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Determination of retrogradation degree in starch by Mid-infrared and Raman spectroscopy during storage

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Abstract

Retrogradation behavior is an important physicochemical property of starch during storage. A fast and sensitive method was developed for determining the retrogradation degree (RD) in corn starch by mid-infrared (MIR), Raman spectroscopy and combination of MIR and Raman. MIR and Raman spectra were collected from different retrogradation starch and then processed by partial least-squares (PLS), interval PLS (iPLS), synergy interval PLS (siPLS), and backward interval PLS (biPLS). Two different levels' fusion data extracted from MIR and Raman spectra were analyzed by partial least-squares (PLS). The developed models demonstrated that both MIR and Raman techniques combined with chemometrics can be used to determine the RD in starch. The PLS model built by medium-level fusion approach achieved the most satisfied performance with a correlation coefficient of 0.9658. Integrating MIR and Raman technique combined with chemometrics improved the prediction performance of RD in comparison with a single technique.

Keywords: Retrogradation degree; Starch; Raman spectroscopy; MIR spectroscopy; Partial least-squares

Introduction

Starch presented in an enormous variety of food products acts as the main materials to supply nutrition and energy, or as an additive to improve the quality of food. Starch retrogradation behavior is an important physicochemical property of starch during storage. Retrogradation could lead to deterioration of starch-based food during storage (Eliasson 2010), while retrogradation also could provide starch food with functional properties. Starch is beginning to retrograde after starch completely gelatinized. During retrogradation, molecular chains in starch begin to reassemble to develop an ordered structure (Ferrero et al. 1994). Generally, starch paste retrogradation is accompanied by gradual increases in rigidity and phase separation between polymer and solvent (Karim et al. 2000). Starch-based foods after retrogradation are indigestible by body enzymes and may make consumer suffer from indigestion (Hayakawa et al. 1997). Therefore, a number of steps were attempted to study and prevent retrogradation (Liu et al. 2007). As we all know, one kind of resistant starch called RS₃ is the retrograded starch forming during cooling of gelatinized starch. As a new resource of dietary fiber, retrogradation starch can provide functional properties and find applications in a variety of foods (Karim et al. 2000; Sajilata et al. 2006). Consumers prefer appropriate retrogradation to no retrogradation in starch-based products. Retrogradation is used to harden products and reduced products stickiness during the manufacturing process of breakfast cereals and parboiled rice (Karim et al. 2000). The retrogradation starch is often said that

retrogradation deteriorates the quality of starch food. It is equally true that the suitable retrogradation starch is a benefit to gastrointestinal digestion. Therefore, the retrogradation degree (RD) in starch is a very important index for monitoring the quality of starch foods.

Various methods have been applied for studying retrogradation starch, such as rheological methods texture profile analysis (TPA) and rapid visco analyzer (RVA) (Mariotti et al. 2009; Olayinka et al. 2011), thermal analysis (differential scanning calorimetry (DSC), differential thermal analysis (DTA) and nuclear magnetic resonance (NMR))(Chang and Liu 1991). The most popular method is enzymatic methods based on acid or amylolytic enzymes (e.g. *α*-amylase and *β*-amylase etc.) for determining RD in starch. Normally, the RD is measured by determining residual non-digestible starch which is not digested to glucose after incubation with amylolytic enzymes (Karim et al. 2000). These methods are complicate, laborious and time-consuming. Rheological methods are used to evaluate the characteristics of retrogradation starch based on viscosity property, hardness and elasticity (Karim et al. 2000). These properties can provide the qualitative description of starch retrogradation while the RD values was not determined specifically (Smits et al. 1998). The enthalpy in the melting endotherm resulting from thermal analysis was used as the index for evaluation of starch retrogradation (Paker and Matak 2016). Samples detected by thermal analysis, enzymatic methods and rheological methods are not reusable by consumers (Chang and Liu 1991). Most NMR instruments are

74 expensive and not available in many labs or industries (Monakhova and Diehl 2016).

75 Spectroscopic methods have been used in study of retrogradation as

76 nondestructive methods, such as near-infrared (NIR) spectroscopy, mid-infrared

77 (MIR) spectroscopy, Raman spectroscopy, and so on (de Peinder et al. 2008; Rocha et

78 al. 2016; Thygesen et al. 2003). Each retrogradation starch has a unique and

79 characteristic spectrum due to their particular molecular component and structure.

80 NIR shows overtones and combination vibrations of the molecule when NIR beam

81 irradiates into samples. The molecular bands observed in NIR spectra are very broad

82 resulting in that it is difficult to ascribe specific bands to specific chemical

83 components (Romano et al. 2016). Both MIR and Raman spectroscopy can generate

84 bands linked to fundamental vibration and supply fingerprints of components that can

85 be used for quantitative and qualitative characterization (Vankeirsbilck et al. 2002).

86 They have been applied to characterize the molecular structural changes of

87 retrogradation starch, and are conducive to comprehending the changes of amylose

88 and amylopectin (Flores-Morales et al. 2012). However, there is no study about

89 quantitative analysis of RD in starch by MIR, Raman and the combination of them.

90 Different types of starch (from pure corn and cassava starch samples, as well with

91 mixtures from both starch types) can be characterized using by using Raman

92 spectroscopy (Almeida et al. 2010). Besides Raman spectroscopy, MIR spectroscopy

93 can also be used for quantitative analysis of RD in starch (Wu et al. 2016). Acting as

94 complementary spectroscopic techniques, both types of measurements, Raman and

MIR, can provide different molecular vibrations. (Thygesen et al. 2003). Previous studies have demonstrated that data fusion technique based on MIR and Raman can increase the prediction ability of chemical components in food (Wu et al. 2016). .

Therefore, the objectives of this paper were: (1) to use MIR, Raman spectroscopy and the combination of two techniques for investigating the retrogradation behavior in starch; (1) to establish a nondestructive and rapid method for measuring the quality, acceptability, and shelf-life of starch-containing foods.

Materials and methods

Retrogradation starch preparation

Corn starch was purchased from Runzhou Starch Company in Zhenjiang. A solution of corn starch (1g) suspended in 19 ml of water was heated at 100°C with constant stirring for 1 hour in order to make starch completely gelatinized. The gelatinized starch paste was stored for different time (0, 1, 2, 3, 4, 5, 10, 15, 20 days) at 4°C. After storage, retrogradation starch with different storage time was dried and kept in a desiccator. Sixteen samples for each retrogradation starch were prepared using same procedures. All samples were obtained from one independent gelatinization experiment.

MIR and Raman spectroscopy

The MIR spectra of retrogradation starch were collected by Nicolet 380 FT-IR spectrometer (Thermo Electron Corporation, USA) in the spectral range of 650 to 4000 cm^{-1} at resolution of 2 cm^{-1} (Flores-Morales et al. 2012). Raman spectra were

recorded with DXR Laser micro-Raman spectrometer (Thermo Electron Corporation, USA) with 532 nm laser source. During collection of Raman spectra, time of integration is 5 seconds. For each spectrum, an average of 32 scans were performed at a resolution of 1 cm^{-1} , over the $100\text{--}3200\text{ cm}^{-1}$ range (Xu et al. 2014). To obtain the most useful spectral information, multiple scans were performed in different points of the sample by moving the substrate on an X-Y stage. And the spectra from same sample were averaged into one spectra. Before collection, the Raman system was calibrated with a silicon semiconductor. The laser power irradiation over the samples was 4 mW. Finally, 144 and 144 spectra for MIR and Raman were obtained, respectively.

Reference analysis of RD in starch

The reference RD in starch was measured by the modified method of *Tsuge et al* (Di Paola et al. 2003). A solution of 25 mg retrogradation starch in 8 ml distilled water was placed into a test tube. 5 ml 0.1 mol L^{-1} phosphate buffer (pH 6.0, 0.3% NaCl) and 2 ml 3.5 U mL^{-1} α -amylase solution was then placed into the test tube. After incubation for 1 hour at 37°C , the enzymatic reaction was stopped by adding 5ml of 4 mol L^{-1} NaOH. The pH of the solution was adjusted to neutrality with 4 mol L^{-1} HCl and the volume was made up to 100 ml with distilled water. 5 ml of iodine solution ($0.2\%\text{ I}_2\text{--}2\%\text{ KI}$) was added to 10 ml of the digested solution, and made up to 100 ml with distilled water. The absorbance of solution at 625 nm was measured after standing for 20 min. The RD (%) is calculated from equation described by *Tsuge et al.*

The values of RD obtained would be used for the construction and validation of model (Kim et al. 1997).

Data analysis

Different pre-processing techniques (standard normal variate (SNV), mean centering (MC) and multiplicative scatter correction (MSC), Savitzky–Golay smoothing (SG)) were applied for eliminating baseline shift and scatter effects etc. By comparing results obtained from four preprocessing methods, SG is much better than SNV, MC and MSC (Chen et al. 2011).

PCA was performed to show the clustering trend of retrogradation starch samples (Yeung and Ruzzo 2001). PCA is a well-known method for feature extraction in spectral analysis. It transforms the original independent variables into new variables (principal components (PCs)). The PCs are orthogonal and can be used as input variables for pattern recognition analysis (Haiyan et al. 2008).

Partial least squares (PLS) is used extensively for it is able to cope with high-dimensional data by extracting latent variables. So far, PLS has been widely used to build multivariate calibration models using the whole spectrum (WS) range (Lin et al. 2016). Therefore, WS-PLS models based on MIR spectra or Raman spectra were established. In application of PLS algorithm, the optimum number of latent variables (LVs) are a critical parameter in calibration model.

Interval variable selection algorithms (iPLS, siPLS, and biPLS) proposed based on the PLS method were used to eliminate uncorrelated variables to improve PLS

model performance(Chen et al. 2008). Literatures have discussion about the important variables selection or unimportant variables elimination(Zou et al. 2007; Ma et al. 2017). The principles of interval variable selection algorithms were described in various papers(Ma et al. 2017). In iPLS algorithm, the *RMSECV* was calculated for every subinterval when the full spectrum was split into 40 intervals. The spectral region with the lowest *RMSECV* was chosen as the best interval for prediction of RD. In siPLS and biPLS algorithm, the combination of intervals with the lowest *RMSECV* is chosen(Chen et al. 2008). This enables us to select the best combination of intervals, generally providing better correlation coefficient (*R*) values and smaller prediction errors than iPLS(Nørgaard et al. 2000).

PLS models based on fusion data extracted from MIR and Raman spectra were investigated. Fusion data were carried out basically at three level: low-level fusion, mid-level fusion and high-level fusion(Borràs et al. 2015). High-level fusion has often provided worse results than the other two levels(Nunes et al. 2016). Thus, low- level fusion and medium-level were investigated in this paper(Wu et al. 2016). Low-level fusion data consists of original variables of MIR and Raman after the preprocessing steps. Medium-level fusion extracts relevant features from MIR and Raman data separately and then merges them into a single matrix, which will be analyzed by chemometrics (Borràs et al. 2015). In this paper, the characteristic intervals of MIR and Raman were combined as fusion data which acted as input data for establishing models.

144 MIR spectra were divided into two subsets which were called calibration set and prediction set. The calibration set contained 90 spectra were used for establishing model, and remaining 54 spectra as prediction set were used to test the performance of predictive models. The 144 Raman spectra were processed like MIR spectra. In this study, WS-PLS, iPLS, siPLS and biPLS models based on MIR or Raman spectra were obtained. The low- and medium-level fusion approaches were applied to combine of MIR and Raman. In all models, optimum number of latent variables (*LVs*) were determined by root mean square error of cross-validation (*RMSECV*). The performance of the final models were evaluated in accordance with the correlated coefficient of determination (*R*), and *RMSEP* values (Varliklioğlu et al. 2016). The data were processed in MATLAB software version 7.10 (Math Works, Natick, MA, USA).

Results and discussion

RD in starch stored for different time

Corn starch was completely gelatinized as described in the experimental section and then the starch pastes stored at 4 °C for 0, 1, 2, 3, 4, 5, 10, 15 and 20 days were dried. Finally, nine kinds of retrogradation starch were obtained and named 0, 1, 2, 3, 4, 5, 10, 15 and 20 d. RD of retrogradation starch was determined by the enzymatic method based on *α-amylase*. Average values of RD for nine kinds of retrogradation starch were 15.87%, 45.64%, 67.21%, 77.39%, 85.30%, 91.05%, 94.91%, 97.07%, and 98.73% shown in Fig. 1. The standard deviations of each kind retrogradation starch

were 1.234, 1.322, 0.9814, 1.097, 1.501, 0.5618, 0.5222, 0.5189, and 0.3777. It is indicated that RD in starch increased with the storage time prolonged. RD at the first five days varied significantly from 15.81% to 85.30%. The speed of retrogradation was decreased gradually after 5 days. Particularly, the RD of 15 d and 20 d were similar. It can be concluded that the structure and component of retrogradation starch at 0, 1, 2, 3, 4 and 5 d alter dramatically while that at 10, 15, and 20 d alter slightly.

Fig. 1 retrogradation degree of corn starch paste stored for 1, 2, 3, 4, 5, 10, 15 and 20 days

Spectral analysis

MIR and Raman spectroscopy can be used to detect properties of retrogradation starch, such as crystallinity and amorphization. Fig. 2 (a) shows the average MIR spectra of retrogradation starch stored for 0, 1, 2, 3, 4, 5, 10, 15, and 20 days. Fig. 2 (b) shows average Raman spectra for the retrogradation starch samples with different storage time.

MIR spectral patterns of retrogradation starch showed almost identical characteristic bands (Fig. 2 (a)). The characteristic bands mainly contain 756, 820,

216 850, 880, 928, 949, 994, 1022, 1067, 1077, 1133, 1150, 1181, 1241, 1340, 1506,
217 1560, 1648, 1654, 1701, 2177, 2926, and 3275 cm^{-1} . These bands mainly resulted
218 from the vibrational modes of molecule in retrogradation starch. The bands around
219 1600 cm^{-1} are attributed to amorphous region of starch (Smits et al. 1998). The band
220 at 1506 cm^{-1} is influenced by the skeletal mode vibration of α -1, 4 glycosidic linkage
221 (C–O–C). The bands at 1022 and 850 cm^{-1} are sensitive to changes in crystallinity.

222 The nine Raman spectra for retrogradation starch also showed almost identical
223 characteristic bands (Fig. 2 (b)). The main vibrational bands of each spectrum are
224 similar because different samples contain the same main components
225 (polysaccharide). The vibrational bands mainly included 373, 408, 437, 480, 577, 860,
226 940, 952, 1051, 1082, 1126, 1260, 1338, 1382, 1462, 1518, 2116, 2870, 2908 cm^{-1} .
227 The band at 2908 cm^{-1} is related to the symmetrical and antisymmetric CH stretching.
228 The unobvious and sharpened characteristic band at 2870 cm^{-1} can be attributed to the
229 amylose and amylopectin presented in starch (Kizil et al. 2002). The region between
230 1200 and 1600 cm^{-1} contain a large supply of structural information. A majority of the
231 bands in this region are due to coupled vibration involving hydrogen atoms. For
232 instance, the band at 1462 is related to CH, CH_2 , and COH deformation. The feature
233 at 1382 cm^{-1} corresponds to coupling of the CCH and COH deformation modes. The
234 bands at 1260 and 1338 cm^{-1} can be mainly attributed to several vibrational modes,
235 such as CO stretching, CC stretching, CCH deformation, COH deformation, and CCH
236 deformation. The region between 1200 and 800 cm^{-1} is highly characteristic bands

owing to CO stretching, CC stretching and COC deformation modes, referring to the glycosidic bond (Mahdad-Benzerdjeb et al. 2007). This region is considered as the fingerprint or anomeric region, and is discussed with high frequency in the previous papers (Baranska et al. 2005; Nikonenko et al. 2005; Yang and Zhang 2009). The vibrations originating from glycosidic linkages can be observed in the 920-960 cm^{-1} region. Particularly, the band observed at 940 cm^{-1} is assigned to the amylose α -1, 4 glycosidic linkage. Raman spectra of retrogradation starch exhibited complex vibrational modes at low wavenumbers (below 800 cm^{-1}) due to the skeletal mode vibrations of the glucose pyranose ring. Among the Raman bands at 437, 480, 577, a strong band at 480 cm^{-1} portraying the rate of polymerization in polysaccharides is one of the prominent and important indication of the presence of pyranose ring due to skeletal vibration mode (Kizil et al. 2002). Characteristic vibrational bands found in retrogradation starch are shown in Table 1 for both IR and Raman.

Each spectrum of retrogradation starch is unique owing to its particular component and structure. MIR peaks at 1047 and 1022 cm^{-1} have been used for investigating changes in starch structure (organized starch and amorphous starch) during starch retrogradation (Flores-Morales et al. 2012). Previous studies have shown the most useful Raman bands which reflect the characteristics of retrogradation starch. For instance, according to W.T. Winter et al (Winter and Kwak 1987), intensity of the Raman band at 480 cm^{-1} and the half-bandwidths of Raman bands at 2800-3000 cm^{-1} were used as excellent indexes for evaluating retrogradation starch. Besides the bands

discussed in the previous work (Kizil et al. 2002), the intensities and shapes of other characteristic bands are also relevant to the starch component and structure. However, these bands were ignored for determination of retrogradation of starch. Therefore, the more spectral feature will be applied for determining retrogradation starch in further analysis.

Fig. 2 mid-infrared and Raman spectra of different retrogradation starch

265

Table 1 IR and Raman wavenumbers and their respective tentative assignments based on literature data

IR (cm ⁻¹)	Assignments	Raman (cm ⁻¹)	Assignments
3275 S	$\nu(\text{OH})$		
2926 M	$\nu(\text{CH})$	2908 S	$\nu(\text{CH})$
1506 W	COC	1462 M, 1518 W	$\delta(\text{CH}) + \delta(\text{COH}) + \delta(\text{CH}_2)$
1241 M	$\delta(\text{CH}) + \delta(\text{OH})$	1338 S, 1260 M, 1382 M	$\delta(\text{CH})$
1150 S	$\nu(\text{CO}) + \nu(\text{CC})$	1126 S	$\delta(\text{COH}) + \nu(\text{CO}) + \nu(\text{CC})$
1077 S, 1022 S	$\nu(\text{CO}) + \nu(\text{OH}) + \nu(\text{CC})$	1051 M, 1082 M	$\delta(\text{COH}) + \nu(\text{CO}) + \nu(\text{CC})$
994, 928 W	$\gamma(\text{COOH}) + \delta(\text{COO})$	940 S, 952 S	$\delta(\text{COC}) + \delta(\text{COH}) + \nu(\text{CO})$
850 W	$\delta(\text{CCH}) + \delta(\text{COH}) + \gamma(\text{COH})$	860 W	$\nu(\text{COC}) + \nu(\text{CCH})$
		577 W	$\delta(\text{CCO}) + \delta(\text{CO})$
		437W, 480 S	$\delta(\text{CCO}) + \delta(\text{CCC})$

266 **General discrimination of Samples**

267 PCA was applied to MIR and Raman spectra to evaluate their ability to

268 differentiate the retrogradation starch with different storage time. The scores plots of

269 PCA for retrogradation starch stored 0, 1, 2, 3, 4, 5, 10, 15, 20 days were shown in

270 Fig. 3 (a) and (b). Fig. 3 (a) shows the scores plot of PCA based on MIR spectra. The

271 retrogradation starch samples were divided into two categories with some

272 overlapping. The first one category located at the left of the scores plot contained

273 retrogradation starch samples stored 0, 1, 2, 3 and 4 days. The starch samples stored 5,

274 10, 15 and 20 days belonged to the second category located at the right of the scores

275 plot. According to the RD determined by enzymatic method, the RD reached 85%

276 when the starch was stored 4 days. The average growth rate of one day was 21.25%.

277 The RD during the first four days increased rapidly. When the starch was stored for 20

278 days the RD of starch achieved 99%. From the 5th to 20th day, the RD increased from

279 85% to 99% with growth rate of 14%. The average growth rate of every day was

0.875%. The RD increased slowly. The starch retrogradation may be divided into two stages i.e. fast and slow stages.

Fig. 3 (b) shows scores plot of PCA based on Raman spectra. All retrogradation starch samples were divided into three categories. The three categories were not completely separated. One of categories located at the middle of the scores plot contained retrogradation starch samples stored for 0, 1, 2, and 3d. The retrogradation starch samples stored 4 and 5 days belonged to the second category and the other retrogradation starch samples (stored 10, 15 and 20 day) constituted the third category. In the first category, the retrogradation starch stored for 0, 1 and 3 d were separated well from the each other except of 2 d overlapping with 1d. According to the results by enzymatic method the RD of them were increased rapidly. The structure or component of retrogradation starch was very fast at the first three days. For the retrogradation starch samples stored for 4 and 5 days, the RD of them is increased more slowly than the retrogradation starch sample stored 0, 1, 2, and 3 d. The change of retrogradation starch was slow. The RD of retrogradation starch stored for 10, 15 and 20 days showed subtle difference by enzymatic method indicating that starch changed very slowly. Structure and component of retrogradation starch were beginning to stabilize. The aggregation of starch samples maybe led by their similar component and structure. The starch retrogradation may be divided into three stage, i.e. fast, slow and stable stage. The stage retrogradation starch categories could be discriminated by PCA. Whereas, the specific RD in starch sample was not

determined. Therefore, the RD in starch would be determined in following research.

Fig. 3 score plot of the first principal component (PC1) versus the second principal

component (PC2) of different retrogradation starch samples: (a) MIR and (b) Raman

Models for determining RD

Results of models based on MIR or Raman spectra

PLS, iPLS, siPLS and biPLS algorithm was used in this paper for establishing

models to determine RD in starch. The results of models based on MIR and Raman spectra were listed in Table 2. From Table 2, good performance was obtained from the PLS models based on MIR or Raman spectra. The prediction values of RD were between 10%~100%. The lowest *RMSECV* value (8.4) was achieved by biPLS model based on Raman spectra. The corresponding correlation coefficient (R_p) of prediction set was 0.9252 which achieved the best performance. The worst model was iPLS model with highest *RMSECV* of based on the single interval in Raman spectra. The optimal Raman spectral interval is the sixth interval in the spectral range of 407 and 485 cm^{-1} when the whole spectrum split into 40 intervals. The characteristic interval variables were attributed to skeletal mode in starch. The MIR biPLS model based on intervals numbered 2, 40, 28, 34, 17, 33, 37, 35, and 38 achieved the best performance in all models of MIR. The selected intervals are located in the spectral ranges of 734-817, 3917-4000, 2911-2994, 3413-3497, 1989-2072, 3330-3413, 3665-3749, 3497-3581, 3749-3832 cm^{-1} (Fig. 4 (a)). Fig. 4 (b) shows a correlation between RD measured by reference analysis and RD predicted by MIR biPLS in prediction set. For models of Raman, biPLS models also showed the best performance. The selected Raman intervals were numbered 1 2 5 6 9 10 15 33 37 38, corresponding to the wavenumbers in the range of 100-254, 410-564, 718-872, 1181-1258, 2570-2647, 2878-3033 cm^{-1} shown in Fig.5 (a). Fig. 5 (b) shows a correlation between RD measured by reference analysis and RD predicted by Raman biPLS in prediction set.

Comparing the models based on MIR and Raman spectra, models based on Raman spectra were better than models based on MIR spectra except iPLS model. IPLS algorithm caused a decline of the model performance when applied to Raman spectra in comparison with full spectrum PLS model. The superiority of Raman spectroscopy may be attributed to specificity and sensitivity (Yuan et al. 2017). The two methods do not offer the identical information about the molecular vibrations and structure. MIR spectroscopy probe the molecular vibrations when the electrical dipole moment changes, while Raman spectroscopy detect molecular vibrations according to the changes of electrical polarizability (Thygesen et al. 2003). The difference between them indicates that molecules tend to be more sensitive to Raman spectroscopy than to MIR spectroscopy. For instance, the C-C or C=C bond is more sensitive to Raman spectroscopy than to MIR spectroscopy. According to the characteristic vibrational bands for MIR and Raman found in food system, the skeletal mode of starch lead to specific vibrations in the Raman spectral regions located at 900-800 and 500-400 cm^{-1} (Thygesen et al. 2003). MIR is strongly dependent on proper sample preparation and moisture content can seriously affect MIR spectra. Conversely, Raman is highly sensitive and do not require special sample treatment.

Fig. 4 (a): the efficient intervals of MIR variables selected by biPLS for predicting RD
 and (b): reference measured values versus MIR predictive values of RD predicted by
 biPLS in prediction set

Fig. 5 (a): the efficient intervals of Raman variables selected by biPLS for predicting RD and (b): reference measured values versus Raman predictive values of RD predicted by biPLS in prediction set.

Results of models based on fusion data

The validity of data fusion method has been demonstrated in the literature. Even though the performance of models based on MIR or Raman spectra is satisfied, the models based on information extracted from MIR and Raman spectra were still

established for verifying that Raman with aid of MIR would be better than Raman spectroscopy.

As listed in Table 2, the prediction performance of PLS model based on low-level data is not improved in comparison with that of models based on MIR or Raman. Our findings are in agreement with results obtained by other researchers (Nunes et al. 2016). This might be due to that the fusion data from MIR and Raman spectra contained too much redundant information which significantly result in decline of PLS model performance.

To overcome too much redundant information, characteristic intervals of MIR and Raman which selected by biPLS in section 3.4.1 were merged as medium-level fusion data. The process of medium-level data fusion was shown in Fig. 6. As a result, PLS model based on characteristic intervals of MIR and Raman (medium-level fusion data) achieved a better performance with the highest R_p of 0.9658 than PLS model based on full raw variables (low-level fusion data). Low-level fusion is simple, just uses merge raw spectra. But high data volume may contain a large number of noise or irrelevant information. The performance of predictive models would be influenced by the negative information. Some limitation of low-level fusion can be partially overcome by medium-level fusion. Characteristic intervals can decrease the data volume and eliminate noise or irrelevant information.

Retrogradation degree are determined only by Raman spectroscopy, and satisfied results are obtained. (Table 2). But by combining MIR and Raman spectroscopy, more

380 accurate and reliable models were obtained. The combination of MIR and Raman
381 spectroscopy proved better in determination of retrogradation degree compared to
382 single Raman spectroscopy. Though Raman spectroscopy and chemometric tools have
383 been successfully used for exploratory analysis of pure corn, cassava starch samples
384 and mixtures of both starches, as well as for the quantification of amylose content in
385 corn and cassava starch samples (Almeida et al. 2010). Both MIR and Raman can
386 generate bands linked to fundamental vibration and supply fingerprints of components
387 that can be used for quantitative and qualitative characterization. Even though both
388 methods probe molecular vibrations and structure, they do not provide exactly the
389 same information (Thygesen et al. 2003). Raman spectroscopy detect molecular
390 vibrations according to the changes of electrical polarizability. While MIR
391 spectroscopy probe the molecular vibrations when the electrical dipole moment
392 changes. They are complementary techniques for the study of molecular vibrations
393 and structure. For example, the C-C group has a strong Raman scattering band in
394 Raman spectra but weak absorption bands in the mid-infrared. O-H vibration is very
395 strong in MIR, but very weak in Raman(Yang and Irudayaraj 2002). The intensities of
396 characteristic bands in Raman and MIR spectra collected from same food are different
397 and the information they contain are not identical (Flores-Morales et al. 2012). Due to
398 their distinct advantages, data fusion, as an emerging technology, is an efficient way
399 for the optimum utilization of data from different sources, and has been successfully
400 used to the rapid measurement of retrogradation starch in this paper. Therefore, a

useful methods based on MIR and Raman spectroscopy were developed for determining retrogradation starch.

Fig. 6 PLS model based on medium fusion data extracted from the MIR and Raman spectra.

Table 2 the results of different predictive models

407

Spectra	Models	LV	Calibration set		Prediction set	
			$RMSECV$	R_c	$RMSEP$	R_p
MIR spectra	WS-PLS	10	12.8	0.8956	16.2	0.8162
	iPLS	7	14.3	0.8538	16.5	0.8101
	siPLS	8	11.5	0.9016	15.1	0.8401
	biPLS	8	10.0	0.9258	14.2	0.8542
Raman spectra	WS-PLS	10	12.9	0.8924	15.9	0.8204
	iPLS	5	14.9	0.8433	17.1	0.808
	siPLS	7	10.0	0.9275	13.5	0.8806
	biPLS	8	8.4	0.9587	10.1	0.9252
Fusion spectra	low-PLS	12	7.2	0.9873	8.7	0.9573
	medium-PLS	7	7.0	0.9887	7.7	0.9658

408 **Conclusion**

409 The RD in starch stored for different storage time have been predicated by MIR
410 and Raman spectroscopy combined with chemometrics. The PLS model based on
411 medium-level fusion data of MIR and Raman spectra had the best prediction
412 performance with correlation coefficient (R) of 0.9658. Retrogradation starch was
413 obtained by chilling gelatinized corn starch for different time (0, 1, 2, 3, 4, 5, 10, 15,
414 20 days). The low-level and medium-level fusion data extracted from MIR and
415 Raman were also analyzed by PLS. In addition, The MIR and Raman spectra of
416 retrogradation starch were analyzed by SG, PCA, PLS, iPLS, siPLS and biPLS for
417 determination of RD in starch. The results demonstrated that the prediction
418 performance of models for Raman are better than those based on the MIR except iPLS
419 model. Variables selection improved the performance of PLS models. PLS model
420 based on medium-level fusion data achieved the best performance in comparison with
421 the models. Prediction of the RD in starch based on combination of MIR and Raman

spectroscopy are more accurately than that based on single technique. This indicates that the developed methodology may be able to forecast the quality, acceptability and shelf-life of starch products or starch-containing products easily damaged by starch retrogradation.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent is not applicable for the nature of this study.

Reference

- Almeida MR, Alves RS, Nascimbem LBLR, Stephani R, Poppi RJ, de Oliveira LFC (2010) Determination of amylose content in starch using Raman spectroscopy and multivariate calibration analysis Analytical and Bioanalytical Chemistry 397:2693-2701 doi:10.1007/s00216-010-3566-2
- Baranska M, Schulz H, Baranski R, Nothnagel T, Christensen LP (2005) In Situ Simultaneous Analysis of Polyacetylenes, Carotenoids and Polysaccharides in Carrot Roots Journal of Agricultural and Food Chemistry 53:6565-6571 doi:10.1021/jf0510440
- Borràs E, Ferré J, Boqué R, Mestres M, Aceña L, Bust o O (2015) Data fusion methodologies for food and beverage authentication and quality assessment – A review Analytica Chimica Acta 891:1-14 doi:http://dx.doi.org/10.1016/j.aca.2015.04.042
- Chang S-M, Liu L-C (1991) Retrogradation of Rice Starches Studied by Differential Scanning Calorimetry and Influence of Sugars, NaCl and Lipids Journal of Food Science 56:564-566 doi:10.1111/j.1365-2621.1991.tb05325.x
- Chen H, Pan T, Chen J, Lu Q (2011) Waveband selection for NIR spectroscopy analysis of soil organic matter based on SG smoothing and MWPLS methods Chemometrics and Intelligent Laboratory Systems 107:139-146 doi:http://dx.doi.org/10.1016/j.chemolab.2011.02.008
- Chen Q, Zhao J, Liu M, Cai J, Liu J (2008) Determination of total polyphenols content in green tea using FT-NIR spectroscopy and different PLS algorithms Journal of Pharmaceutical and Biomedical Analysis 46:568-573 doi:http://dx.doi.org/10.1016/j.jpba.2007.10.031
- de Peinder P, Vredenburg MJ, Visser T, de Kaste D (2008) Detection of Lipitor® counterfeits: A comparison of NIR and Raman spectroscopy in combination with chemometrics Journal of Pharmaceutical and Biomedical Analysis 47:688-694 doi:http://dx.doi.org/10.1016/j.jpba.2008.02.016
- Di Paola RD, Asis R, Aldao MAJ (2003) Evaluation of the Degree of Starch Gelatinization by a New Enzymatic Method Starch - Stärke 55:403-409 doi:10.1002/star.200300167
- Eliasson AC (2010) Gelatinization and retrogradation of starch in foods and its implications for food quality. In: Skibsted LH, Risbo J, Andersen ML (eds) Chemical deterioration and physical instability of food and beverages. Woodhead Publishing Limited, Oxford, U.K., pp 296-323
- Ferrero C, Martino MN, Zaritzky NE (1994) Corn Starch-Xanthan Gum Interaction and Its Effect on the Stability During Storage of Frozen Gelatinized Suspension Starch - Stärke 46:300-308 doi:10.1002/star.19940460805
- Flores-Morales A, Jiménez-Estrada M, Mora-Escobedo R (2012) Determination of the structural changes by FT-IR, Raman, and CP/MAS 13C NMR spectroscopy on retrograded starch of maize tortillas Carbohydrate Polymers 87:61-68 doi:http://dx.doi.org/10.1016/j.carbpol.2011.07.011
- Haiyan Y, Xiaoying N, Yibin Y, Xingxiang P (2008) Non-invasive determination of enological parameters of rice wine by Vis-NIR spectroscopy and least-squares support vector machines. Paper presented at the 2008 Providence, Rhode Island, June 29 – July 2, 2008, St. Joseph, Mich.,
- Hayakawa K, Tanaka K, Nakamura T, Endo S, Hoshino T (1997) Quality Characteristics of Waxy

483 Hexaploid Wheat (*Triticum aestivum* L.): Properties of Starch Gelatinization and
 484 Retrogradation Cereal Chemistry Journal 74:576-580 doi:10.1094/CCHEM.1997.74.5.576
 485 Karim AA, Norziah MH, Seow CC (2000) Methods for the study of starch retrogradation Food Chem
 486 71:9-36 doi:http://dx.doi.org/10.1016/S0308-8146(00)00130-8
 487 Kim J-O, Kim W-S, Shin M-S (1997) A Comparative Study on Retrogradation of Rice Starch Gels by DSC,
 488 X-Ray and α -Amylase Methods Starch - Stärke 49:71-75 doi:10.1002/star.19970490207
 489 Kizil R, Irudayaraj J, Seetharaman K (2002) Characterization of Irradiated Starches by Using FT-Raman
 490 and FTIR Spectroscopy Journal of Agricultural and Food Chemistry 50:3912-3918
 491 doi:10.1021/jf011652p
 492 Lin Y-W, Deng B-C, Wang L-L, Xu Q-S, Liu L, Liang Y-Z (2016) Fisher optimal subspace shrinkage for block
 493 variable selection with applications to NIR spectroscopic analysis Chemometrics and
 494 Intelligent Laboratory Systems 159:196-204
 495 doi:http://dx.doi.org/10.1016/j.chemolab.2016.11.002
 496 Liu H, Yu L, Chen L, Li L (2007) Retrogradation of corn starch after thermal treatment at different
 497 temperatures Carbohydrate Polymers 69:756-762
 498 doi:http://dx.doi.org/10.1016/j.carbpol.2007.02.011
 499 Ma H-I, Wang J-w, Chen Y-j, Cheng J-I, Lai Z-t (2017) Rapid authentication of starch adulterations in
 500 ultrafine granular powder of Shanyao by near-infrared spectroscopy coupled with
 501 chemometric methods Food Chem 215:108-115
 502 doi:http://dx.doi.org/10.1016/j.foodchem.2016.07.156
 503 Mahdad-Benzerdjeb A, Taleb-Mokhtari IN, Sekkal-Rahal M (2007) Normal coordinates analyses of
 504 disaccharides constituted by d-glucose, d-galactose and d-fructose units Spectrochimica Acta
 505 Part A: Molecular and Biomolecular Spectroscopy 68:284-299
 506 doi:http://dx.doi.org/10.1016/j.saa.2006.11.032
 507 Mariotti M, Sinelli N, Catenacci F, Pagani MA, Lucisano M (2009) Retrogradation behaviour of milled
 508 and brown rice pastes during ageing Journal of Cereal Science 49:171-177
 509 doi:http://dx.doi.org/10.1016/j.jcs.2008.09.005
 510 Monakhova YB, Diehl BWK (2016) Authentication of the origin of sucrose-based sugar products using
 511 quantitative natural abundance ^{13}C NMR Journal of the Science of Food and Agriculture
 512 96:2861-2866 doi:10.1002/jsfa.7456
 513 Nørgaard L, Saudland A, Wagner J, Nielsen JP, Munck L, Engelsen SB (2000) Interval Partial Least-
 514 Squares Regression (iPLS): A Comparative Chemometric Study with an Example from Near-
 515 Infrared Spectroscopy Applied Spectroscopy 54:413-419
 516 Nikonenko NA, Buslov DK, Sushko NI, Zhibankov RG (2005) Spectroscopic manifestation of stretching
 517 vibrations of glycosidic linkage in polysaccharides Journal of Molecular Structure 752:20-24
 518 doi:http://dx.doi.org/10.1016/j.molstruc.2005.05.015
 519 Nunes KM, Andrade MVO, Santos Filho AMP, Lasmar MC, Sena MM (2016) Detection and
 520 characterisation of frauds in bovine meat in natura by non-meat ingredient additions using
 521 data fusion of chemical parameters and ATR-FTIR spectroscopy Food Chem 205:14-22
 522 doi:http://dx.doi.org/10.1016/j.foodchem.2016.02.158
 523 Olayinka OO, Olu-Owolabi BI, Adebawale KO (2011) Effect of succinylation on the physicochemical,
 524 rheological, thermal and retrogradation properties of red and white sorghum starches Food

Hydrocolloids 25:515-520 doi:<http://dx.doi.org/10.1016/j.foodhyd.2010.08.002>

Paker I, Matak KE (2016) Influence of pre-cooking protein paste gelation conditions and post-cooking gel storage conditions on gel texture Journal of the Science of Food and Agriculture 96:280-286 doi:10.1002/jsfa.7091

Rocha JTC et al. (2016) Sulfur Determination in Brazilian Petroleum Fractions by Mid-infrared and Near-infrared Spectroscopy and Partial Least Squares Associated with Variable Selection Methods Energy & Fuels 30:698-705 doi:10.1021/acs.energyfuels.5b02463

Romano G, Nagle M, Müller J (2016) Two-parameter Lorentzian distribution for monitoring physical parameters of golden colored fruits during drying by application of laser light in the Vis/NIR spectrum Innovative Food Science & Emerging Technologies 33:498-505 doi:<http://dx.doi.org/10.1016/j.ifset.2015.11.007>

Sajilata MG, Singhal RS, Kulkarni PR (2006) Resistant Starch—A Review Comprehensive Reviews in Food Science and Food Safety 5:1-17 doi:10.1111/j.1541-4337.2006.tb00076.x

Smits ALM, Ruhnau FC, Vliegthart JFG, van Soest JGG (1998) Ageing of Starch Based Systems as Observed with FT-IR and Solid State NMR Spectroscopy Starch - Stärke 50:478-483 doi:10.1002/(SICI)1521-379X(199812)50:11/12<478::AID-STAR478>3.0.CO;2-P

Synytsya A, ČopíKová J, Matějka P, Machovič V (2003) Fourier transform Raman and infrared spectroscopy of pectins Carbohydrate Polymers 54:97-106

Thygesen LG, Løkke MM, Micklander E, Engelsen SB (2003) Vibrational microspectroscopy of food. Raman vs. FT-IR Trends in Food Science & Technology 14:50-57 doi:[http://dx.doi.org/10.1016/S0924-2244\(02\)00243-1](http://dx.doi.org/10.1016/S0924-2244(02)00243-1)

Vankeirsbilck T, Vercauteren A, Baeyens W, Van der Weken G, Verpoort F, Vergote G, Remon JP (2002) Applications of Raman spectroscopy in pharmaceutical analysis TrAC Trends in Analytical Chemistry 21:869-877 doi:[http://dx.doi.org/10.1016/S0165-9936\(02\)01208-6](http://dx.doi.org/10.1016/S0165-9936(02)01208-6)

Varliklioz Er S, Eksi-Kocak H, Yetim H, Boyaci IH (2016) Novel Spectroscopic Method for Determination and Quantification of Saffron Adulteration Food Analytical Methods:1-9 doi:10.1007/s12161-016-0710-4

Winter WT, Kwak YT (1987) Rapid-scanning Raman spectroscopy: a novel approach to starch retrogradation Food Hydrocolloids 1:461-463 doi:[http://dx.doi.org/10.1016/S0268-005X\(87\)80041-3](http://dx.doi.org/10.1016/S0268-005X(87)80041-3)

Wu Z, Xu E, Long J, Pan X, Xu X, Jin Z, Jiao A (2016) Comparison between ATR-IR, Raman, concatenated ATR-IR and Raman spectroscopy for the determination of total antioxidant capacity and total phenolic content of Chinese rice wine Food Chem 194:671-679 doi:<http://dx.doi.org/10.1016/j.foodchem.2015.08.071>

Xu W, Liu X, Xie L, Ying Y (2014) Comparison of Fourier Transform Near-Infrared, Visible Near-Infrared, Mid-Infrared, and Raman Spectroscopy as Non-Invasive Tools for Transgenic Rice Discrimination Transactions of the Asabe 57:141-150

Yang L, Zhang L-M (2009) Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources Carbohydrate Polymers 76:349-361 doi:<http://dx.doi.org/10.1016/j.carbpol.2008.12.015>

Yeung KY, Ruzzo WL (2001) Principal component analysis for clustering gene expression data Bioinformatics 17:763-774 doi:10.1093/bioinformatics/17.9.763

567 Yuan J et al. (2017) A rapid Raman detection of deoxynivalenol in agricultural products Food Chem
568 221:797-802 doi:<http://dx.doi.org/10.1016/j.foodchem.2016.11.101>
569 Zou X, Zhao J, Li Y (2007) Selection of the efficient wavelength regions in FT-NIR spectroscopy for
570 determination of SSC of 'Fuji' apple based on BiPLS and FiPLS models Vibrational
571 Spectroscopy 44:220-227 doi:<http://dx.doi.org/10.1016/j.vibspec.2006.11.005>
572
573