Original research article

Changes in the phenolic acids composition during pancake preparation: Whole and refined grain flour and processed food classification by UV and NIR spectral fingerprinting method—Proof of concept

Yingjian Lu^a, Ayaz Memon^a, Patrick Fuerst^{b,c}, Alecia Kizonas^b, Craig Morris^c,
Devanand Luthria^{a,*}

^a Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, United States

^b Department of Crop and Soil Sciences, Washington State University, Pullman, WA, 99164, United States

^c Western Wheat Quality Lab., Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA, 99164, United States

ARTICLE INFO

Article history:

Received 26 September 2016

Received in revised form 24 February 2017

Accepted 1 March 2017

Available online 4 March 2017

Keywords:

Food analysis

Wheat (*Triticum*)

Pancake preparation

Phenolic acids

Spectral fingerprinting

PCA

Two wheat cultivars

Whole and refined grain

Flour to batter to pancake

Proof of concept

ABSTRACT

We investigated the changes in the levels of phenolic acids during pancake preparation (flour to batter-pancake) from two refined and whole-wheat wheat varieties. In addition, we evaluated if multivariate analysis of ultraviolet (UV) and near infrared (NIR) spectral fingerprinting data can be used for classification of samples based on preparation stage, wheat varieties, and flour types (whole and refined). Results indicated that total phenolic acids did not significantly change (< 10%) during preparation of pancake from the refined and whole wheat flours. Most (> 90%) of the phenolic acids existed in insoluble bound form and ferulic acid (82–92%) was the most abundant phenolic acid. Correlation between simple UV spectral scan and HPLC analysis for the assay of phenolic acids ranged from 0.771 to 1.00. Principle component analysis (PCA) of NIR spectral data from 4900 to 5900 cm⁻¹ showed clear separation between flour, batter, and pancake. Additionally, the PCA of UV spectral data between 250 and 350 nm separated two into clusters well (refined and whole-wheat samples). The results presented in this manuscript with limited number of samples illustrate the proof of concept that spectral fingerprinting techniques show promising potential for whole and refined grains sample classification and their products.

© 2017 Published by Elsevier Inc.

1. Introduction

Batter-based wheat products such as cakes, ice cream cones, pancakes, and waffles are consumed globally and are important sources of energy, carbohydrates, and other bioactive phytochemicals. A batter is made by mixing dry flours with ingredients such as water, milk, or eggs to the point that a pourable viscous flow is obtained. In the United States, pancakes or griddle cakes are commonly served at breakfast. Pancakes provide an effective model of batter-based products because its batter is fried or cooked on a hot griddle, this allows us to investigate the influence of processing on bioactive phytochemical compositions (Morris and Rose, 1996).

Phenolic acids are widely distributed in grains and are present in high concentrations in whole-wheat products. Ferulic acid is the primary and most abundant phenolic acid in wheat grain. Smaller concentrations of caffeic, *p*-coumaric, sinapic acid, and other phenolic acids are also observed in wheat (Verma et al., 2009). Phenolic acids are present in comparatively higher concentrations in the germ and bran fractions as compared to the endosperm (Lu et al., 2014a). They are thought to be one of the important groups of compounds responsible for health promoting properties of whole wheat products (Slavin et al., 2000; Truswell, 2002).

There are number of research investigation on nutrients on whole grain foods due to health benefits associated with the consumption of whole grains (Vitaglione et al., 2015). Dietary guidelines for Americans suggests at least half the grain consumed is whole grain (USDA, 2010). There are large number of studies showing the phytochemical content of whole grain is significantly higher as compared to the refined grain. Recently, Geng et al. (2015) showed the application of fuzzy chromatography mass

* Corresponding author.

E-mail address: Dave.Luthria@ars.usda.gov (D. Luthria).

chromatographic followed by chemometrics analysis (principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA)) protocols for differentiation of whole and refined grains.

There have been a number of recent studies evaluating the effect of thermal treatments (cooking, frying, and baking) on the concentration of bioactive phytochemicals in the wheat-based products. Abdel-Aal and Rabalski (2013) found that baking increased the free phenolic acid while bound phenolic acids decreased or changed slightly in three whole grains cookies, muffins, and bread. In another study, baking decreased the content of total ester-bound phenolic acids and ferulic acid dehydrodimers in whole rye bread (Hansen et al., 2002). Additionally, Lu et al. (2014b) indicated that the total quantified phenolic acids did not change significantly when breads were prepared from refined and whole wheat flour. In another study by Lu et al. (2015) the authors pointed out that phenolic acids were relatively stable during baking, whereas tocopherols (~25–80%) and carotenoids (~20–80%), were partially degraded. Furthermore, Kiszona et al. (2015) carried out a study to investigate changes in the total arabinoxylans (TAX) and water-extractable arabinoxylans (WEAX) content during the making of pancakes. The authors found moderate changes in AX content during processing and a positive association of WEAX levels with end-product pancake volume.

As per our knowledge, there is no study reported in the literature on the changes in phenolic acids content during pancake preparation from whole and refined grain. The objective of our study was to investigate the changes in the levels of phenolic acids content during pancake preparation. We also determined the correlation between rapid UV spectral absorbance and the detailed LC–MS analysis for the determination of TPA in wheat-based matrix. Furthermore, we evaluated the application of simple ultraviolet and near infrared spectral fingerprints as a proof of concept to classify flour, batter, and pancake samples made from two wheat varieties, ‘Louise’ (soft white spring wheat) and ‘Westbred 936’ (WB 936, hard red spring wheat) using two types of wheat flours, refined (RF) and whole wheat (WW).

2. Materials and methods

2.1. Wheat samples

Two wheat varieties (Louise – soft white spring and Westbred 936 – hard red spring), grown in 2009 were used in the present study. Pancakes were prepared from both the refined (RF) and whole-wheat (WW) flours for each wheat variety. Grain was tempered and milled to approximately 70% extraction (refined flour) in a Bühler MLU-202 flour mill (Bühler, Uzwil, Switzerland) (AACCI 1988a, 1988b). Whole wheat flour was produced by reducing the particle size of bran with a pin mill (model 91, Blendtec, Orem, UT, USA) so that $\geq 70\%$ passed through a 180 μm sieve and then blending with the refined flour. All the flour samples were stored at -20°C prior to use.

2.2. Pancake sample preparation method

The pancakes were prepared as described in our earlier publications (AACCI 2015; Kiszona et al., 2015). In short, dry pancake mixture consisted of 175 g of flour, 17.0 g of granulated sucrose, 5.5 g of dextrose, 3.2 g of sodium bicarbonate, 0.8 g of mono calcium phosphate, 3.5 g of sodium acid pyrophosphate, and 2.6 g of sodium chloride. Thus, the flour proportion in the dried batter and pancake was 80.1% of the total weight of dry ingredients plus oil. The leavening acids and sodium bicarbonate 25% of dextrose, 3.2 g of sodium bicarbonate, 0.8 g of mono calcium phosphate, 3.5 g of sodium acid pyrophosphate, and 2.6 g of

sodium chloride. Thus, the flour proportion in the dried batter and pancake was 80.1% of the total weight of dry ingredients plus oil. The leavening acids and sodium bicarbonate were kept separate from the other ingredients as well as from each other until combined with the other dry ingredients prior to use to prevent pre-reaction. The dry ingredients were mixed together at low speed with a Hobart N5 mixer from Hobart Corporation (Troy, OH, USA) or at medium speed with a KitchenAid 600 Series mixer from KitchenAid Corporation (St. Joseph, MI, USA) with a whisk attachment for 1 min. Preparation of pancake is described in our earlier publication (Kiszona et al., 2015). All analyses were performed on dried flour, batter, and pancake samples.

2.3. Chemicals and reagents

Phenolic acid (vanillic, caffeic, syringic, *p*-coumaric, ferulic, and sinapic acids) standards and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide and all solvents (ethyl ether, ethyl acetate, and methanol) were obtained from Fisher Chemicals (Fair Lawn, NJ, USA). All chemicals and solvents were either analytical grade or HPLC grade and were used directly without further purification. EDTA (ethylene diamine tetra acetic acid) was purchased from EMD Chemicals (Gibbstown, NJ, USA). Polyvinylidene difluoride (PVDF) syringe filters with pore size 0.45 μm were purchased from National Scientific Company (Duluth, GA, USA).

2.4. Phenolic acids analysis

The ground flour, batter, or pancake sample (250 ± 1 mg) was separately extracted for soluble (free and conjugated) and insoluble bound phenolic acids using ultrasonic assisted extraction as described previously (Lu et al., 2015). After evaporating the organic phase under nitrogen, the residue was re-dissolved in methanol: water (75:25, v/v) and filtered through a 0.45 μm membrane filter. The filtered extract was used for the analysis of soluble (free and conjugated) and insoluble bound phenolic acids by LC–MS.

2.5. Analysis of phenolic acids by HPLC–ESI–MS

Quantification of phenolic acids from all samples was carried out using an Agilent 1290 Infinity LC system coupled to a diode array (DA) and a mass spectrometry (MS) detectors from Agilent Technologies (Palo Alto, CA, USA) as previously described (Luthria et al., 2012). Identification of phenolic acids was achieved by comparison of mass and ultraviolet spectral data as previously reported and by comparison of retention time with authentic commercial phenolic acids standard (Luthria et al., 2006). Briefly, separation of phenolic acids was achieved using a reversed phase C18 Luna column (150 $\times$ 4.6 mm; particle size 5 μm) from Phenomenex (Torrance, CA, USA) at ambient temperature (between 22 and 27 $^\circ\text{C}$). Separation was achieved using a binary gradient with two solvents. Solvent A was 0.1% (v/v) formic acid in water and solvent B was 0.1% (v/v) formic acid in methanol. The flow rate was set to 1 mL/min and 20 μL of sample was used for injection. The linear gradient consisted of 5% (B) to 30% (B) for 25 min, followed by 30% (B) for 35 min, then ramped from 30% (B) to 100% (B) for 10 min and held at 100% (B) for 5 min. The mobile phase concentration was returned to 5% (B) and held for 10 min before the next injection. The mass spectral data were collected in both positive and negative ion modes at low and high fragment voltages (70 V and 250 V). The instrument was set to scan from 100 to 750 mass units. The temperature of the drying gas was 300 $^\circ\text{C}$ at a flow rate of 12 L/min, capillary voltage for both positive and negative ion modes was adjusted at 3000 V and a nebulizer

pressure of 345 kPa. The LC system was directly connected to a mass spectrometer with no stream splitting. All Samples were extracted and analyzed in four replicates.

2.6. UV spectral fingerprinting

Flour, batter, and pancake extracts from HPLC analysis of phenolic acids (200 μ L) were placed in a 96 well Costar plate with a UV transparent flat bottom (Corning Inc., NY, USA). The extracts were scanned from 250 and 500 nm in the Spectramax Plus 384 plate reader (Molecular Devices, CA, USA). Columns 1 and 12 in 96 well plates were filled with MeOH:H₂O (80:20, v/v, blank) and four replicates extraction and analysis was carried out with samples from different pancake preparation stages. The spectral absorbance data for each extract was converted into an Excel (Microsoft, Inc., Bellevue, WA, USA) format for statistical analysis. Spectra were from 250 to 500 nm at 1 nm intervals. Each extract was analyzed in quadruplicate. The resultant data matrix, 48 samples by 251 absorption intensities were used for statistical analysis.

2.7. NIR spectral fingerprinting

Approximately 1 g of the dried powdered flour, batter, or pancake sample was placed in an 8 mL glass vial and tamped on a solid surface to ensure that the powder was settled firmly. The powdered samples were scanned between 4000 and 10,000 cm^{-1} at approximately 2 cm^{-1} intervals in the NIR spectrometer – Nicolet 6700 (Thermo-Electron, Waltham, MA, USA). Each sample was analyzed six times after remixing the samples by shaking resulting in 72 spectral fingerprints. Spectra were exported from the NIR spectrometer to Excel (Microsoft, Inc., Bellevue, WA, USA). The resultant data matrix, was further analyzed using standalone chemometrics software: Solo (Eigenvector Research, Inc., Manson, WA, USA). Preprocessing in Solo consisted of normalization (normalized to unit vector) and mean centering.

2.8. Statistical analysis

Data were reported as means $\pm$ standard deviations (SD) for four replicates treatments. One-way analysis of variance (ANOVA) and Tukey's tests were performed using SPSS (SPSS for Windows, version 10.0.5, SPSS Inc., Chicago, IL, USA). Correlation analyses were performed using a two-tailed Pearson's correlation test. Statistical significance was declared at $P < 0.05$.

3. Results and discussion

3.1. Comparison of phenolic acids in refined vs. whole wheat samples from different pancake preparation stages

Six point calibration curves for the four commercial phenolic acids identified in all samples were constructed using diode-array detection method. The linear regression equation and the correlation coefficient for each identified phenolic acid was determined (caffeic acid – Eq $y = 1896.4x - 47.617$, R^2 0.9999; *p*-coumaric acid – Eq $y = 499.31x + 5.419$, R^2 0.9969; ferulic acid – Eq $y = 2680.3x + 16.041$, R^2 0.9995; sinapic acid – Eq $y = 1917.3x - 3.165$, R^2 0.9997). The limit of detection and the limit of quantification for phenolic acids was determined as 0.05 $\mu\text{g/mL}$ and 0.2 $\mu\text{g/mL}$ respectively. The quantities of total phenolic acids extracted from the RF flour samples were significantly lower than those from the WW flour samples (Table 1). This is in agreement with the previously published reports from our lab and other authors where only about one tenth of the total phenolic acids content in RF wheat flour was observed as compared to the WW flour (Beta et al., 2005; Lu et al., 2015; Mattila et al., 2005).

The phenolic acid composition and content in RF and WW flour, batter, and pancake are presented in Table 1. In terms of overall abundance, ferulic acid (FA) was the most abundant phenolic acid, accounting for a mean of 85% (ranged from 84 to 87%) of TPA among RF samples and a mean of 90% (ranged from 89 to 90%) among WW samples. Sinapic acid was second most abundant phenolic acid, accounting for a mean of 9% and 6% of TPA in RF and WW samples, respectively. Among all samples evaluated, TPA ranged from 10.2- to 12.9-fold greater in WW samples than in RF samples (Table 1). All individual phenolic acids were also higher in WW than in RF samples (Table 1). The greatest increases came from FA, which had at most a 13.2-fold increase, in WW compared to RF flour when averaged across all samples in different pancake stages and varieties. Our results are consistent with previous studies demonstrating that total phenolic acids were around 10-fold greater in whole wheat than in refined wheat products (Mattila et al., 2005).

The huge increase in FA in WW compared to RF samples correlated well with previous study where higher level (8–15-fold) of FA was detected in bran than in RF flour samples (Andreasen et al., 2000). Ferulates were also far more abundant (about 100-fold) in outer layer of the wheat kernel, including bran and germ, compared to starchy endosperm tissue (Barron et al., 2007).

Table 1

Individual and total phenolic acids (TPA) of the flour, batter, and pancake prepared from Louise (soft white wheat) and WB936 (hard red wheat) variety*.

Type	Preparation stage	CA ($\mu\text{g/g}$)	<i>p</i> -CA ($\mu\text{g/g}$)	FA ($\mu\text{g/g}$)	SiA ($\mu\text{g/g}$)	TPAs ($\mu\text{g/g}$)
Louise RF	Flour	2.17 $\pm$ 0.04a	1.70 $\pm$ 0.07a	48.75 $\pm$ 1.70a	3.50 $\pm$ 0.06a	56.13 $\pm$ 2.00a
Louise RF	Batter	2.63 $\pm$ 0.09a	2.29 $\pm$ 0.12a	48.45 $\pm$ 2.43a	4.07 $\pm$ 0.45a	57.43 $\pm$ 0.85a
Louise RF	Pancake	2.72 $\pm$ 0.13a	2.40 $\pm$ 0.22a	50.81 $\pm$ 1.06a	4.50 $\pm$ 0.35a	60.44 $\pm$ 1.21a
Louise WW	Flour	12.12 $\pm$ 1.72bc	24.67 $\pm$ 1.01c	657.13 $\pm$ 19.14b	58.19 $\pm$ 13.70d	752.11 $\pm$ 22.31bc
Louise WW	Batter	11.15 $\pm$ 1.17b	24.07 $\pm$ 1.94c	670.76 $\pm$ 22.57b	33.01 $\pm$ 1.92b	738.98 $\pm$ 23.63b
Louise WW	Pancake	13.18 $\pm$ 1.78bc	24.67 $\pm$ 1.21c	683.52 $\pm$ 37.14b	51.34 $\pm$ 16.74cd	772.71 $\pm$ 27.64c
WB936 RF	Flour	2.45 $\pm$ 0.10a	2.46 $\pm$ 0.20a	59.93 $\pm$ 1.64a	6.94 $\pm$ 2.57a	71.78 $\pm$ 2.23a
WB936 RF	Batter	2.78 $\pm$ 0.10a	2.18 $\pm$ 0.27a	51.67 $\pm$ 3.08a	4.27 $\pm$ 1.72a	60.90 $\pm$ 2.80a
WB936 RF	Pancake	3.09 $\pm$ 0.13a	2.49 $\pm$ 0.14a	60.61 $\pm$ 5.09a	7.30 $\pm$ 2.08a	73.49 $\pm$ 3.95a
WB936 WW	Flour	13.69 $\pm$ 0.90c	15.62 $\pm$ 0.61b	714.31 $\pm$ 15.51c	64.94 $\pm$ 4.16d	808.57 $\pm$ 11.49d
WB936 WW	Batter	12.21 $\pm$ 2.18bc	15.77 $\pm$ 0.36b	763.72 $\pm$ 16.28c	40.16 $\pm$ 2.16bc	831.85 $\pm$ 16.66d
WB936 WW	Pancake	16.37 $\pm$ 1.17d	15.05 $\pm$ 0.76b	756.46 $\pm$ 7.34c	54.43 $\pm$ 4.42d	842.31 $\pm$ 9.90d

Note: RF, refined; WW, whole wheat; CA, caffeic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; SiA, sinapic acid; TPAs, total phenolic acids. The results were expressed as Mean $\pm$ SD of four replicate extraction and analysis ($n=4$) followed by a letter. Same letter means no statistical difference whereas different letter stands for significant statistical difference ($P > 0.05$).

3.2. Significance of processing on phenolic acids during pancake preparation

TPA and FA contents in pancakes (60.44 and 73.49 $\mu\text{g/g}$ dry weight (DW)) were similar or marginally higher than the batter and flour in the refined samples for both wheat varieties (Table 1). The pancake made from refined flours also had slightly higher amounts of caffeic, *p*-coumaric, ferulic, and TPA in both Louise and WB936 wheat varieties. Similarly, TPA and FA trends among WW (flour, batter, and pancake) samples were observed. The WW pancake showed similar or marginal increase in most individual phenolic acids and TPA content than the corresponding flour and batter samples. The above results indicate that thermal processing did not reduce the levels of phenolic acids in prepared pancake samples.

3.3. Comparison of phenolic acid content in two wheat varieties

When comparing RF flour samples for the two varieties, caffeic, ferulic, sinapic, and TPA content were always higher in WB936 than in Louise (Table 1). In the WW samples, caffeic, ferulic, sinapic, and total phenolic acids contents in WB936 were consistently higher as expected, but the levels of *p*-coumaric acid was greater in Louise than in WB936 wheat variety. In WW pancake sample, FA and TPA content was significantly higher in WB936 (756.46 $\mu\text{g/g}$ DW; 842.31 $\mu\text{g/g}$ DW) than in Louise (683.52 $\mu\text{g/g}$ DW; 772.71 $\mu\text{g/g}$ DW) (Table 1). These were in agreement with previously reported results (Whent et al., 2012).

3.4. NIR and UV spectral fingerprints

Fig. 1A shows the UV spectral fingerprints (250–350 nm) and Fig. 1B depicts near infrared spectral fingerprints (4900–5900 cm^{-1}) of the flour, batter, and pancake made from two refined and whole wheat cultivars. For UV analysis each sample was extracted four times and analyzed and for NIR analysis each sample was analyzed in six replicates. The relative standard

Fig. 1. A. UV spectral fingerprints (250–350 nm) and B. Near infrared spectral fingerprints (4900–5900 cm^{-1}) of the flour, batter, and pancake made from two refined and whole wheat cultivars. For UV analysis each sample was extracted four times ($n=4$) and analyzed and for NIR analysis each sample was analyzed in six replicates ($n=6$).

deviation was less than 5% for all UV and NIR spectral fingerprint at three different wavelengths or wavenumber. The UV and NIR spectral fingerprints Spectral fingerprinting is an un-targeted method used for classification of samples based on spectral patterns without identification or quantification of specific components. NIR spectral fingerprints are obtained on powdered solid material and UV spectral fingerprints were acquired in MeOH:H₂O (80:20, v/v) extract of fraction obtained after base hydrolysis. Pattern recognition for the NIR and UV spectral data for the classification was achieved used unsupervised PCA. The first and the second derivative plots of the spectral data showed that maximum variation in the UV spectral data was between 250 and 350 nm and 4900–5900 cm^{-1} for the near infrared spectral data. Therefore these regions were selected for classification of sample using PCA.

The PCA score plots for NIR spectral data between 4900 and 5900 cm^{-1} is shown in Fig. 2. The abbreviations used in the score plot for sample identification are described in Table 2. The score plot with limited number of samples showed distinct separation as a proof of concept between the 12 different sample sets. Over 99% of the variance was captured by PC1, PC2, and PC3. The two wheat cultivars, Louise and WB936 were well separated along component 3. All flour samples were located on the left side in the negative PC1 area, and were separated from the processed samples, batter and pancakes located on the right side in the positive region of PC1. In both wheat cultivars, refined and whole, flour, batter, and pancake samples were again grouped together. Thus simple principal component analysis of the NIR spectral data showed significant potential for analysis refined and whole wheat processed product prepared from different wheat cultivars. Similar separation of whole and refined wheat samples was recently reported by Geng et al. (2015) using fuzzy chromatography mass chromatographic using multivariate analysis tools namely, PCA and SIMCA. In addition, Fig. 2 also showed that the score plots might separate well between flour types (RF based vs. WW based).

The PCA score plots of the UV spectral data analysis showed distinct separation between all refined and whole-wheat samples (Fig. 3). All whole-wheat samples were located on the left lower side in the negative PC1 area, and all refined samples were on the opposite side (positive PC1). This can be attributed to the phenolic acid content which show strong absorption between 250 and 350 nm (Robbins, 2003). In addition, the results presented in Table 2 on phenolic acid analysis shows over 10-folds greater TPA content in WW samples as compared to RF samples (Table 1) as reported in the present and earlier reported studies (Mattila et al., 2005). Recently, Martelo-Vidal and Vazquez (2014) used similar UV-VIS-NIR spectroscopy method to analyze wine based on phenolic content. The distinction between flour, batter, and pancake within each subgroup (whole or refined) was not clear as compared to the NIR data. Thus simple UV spectral studies showed similar classification between whole and refined wheat sample as observed in recently published study (Geng et al., 2015).

3.5. Correlations between UV spectral absorbance and HPLC analysis for phenolic acids

Simple UV spectral methodology provides rapid assay of TPA. This method was used for estimation of chlorogenic acid in eggplants (Luthria, 2012). According to Robbins (2003), the optimal spectral absorbance for phenolic acids (hydroxybenzoic acid and hydroxycinnamic acid derivatives) range between 270 and 360 nm. In the present study, we compared that application of simplified spectral method with detailed LC–MS analysis for the estimation of TPA. Pearson's correlation coefficients between UV spectral absorbance (λ_{max} 280 nm and 310 nm) and detailed HPLC analysis of individual and total phenolic acids are shown in Table 3.

Fig. 2. Principal component analysis of Near Infrared spectral fingerprints of flour, batter, and pancake made from two refined and whole-wheat wheat varieties. Each sample was analyzed in six replicate ($n = 6$). Abbreviations: L: Louise wheat cultivar; W: Westbred 936 wheat cultivar; ref: refined wheat; whole: whole wheat; F: Flour; B: Batter; P: Pancake.

Table 2
Sample ID for 12 wheat samples.

Sample ID	Wheat cultivar	Wheat Fraction	Flour Type
LRef	Louise	Flour	Refined Wheat Flour
LBref	Louise	Batter	Refined Wheat Flour
LPref	Louise	Pancake	Refined Wheat Flour
LFwhole	Louise	Flour	Whole Wheat Flour
LBwhole	Louise	Batter	Whole Wheat Flour
LPwhole	Louise	Pancake	Whole Wheat Flour
WBFref	Westbred 936	Flour	Refined Wheat Flour
WBBref	Westbred 936	Batter	Refined Wheat Flour
WBPref	Westbred 936	Pancake	Refined Wheat Flour
WBFwhole	Westbred 936	Flour	Whole Wheat Flour
WBBwhole	Westbred 936	Batter	Whole Wheat Flour
WBPwhole	Westbred 936	Pancake	Whole Wheat Flour

UV spectral data showed significantly strong correlations ($r \geq 0.970$) between UV spectral data and ferulic acid (predominant phenolic acid) content as determined by LC–MS analysis in different flour, batter, and pancake samples. These results suggest that a simple UV spectral method can be used to estimate the concentration of phenolic acids in base hydrolyzed flour, batter, and pancake extracts. However, this positive correlation cannot be directly extended or used for the assay of other phenolic acids in different matrices as other phytochemicals may have absorbance coefficient in this region and may potentially interfere with direct quantification.

4. Conclusions

The results presented in the current study showed that the concentration and distribution of phenolic acids varied with type of wheat flours (whole and refined) and cultivars (Louise – soft

Fig. 3. Principal component analysis of ultraviolet spectral fingerprints of flour, batter, and pancake extracts made from two refined and whole-wheat wheat varieties. Each sample was extracted and analyzed in four replicates ($n=4$). Abbreviations: L: Louise wheat cultivar; W: Westbred 936 wheat cultivar; ref: refined wheat; whole: whole wheat; F: Flour; B: Batter; P: Pancake.

Table 3

Correlation between UV spectroscopy and HPLC analysis methods for the assay of phenolic acids.

	280 nm	310 nm	CA	p-CA	FA	SiA
310 nm	0.998**					
CA	0.952**	0.968**				
p-CA	0.965**	0.956**	0.885**			
FA	0.970**	0.980**	0.985**	0.915**		
SiA	0.871**	0.891**	0.953**	0.771**	0.928**	
TPA	0.970**	0.981**	0.987**	0.915**	1.000**	0.933**

Results without asterisks were not significant at $p < 0.05$. 280 nm, UV spectral at 280 nm; 310 nm, UV spectral at 310 nm; CA, Caffeic acid; p-CA, p-Coumaric acid; FA, Ferulic acid; SiA – Sinapic acid; TPA – Total phenolic acids; The results were expressed as Pearson correlation coefficients (r value).

* $p < 0.05$.

** $p < 0.01$.

white spring and Westbred 936 – hard red spring). Concentrations of phenolic acids were higher in all of the whole wheat (WW) samples than in the refined (RF) wheat samples. Westbred 936 had higher phenolic acid concentrations as compared to Louise cultivar. PCA of NIR and UV spectral data with limited number of samples provides the proof of concept that spectral fingerprinting techniques shows promising potential for sample classification. Multivariate analysis of the NIR spectral fingerprints

separated flour, batter, and pancake samples made from refined and whole wheat. PCA of UV spectral data classified refined and whole-wheat samples due to higher concentration of phenolic acid present in the bran part of the whole wheat grain. However, flour, batter, and pancake fractions within each subgroup (whole and refined) were not well separated. In addition, a simple UV spectral scan can differentiate whole and refined grain products based on simple absorption scan between 260 and 330 nm. Significant correlation of individual and total phenolic acids between UV spectral scan method and HPLC analysis was observed.

Acknowledgements

The authors wish to acknowledge Mrs. Samina Shami of USDA-ARS for her assistance in carrying out experimentation. This project was supported by the Agriculture and Food Research Initiative Grant no. 2009-02347 from the USDA National Institute of Food and Agriculture.

References

- American Association of Cereal Chemists International – AACCI, 1988a. Approved Methods 26–21.02. Experimental Milling—Bühler Method for Hard Wheat. AACC International, St. Paul, MN (Approved October 12).
- American Association of Cereal Chemists International – AACCI, 1988b. Approved Methods 26–31.01. Experimental Milling—Bühler Method for Soft Wheat

- Straight-Grade Flour. AACC International, St. Paul, MN Approved October 12, Available online only.
- American Association of Cereal Chemists International – AACCI, 2015. Approved Methods 10–80.01. Flour Quality in a Pancake-Making Method, 11th ed. The Association, St. Paul, MN (Approved December).
- Abdel-Aal, E.S.M., Rabalski, I., 2013. Effect of baking on free and bound phenolic acids in whole grain bakery products. *J. Cereal Sci.* 57, 312–318.
- Andreasen, M.F., Christensen, L.P., Meyer, A.S., Hansen, Å., 2000. Ferulic acid dehydrodimers in rye (*Secale cereal L.*). *J. Cereal Sci.* 31, 303–307.
- Barron, C., Surget, A., Rouau, X., 2007. Relative amounts of tissue in mature wheat (*Triticum aestivum L.*) grain and their carbohydrate and phenolic acid composition. *J. Cereal Sci.* 45, 88–96.
- Beta, T., Shin, N., Dexter, J.E., Sapirstein, H.D., 2005. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chem.* 82, 390–393.
- Geng, P., Zhang, M., Harnly, J.M., Luthria, D.L., Chen, P., 2015. Use of fuzzy chromatography mass spectrometric (FCMS) fingerprinting and chemometrics analysis for differentiation of whole-grain and refined wheat (*T. aestivum*) flour. *Anal. Bioanal. Chem.* 407, 7875–7888.
- Hansen, H.B., Andresen, M.F., Nielsen, M.M., Larsen, L.M., Knudsen, K.E.B., Meyer, A. S., Christensen, L.P., Hansen, Å., 2002. Changes in dietary fiber, phenolic acids and activity of endogenous enzymes during rye bread-making. *J. Eur. Food Res. Technol.* 214, 33–42.
- Kiszonas, A.M., Fuerst, E.P., Luthria, D., Morris, C.F., 2015. Tracking arabinoxylans through the preparation of pancakes. *Cereal Chem.* 92, 37–43.
- Lu, Y., Lv, J., Yu, L., Bayard, A., Costa, J., Yu, L., Luthria, D., 2014a. Phytochemical compositions and antiproliferative activities of bran fraction of ten Maryland-grown soft winter wheat cultivars: comparison of different radical scavenging assays. *J. Food Compos. Anal.* 36, 51–58.
- Lu, Y., Luthria, D., Fuerst, E.P., Kiszonas, A.M., Yu, L., Morris, C.F., 2014b. Effect of processing on phenolic composition of dough and bread fractions made from refined and whole wheat flour of three wheat varieties. *J. Agric. Food. Chem.* 62, 10431–10436.
- Lu, Y., Fuerst, E.P., Lv, J., Morris, C.F., Yu, L., Fletcher, A., Kiszonas, A.M., Yu, L., Luthria, D., 2015. Phytochemical profile and antiproliferative activity of dough and bread fractions made from refined and whole wheat flours. *Cereal Chem.* 92, 271–277.
- Luthria, D.L., Mukhopadhyay, S., Krizek, D.T., 2006. Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *J. Food Compos. Anal.* 19, 771–777.
- Luthria, D.L., Liu, K., Memon, A.A., 2012. Phenolic acids and antioxidant capacity of distillers dried grains with solubles (DDGS) as compared with corn. *J. Am. Oil Chem. Soc.* 89, 1297–1304.
- Luthria, D.L., 2012. A simplified 96-well method for the estimation of phenolic acids and antioxidant capacity from eggplant pulp extracts using UV spectral scan data. *J. Funct. Foods* 4, 238–242.
- Martelo-Vidal, M.J., Vazquez, M., 2014. Determination of polyphenolic compounds of red wines by UV–VIS–NIR spectroscopy and chemometrics tools. *Food Chem.* 158, 28–34.
- Mattila, P., Pihlava, J., Hellström, J., 2005. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *J. Agric. Food. Chem.* 53, 8290–8295.
- Morris, C.F., Rose, S.P., 1996. Wheat – Cereal Grain Quality. In: Henry, R.J., Kettlwell, P.S. (Eds.), Chapman and Hall, U.K, pp. 3–54.
- Robbins, R.J., 2003. Phenolic acids in foods: an overview of analytical methodology. *J. Agric. Food. Chem.* 51, 2866–2887.
- Slavin, J., Marquart, L., Jacobs, D., 2000. Consumption of whole-grain foods and decreased risk of cancer: proposed mechanisms. *Cereal Food World* 45, 54–58.
- Truswell, A.S., 2002. Cereal grains and coronary heart disease. *Eur. J. Clin. Nutr.* 56, 11–14.
- USDA, United States Department of Agriculture, 2010. Dietary Guidelines for Americans 2010. . http://www.cnpp.usda.gov/sites/default/files/dietary_guidelines_for_americans/PolicyDoc.pdf.
- Verma, B., Hucl, P., Chibbar, R.N., 2009. Phenolic acid composition and antioxidant capacity of acid and alkali hydrolyzed wheat bran fractions. *Food Chem.* 116, 947–954.
- Vitaglione, P., Mennella, I., Ferracane, R., Rivellese, A.A., Giacco, R., Ercolini, D., Gibbons, S.M., La Stora, A., Gilbert, J.A., Jonnalagadda, S., Thielecke, F., Gallo, M. A., Scalfi, L., Fogliano, V., 2015. Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. *Am. J. Clin. Nutr.* 101, 251–261.
- Whent, M., Huang, H., Xie, Z., Lutterodt, H., Yu, L., Fuerst, E., Morris, C., Yu, L., Luthria, D., 2012. Phytochemical composition, anti-inflammatory, and antiproliferative activity of whole wheat flour. *J. Agric. Food. Chem.* 60, 2129–2135.