of the C–H stretching vibrations of lipids. The CH groups are attached to the *cis* double bonds (*cis* RHC=CHR) in unsaturated fatty acids. A relatively weak peak observed at 3015 cm^{-1} in the 785 nm Raman spectrum and at 3006 cm^{-1} in the 1064 nm NIR FT-Raman spectrum is attributed to =C–H symmetric stretching mode. The stretching vibrations of C=C located at 1661 cm^{-1} in the 785 nm Raman spectrum and 1660 cm^{-1} in the 1064 nm NIR FT-Raman spectrum are easy to identify because of their relatively high intensities and characteristic frequencies. Furthermore, shoulder peaks in the 785 nm Raman spectrum and 1064 nm NIR FT-Raman spectrum presented at 1270 and 1265 cm^{-1}, respectively, are assigned to the =C–H bending (symmetric rock). The intensities of these characteristic bands indicate the total unsaturation and the primary differences for the spectra differing from other analogues. *Trans* C=C bonds (*trans* RHC=CHR) can be presented as a stretch at approximately 1670 cm^{-1} and a bend at approximately 966 cm^{-1} in the Raman spectra. The absence of the

Table 1 Proposed assignments of Raman bands of black peanut cotyledons in 3200 cm^{-1} to 200 cm^{-1}.

785 nm	1064 nm	Assignments
3015w	3006m	=C–H sym. stretch in *cis* RHC=CHR
2934s	2930vs	C–H asym. stretch in $–CH_2$
2901s	2901vs	C–H sym. stretch in $–CH_3$
2858s	2858sh	C–H sym. stretch in $–CH_2$
2731w	2725w	C–H stretch in $–(CH_2)_n–$
1751w	1750w	C=O stretch in RC=OOR
1661vs	1660s	C=C stretch in *cis* RHC=CHR; NH_3^+, COO^- asym. stretch
1607s	1606w	NH_3^+, COO^- asym. Stretch
1444vs	1444vs	C–H bend (scissoring) in $–CH_2$; NH_3^+, COO^- sym. stretch
1306vs	1306s	C–H bend (twisting) in $–CH_2$
1270sh	1265sh	=C–H bend (sym. rocking) in *cis* RHC=CHR
1210w		C–H bend in $–CH_2$
1174w		C–H bend in $–CH_2$, C–O stretch in –CO–O–
1087m	1080m	C–H bend in $–CH_2$; C–C stretch; C–O stretch in –CO–O–
1006w		C–C stretch
975w	970w	C=C bend in *trans* RHC=CHR
941w	934w	C–C stretch
859m	868m	C–C stretch
722w		C–H bend (rocking) in $–(CH_2)_n–$
480w	480w	C–C bend (torsion)

w, weak; m, medium; s, strong; sh, shoulder; v, very; sym., symmetric; asym., asymmetric.

trans C=C stretching mode in Fig. 3 may be attributed to the overlap of a much larger and wider band nearby at 1660 cm^{-1} (*cis* C=C stretching) as a result of the high concentration ratio of unsaturated fatty acids in black peanut cotyledons. By contrast, the C=C bending

vibration was measured at 975 cm^{-1} in the 785 nm Raman spectrum and 970 cm^{-1} in the 1064 nm NIR FT-Raman spectrum.

As for the C–H stretching vibrations in methyl and aliphatic methylene functional groups, those modes shift with the length of the aliphatic chain in fatty acid molecules and generally appear at 3000 cm^{-1} to 2800 cm^{-1}. We have assigned the $-CH_2$ asymmetrical and symmetrical stretching vibrations for black peanut cotyledon corresponding to 785 nm Raman spectrum presented at 2934 and 2858 cm^{-1}, respectively, and 1064 nm NIR FT-Raman spectrum observed at 2930 and 2858 cm^{-1}, respectively. Moreover, the band at 2901 cm^{-1} in both 785 nm Raman spectrum and 1064 nm NIR FT-Raman spectrum can be assigned to C–H symmetric stretching in $-CH_3$. These spectral peaks mutually influence and form an intense, overlapping and broad band in this high-wavenumber region.

Apart from the band nearby at 1660 cm^{-1} (*cis* C=C stretching) that has been assigned in the spectral medium-frequency region, two prominent, strong and broad bands are presented at 1444 cm^{-1} (C–H scissoring mode of methylene) and 1306 cm^{-1} (in-phase methylene twist) by both 785 nm Raman spectrum and 1064 nm NIR FT-Raman spectrum. These two bands can be used as the characteristic peaks of black peanut cotyledon. A very strong band that appeared at approximately 1660 cm^{-1} could also couple with NH_3^+, COO^- asymmetrical stretching vibrations. Another band at approximately 1440 cm^{-1} is influenced by NH_3^+, COO^- symmetrical stretching vibrations, which may expose the existence of peptide bonds. In addition, the –C=O ester Fermi resonance at 1751 cm^{-1} in the 785 nm Raman spectrum or 1750 cm^{-1} in the 1064 nm NIR FT-Raman spectrum is worth mentioning despite its weak band.

The Raman spectral peaks below 1210 cm^{-1} are principally caused by the C–C stretching and C–H bending modes of some groups, such as long-chain methylene $[-(CH_2)_n-]$, along with the C–O stretching mode of –CO–O–. As listed in Table 1, a series of relatively weak peaks observed at 1210, 1174, 1087, 1006, 941 and 859 cm^{-1} in the 785 nm Raman spectrum and at 1080, 934 and 868 cm^{-1} in the 1064 nm NIR FT-Raman spectrum is assigned to the C–C stretching or C–H bending vibrations, even attaching to the C–O stretching mode of –CO–O–. Identification of the peaks is sometimes difficult because of the low intensities and changes of the bands. Moreover, some of them even form conjugated systems. In particular, the weak but characteristic band at 722 cm^{-1} in the 785 nm Raman spectrum is attributable to C–H bending (rocking) in long-chain methylene $[-(CH_2)_n-, n\geq 4]$.

Table 2 Proposed assignments of 1064 nm NIR FT-Raman bands of the four peanut cultivar skins in 3700 cm^{-1} to 200 cm^{-1}.

Black	Red	White	Ordinary	Assignments
3061w	3070w		3065w	aromatic C–H stretch, =C–H sym. stretch in *cis* RHC=CHR
2931s	2926s	2930s	2920s	C–H asym. stretch in $-CH_2$
		2899s	2895s	C–H sym. stretch in $-CH_2$
	1655m	1654m	1654m	C=C stretch in *cis* RHC=CHR; NH_3^+, COO^- asym. stretch
1611s	1611s		1615m	aromatic C=C stretch; NH_3^+, COO^- asym. stretch
	1445s	1445s	1445s	C–H bend (scissoring) in $-CH_2$
1365s				O–H bend, COO^- sym. stretch
1335s				C–H bend (twisting) in $-CH_2$
	1310s	1305m	1305s	C–H bend (twisting) in $-CH_2$

See Table 1 for abbreviations.

3.3 Peanut skin

3.3.1. Raman spectra of skins of the four peanut cultivars in 1064 nm NIR FT-Raman spectroscopy. The Raman spectra of skins of four peanut cultivars in the region 3200 cm^{-1} to 200 cm^{-1} were measured by a 1064 nm Nd:YVO_4 laser **(**Fig. 4). The Raman spectra of peanut skins obtained from the micro-Raman with the 785 nm and the 514 nm laser were covered with extremely high fluorescence, which mostly originated from the samples themselves. No obvious, recognisable and characteristic peaks were observed. The high signal-to-noise ratio Raman spectra were measured without difficulty in the case of 1064 nm excitation. Thus, 1064 nm NIR FT-Raman spectroscopy is more suitable for the identification of different peanut skins. Vibration bands presented in the Raman spectra of peanut skins arise from vibrations of C–O, O–H, C–H, C–C, C=C and aromatic C–H bonds (Table 2). These vibration signals from the various constituents containing essential amino acids, fatty acids, fibre, carbohydrates, ash and plant pigments such as anthocyanins that are predominant part of deciding the colours and pharmacodynamic effects of peanut skins are parallel and highly overlapping. Thus, no visible differences can be well assigned to be immediately related to the chemical abundance of a single peanut skin constituent. Basing on their prime composition and characteristic vibration frequencies of functional groups and chemical bonds, we proposed assignments corresponding to these vibrational bands and comparisons between the black peanut skin and three peanut cultivar skins.

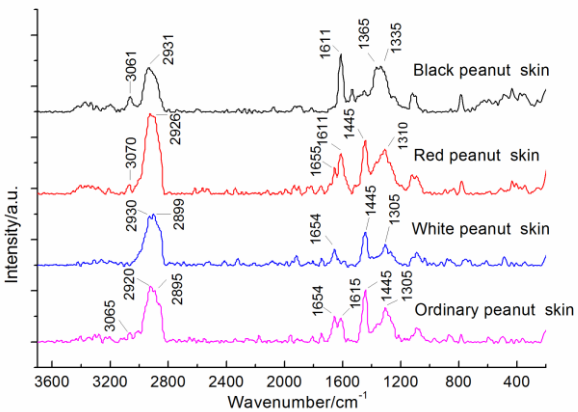

Fig. 4 Raman spectra of skins of black peanut, red peanut, white peanut and ordinary peanut in a 1064 nm Nd:YVO_4 laser.

Different characteristic peaks concerning the four peanut varieties skins are clearly shown in Fig. 4. Initially, the weak band at approximately 3065 cm^{-1} is attributed to the =C–H stretching mode

and C–H stretching of aromatic groups, which are observed in the 1064 nm NIR FT-Raman spectra of the black, red and ordinary peanut skins but not in the white ones. In addition, The C–H asymmetrical and symmetrical stretching vibrations in the CH_2/CH_3 groups generally located at 3000 cm^{-1} to 2800 cm^{-1}, the 1064 nm NIR FT-Raman spectra of white peanut skin presented double peaks at 2930 and 2899 cm^{-1} and ordinary peanut skin at 2920 and 2895 cm^{-1} are assigned to the methylene asymmetrical and symmetrical stretching vibrations. However, the other two are only recognised in the strong and broad peaks at 2931 and 2926 cm^{-1}, respectively, which are assigned to the methylene asymmetrical stretching vibration, because of the overlapping and coverage of vibration modes.

The 1064 nm NIR FT-Raman spectra are obvious distinctions in the region from 1700 cm^{-1} to 1000 cm^{-1} (Fig. 4). The band within the vicinity of 1655 cm^{-1} is frequently assigned to the coupling effects of COO^- and NH_3^+ asymmetrical stretching vibrations in protein and *cis* C=C stretching vibration in fatty acid in all spectra of the peanut cultivars except black peanut skin. However, a sharp and intensive peak presented at 1611 cm^{-1} in the spectrum of black peanut skin principally indicates the coupling effects of the COO^- and NH_3^+ asymmetrical stretching modes and C=C skeletal stretching of aromatic groups. These observations indicate that black peanut skin contains abundant amino acids and pigments. The absence of these characteristic peaks in white peanut skin containing a few pigments further confirms the results. The largest difference is the two strong and broad double-humped bands at 1365 and 1335 cm^{-1} in the spectrum of black peanut skin that are attributed to COO^- symmetrical stretching and O–H bending vibration because of polyphenols and C–H bending (twisting). Other varieties are all composed of two relatively high intensities of peaks at 1445 and 1305 cm^{-1}, which are caused by C–H bending (scissoring and twisting, respectively) in CH_2.

3.4 Application of testing fakes

3.4.1. Black peanut seeds and coloured black peanut seeds. Fig. 5 demonstrates the shapes of black peanut seeds and coloured black peanut seeds dyed by using synthetic pigments. Carmine pigment and compound pigment brilliant black, primarily made up of sunset yellow, lemon yellow, indigo and amaranth red, were used (see Fig. 6 for their chemical structures). These synthetic pigments except indigo are azo compounds. As one of food additives, they are limited the maximum or even forbidden because of their toxic effects on human health when administered in excess. Subtle differences between the black peanut seeds and the coloured black peanut seeds

Fig. 5 Shapes of black peanut seeds and coloured black peanut seeds.

could hardly be found when they are mixed together or when the fake ones are immediately sold to consumers. In this work, the Raman technique was applied to test peanutskins and then distinguish true from fake peanut seeds. Furthermore, the results of our study provide basis for food safety.

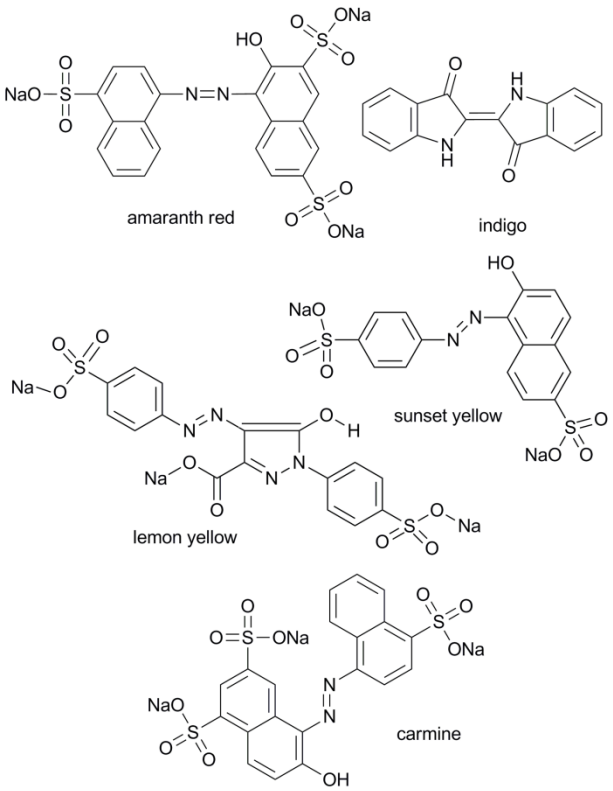

Fig. 6 Chemical structures of the main chromophores of compound pigment brilliant black (including sunset yellow, lemon yellow, indigo and amaranth red) and carmine.

3.4.2 Raman spectra of black peanut seeds and coloured black peanut seeds by the 1064 nm Nd:YVO_4 laser. Clear differences between the black peanut skin and coloured black peanut skin can be observed at the 1650 cm^{-1} to 1100 cm^{-1} region through 1064 nm NIR FT-Raman spectroscopy (Fig. 7). The Micro-Raman[27] and NIR FT-Raman spectra[28] of indigo and Fourier transform infrared spectrum of sunset yellow E110[29] or other analogous azo dyes[30,31] have been previously obtained. The Raman spectrum of the coloured black peanut skin have more and stronger peaks compared with those of the black peanut skin because of the synthetic pigments used. These synthetic pigments have characteristic vibration frequencies, such as aromatic C–H, C=C bonds, N=N, C–N, C–H, O–H, C–O and $S(=O)_2$.

Two sharp and strong bands appeared at 1231 and 1180 cm^{-1} in the spectrum of the coloured black peanut skin, which are not found in the spectrum of the black peanut skin. The former is assigned to C–O stretching in naphthol, coupling with C–N stretching mode. The latter is assigned to $S(=O)_2$ symmetric stretching of sulphone. In addition, the bands appearing between 1450 and 1380 cm^{-1} are attributed to the N=N stretching of azo compounds.[30,31] Thus, the peak at 1430 cm^{-1} in the 1064 nm NIR FT-Raman spectrum is assigned to N=N stretching vibration. The most powerful band in the coloured black peanut skin is 1361 cm^{-1}, which is attributed to the $S(=O)_2$ asymmetric stretching of sulphone, as well as C–N stretching and O–H bending vibrations. By contrast, broad and double-humped bands at 1365 and 1335 cm^{-1} appeared in the spectrum of the black peanut skin. A series of peaks at 1598, 1510 and 1485 cm^{-1} assigned

to the C=C skeletal stretching of aromatic groups, integrating with the peak at 3067 cm^{-1} attributed to the C–H stretching of aromatic groups (Ar–H), supports the presence of the aromatic ring in the Raman spectrum of the coloured black peanut skin. Substituents on conjugated benzene groups affecting the peak wavenumbers and intensities should not be ignored. In conclusion, the black peanut seeds and coloured black peanut seeds can be distinguished by analysing and comparing their characteristic peaks through 1064 nm NIR FT-Raman spectroscopy.

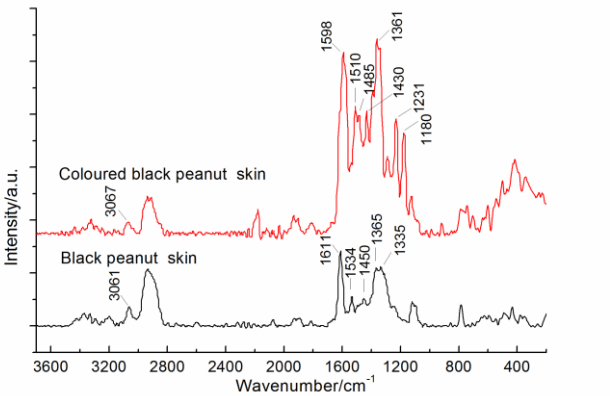

Fig. 7 Raman spectra of the black peanut skin and coloured black peanut skin in a 1064 nm Nd:YVO_4 laser.

3.4.3. Raman spectra of black peanut seeds and coloured black peanut seeds by the 514 nm Ar^+ laser. We also attempted to use the 514 nm Ar^+ laser in studying the peanut seeds apart from the 785 nm semiconductor laser and 1064 nm Nd:YVO_4 laser. It is particularly interesting to note that the Raman spectra for both skins and cotyledons of peanuts by the 514 nm Ar^+ laser are inconspicuous, i.e., almost no bands were found because of the covering fluorescence. However, a series of remarkable peaks in the range of 1800 cm^{-1} to 600 cm^{-1} is present in the coloured black peanut skin (Fig. 8). Thus, distinguishing black peanut seeds from coloured black peanut seeds is tremendously apparent.

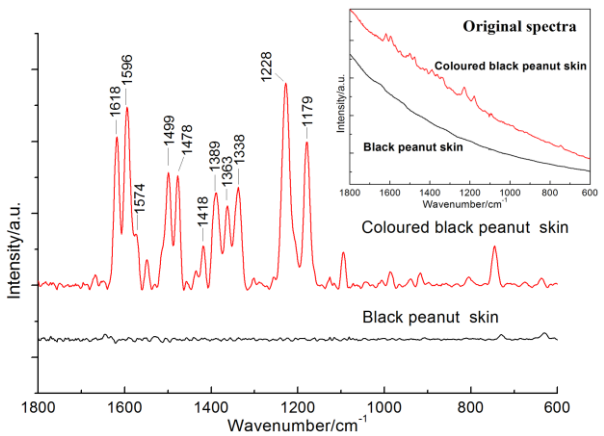

Fig. 8 Raman spectra of the black peanut skin and coloured black peanut skin in a 514 nm Ar^+ laser.

In comparison with the colored black peanut skin measured by 1064 nm NIR FT-Raman spectroscopy, the existence of the aromatic ring in the 514 nm Raman spectrum can also be proven by the peaks at 1596, 1574, 1499 and 1478 cm^{-1}. These peaks are attributed to the C=C stretching of aromatic groups. The sharp and high-intensity bands at 1228 and 1179 cm^{-1} in the 514 nm Raman spectrum are consistent with the peaks at 1231 and 1180 cm^{-1} in the 1064 nm NIR FT-Raman spectrum. However, the most powerful peak at 1361 cm^{-1} in the 1064 nm NIR FT-Raman spectrum is divided into three strong peaks, namely, 1389, 1363 and 1338 cm^{-1} under the 514 nm excitation. In addition, it's worth noting that the azo compounds have aromatic ring and N=N bond, which are easily photodegraded and destroyed under UV/Vis light irradiation, especially with the help of photocatalysts, such as TiO_2, O_3 and H_2O_2.[32] In addition, high laser powers or an excitation laser with short wavelength such as 448 or 514.5 nm may also lead to photodegradation of azo dyes. Although the 514 nm laser power at the surface of the samples in our experiments is only approximately 0.15 mW during 1 s exposure on peanut skins and coloured black peanut skins, we cannot rule out the possibility that these sharp bands may be formed partly by photoproducts of azo dyes; some of the photoproducts are intermediates, such as aromatic amine, phenolic compounds and small organic molecules.[33]

Unknown peanut samples could be identified as fake based on the presence of some characteristic peaks in Raman spectra with the 514 nm excitation. Hence, a good and rapid way to distinguish black peanut seeds from coloured black peanut seeds is by obtaining Raman spectra from the 514 nm Ar^+ laser.

4 Conclusions

Raman spectra obtained by confocal micro-Raman and NIR FT-Raman spectroscopy of the cotyledons and skins of black peanut and other three peanut varieties, as well as fake black peanut seeds and shoddy goods, were investigated. We verified the characteristic vibration peaks from the 785 nm Raman spectrum and 1064 nm NIR FT-Raman spectrum within the scope of 3200 cm^{-1} to 200 cm^{-1} for black peanut cotyledons. Raman spectra obtained by 1064 nm NIR FT-Raman spectroscopy manifested remarkable Raman vibration peaks for black peanut skin. Moreover, three main different Raman bands allowed us to distinguish black peanut skin from the other three peanut cultivars. Furthermore, we successfully discriminated black peanut seeds from coloured black peanut seeds through the two rapid and simple spectral identifiable methods. Such discrimination is important to ensure food quality and safety. The high efficiency and reliability of Raman technologies in this study offer the tremendous possibility for further investigation and identification of correlative peanuts or other plant products, including their nutritional ingredients and health-promoting potential.

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Notes and references

1 V. S. Sobolev and R. J. Cole, *J. Agric. Food Chem.*, 1999, **47**, 1435-1439.
2 H. Lou, H. Yuan, B. Ma, D. Ren, M. Ji and S. Oka, *Phytochemistry.*, 2004, **65**, 2391-2399.
3 T. Tatsuno, M. Jinno, Y. Arima, T. Kawabata, T. Hasegawa, N. Yahagi, F. Takano and T. Ohtaa, *Biol. Pharm. Bull.*, 2012, **35**, 909–916.
4 Z. Liu, J. Wu and D. Huang, *J. Agric. Food Chem.*, 2013, **61**, 4155−4161.
5 J. C. Chang, Y. H. Lai, B. Djoko, P. L. Wu, C. D. Liu, Y. W. Liu

and R. Y. Y. Chiou, *J. Agric. Food Chem.*, 2006, **54**, 10281-10287.
6 C. C. Udenigwe, V. R. Ramprasath, R. E. Aluko and P. J. Jones, *Nutr. Rev.*, 2008, **66**, 445-454.
7 R. M. Lopes, S. Agostini-Costa Tda, M. A. Gimenes and D. Silveira, *J. Agric. Food Chem.*, 2011, **59**, 4321-4330.
8 A. M. Stephens, L. L. Dean, J. P. Davis, J. A. Osborne and T. H. Sanders, *J. Food Sci.*, 2010, **75**, H116-122.
9 Y. Chukwumah, L. T. Walker and M. Verghese, *Int. J. Mol. Sci.*, 2009, **10**, 4941-4952.
10 K. E. Constanza, B. L. White, J. P. Davis, T. H. Sanders and L. L. Dean, *J. Agric. Food Chem.*, 2012, **60**, 10776-10783.
11 http://www.hhtip.com/the-black-peanuts-nutritional-value/
12 A. O. Cherif, H. Trabelsi, M. Ben Messaouda, B. Kaabi, I. Pellerin, S. Boukhchina, H. Kallel and C. Pepe, *J. Agric. Food Chem.*, 2010, **58**, 8709-8714.
13 R. Su, X. Xu, X. Wang, D. Li, X. Li, H. Zhang and A. Yu, *J. Chromatog., B*, 2011, **879**, 3423-3428.
14 J. C. Cheng, L. S. Kan, J. T. Chen, L. G. Chen, H. C. Lu, S. M. Lin, S. H. Wang, K. H. Yang and R. Y. Chiou, *J. Agric. Food Chem.*, 2009, **57**, 8805-8811.
15 P. J. Sarnoski, J. V. Johnson, K. A. Reed, J. M. Tanko and S. F. O'Keefe, *Food Chem.*, 2012, **131**, 927-939.
16 K. R. Kottapalli, P. Payton, R. Rakwal, G. K. Agrawal, J. Shibato, M. Burow and N. Puppala, *Plant Sci.*, 2008, **175**, 321-329.
17 L. Wang, Q. Wang, H. Liu, L. Liu and Y. Du, *J. Sci. Food agric.*, 2013, **93**, 118-124.
18 A. A. Christy and P. K. Egeberg, *Chemom. Intell. Lab. Syst.*, 2006, **82**, 130-136.
19 B. Chen, P. Tian, D.-L. Lu, Z.-Q. Zhou and M.-L. Shao, *Anal. Methods*, 2012, **4**, 4310-4315.
20 B. Muik, B. Lendl, A. Molina-Diaz and M. J. Ayora-Canada, *Chem. Phys. Lipids*, 2005, **134**, 173-182.
21 I. R. Lewis, N. W. Daniel Jr., N. C. Chaffin, P. R. Griffiths and M. W. Tungol, *Spectrochim. Acta A*, 1995, **51**, 1985-2000.
22 T. Hirschfeld and B. Chase, *Appl. Spectrosc.*, 1986, **40**, 133-137.
23 H. Yang and J. Irudayaraj, *J. Am. Oil Chem. Soc.*, 2001, **78**, 889-895.
24 G. Zhu, X. Zhu, Q. Fan and X. Wan, *Spectrochim. Acta A*, 2011, **78**, 1187-1195.
25 S. Kumar, A. Kumar Rai, S. B. Rai, D. K. Rai, A. N. Singh and V. B. Singh, *J. Mol. Struc.*, 2006, **791**, 23-29.
26 E. Smith and G. Dent, *Modern Raman Spectroscopy–A Practical Approach*, Wiley, Chichester, 2005.
27 P. Vandenabeele and L. Moens, *Analyst*, 2003, **128**, 187-193.
28 E. Tatsch and B. Schrader, *J. Raman Spectrosc.*, 1995, **26**, 467-473.
29 M. Snehalatha, N. Sekar, V. S. Jayakumar and I. H. Joe, *Spectrochim. Acta A*, 2008, **69**, 82-90.
30 P. Vandenabeele, L. Moens, H. G. M. Edwards and R. Dams, *J. Raman Spectrosc.*, 2000, **31**, 509-517.
31 P. J. Trotter, *Appl. Spectrosc.*, 1977, **31**, 30-35.
32 L. Wojnárovits and E. Takács, *Radiat. Phys. Chem.*, 2008, **77**, 225-244.
33 K. Tanaka, K. Padermpole and T. Hisanaga. *Water Res.* 34, 327-333.

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