Characterization of microstructure, physicochemical and functional properties of corn varieties using different analytical techniques

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ABSTRACT

Recently, the role of corn and its properties is a field of active research with a view to improving the quality of products being produced from corn flour. In the current study, scanning electron micrographs and textural attributes of three commercially available corn varieties (Xianyu 335, Zhengdan 958, Jade 3) were measured. The present study clearly explored a significant variation in the nutritional and chemical profile of corn varieties. The change in the chemical and molecular structure of different corn flours was recorded through scanning the flour pellet using FTIR. The results illustrated that the chemical attributes showed significant variation in corn flours, but Jade 3 ranked higher followed by Xianyu 335 and zhengdan 958, respectively. The results of SEM revealed more intense bonding and round to oval shape with soft and sharp edges results in more water absorption capacity. In the current study, variations in storage modulus and loss modulus were observed corresponding to quality and process-ability of dough. Water was determined on the basis of H and OH group in the range of 1637 cm$^{-1}$ and 3000–3700 cm$^{-1}$ while protein was measured on the basis of amides (I & II) in the range of 1550 cm$^{-1}$ and 1600 cm$^{-1}$ respectively. The study concludes that analytical techniques (FTIR, NIR) are more reliable to analyze physicochemical mapping and molecular structure.

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Introduction

Cereals are the most widely used crops and a key component of the human diet in the world. Corn is deliberated to be novel amongst other cereals due to its physicochemical and rheological properties. It is known to be the 3rd biggest grown crop of the world.$^1$ Although corn is a rich source of carbohydrates, but it also contains a valuable amount of other macronutrients like protein, fat, minerals and vitamins.$^2$ Owing to its property of being food and feed as well, corn is considered to be multi-purpose cereal crop. However, some limitations such as mucedin deficiency, low shear, and thermal resistance, thermal decomposition and higher retrogradation, which limits its functional properties in the industry and approximately 5% of total production is consumed and rest is converted to fodder.$^3$ Owing to a high nutritional value of corn, its grains and flour can be used in many industrial products as a raw material. Several modifications such as esterification, acidic hydrolysis, carbamation, etherification, polymerization, extrusion, enzymolysis, and addition of gums have been used to improve the applicability of corn flour.$^4$
Several techniques are employed to check the variation among different varieties of corn. These techniques include FTIR, NIR, Rheometer, TPA, RVA, AAS and other comparative techniques. FTIR and NIR are rapid, accurate and non-destructive techniques to measure various parameters. Physicochemical profile and molecular spectra can be well elaborated through spectroscopic methods. Corn is a rich source of vitamins (B-complex, A, C, K), beta-carotene and selenium, which plays a key role to protect the immune system and thyroid gland. It can be used as potent anti-oxidant and to pacify ulcer, cancer, anorexia, infections and sex-related hormones. It is also helpful in wound healing, swelling and liver detoxification. Corn oil is an important source of linoleic acid and helpful in regulating cholesterol, blood pressure and preventing CVDs. A small amount of corn oil in diet can fulfill the daily requirement of essential fatty acids.

Corn can be considered as a complete food having nutraceutical effect that fulfills the human demand, therefore owing to the current situation of food and increased population, the study on corn imparts a positive result. Thus, the aim of present study was to investigate and compare the whole nutritional profile like physicochemical, rheological, molecular and morphological properties of different corn varieties and to assess the advantages of advanced techniques over conventional techniques.

Recently, the role of corn and its properties is a field of active research with a view to improving the quality of products being produced from corn flour. Corn gradually deteriorated due to its mucedin deficiency and its transformation into alcohol and fodder that made corn processing very limited. Characterization of corn varieties enables us to understand the drawbacks for further processing and product development. Characterization improves the applicability of corn flour through a proper understanding of the whole profile. Application of modern techniques save time, and more accuracy can be attained. After successful comprehensive study, the results can be used at an industrial level to enhance utilization of corn for the development of different products.

Materials and methods

Collection of raw materials

Three commercial corn varieties were procured from Jilin province, China. The grains were sorted manually to remove the impurities and foreign material. Milling was done by using laboratory miller (TAISITE, FW100) and then pass through a mesh (80 number). All the chemicals and reagents were purchased from Sigma, USA.

Proximate composition

Grains were milled into flour for further analysis. Proximate profile (MC, ash, CP, fat, CF and NFE) of each corn variety was analyzed according to the method as given in AACC. Each sample was tested in triplicates for proximate analysis.

Near-infrared spectroscopy

Physicochemical profile of corn grains was determined by scanning the grains in a cup through NIR. Indicators including MC, fat, protein and starch with their dry matter (DM) content and test weight as well, were measured according to the method as described by Yang et al. Each sample was tested in triplicates.

Quantification of minerals in corn flour

The mineral contents (Ca, Mg, Fe, K, and Na) were determined by using atomic absorption spectrophotometer and flame photometer by the method presented by Ikhtiar et al. while phosphorus was measured through titrimetric method.
Pasting properties of flour

The pasting properties of corn flour were analyzed using Rapid ViscoAnalyzer (Perten) according to the method presented by Wani et al. [14] 3 g of flour was added in 25 ml of water and placed on RVA cup. The average values for peak viscosity (PV), Trough viscosity (TV), Final viscosity (FV), Breakdown, setback and pasting temperature (PT) were obtained for each sample.

Micromorphology of corn

Scanning electron micrographs (Phenom™) of each sample were obtained at magnifications of x5000 and x1000. Flour samples were fixed on the holders with double spread, and gold layer was sputtered on it and then scanned in a vacuum of the 5 kv potential difference.

Textural analysis of corn flour

Fifty grams of corn flour was kneaded with 50 ml of water in order to make a standard dough and allowed to cool for 60 min at room temperature. The textural analysis comprises hardness, adhesiveness, springiness, cohesiveness, chewiness, gumminess, and resilience were measured by a textural analyzer (TA.XT plus). Maximum force applied can be considered as hardness. The conditions were as follows: test distance 10 mm, probe P/0.5, and test velocity were 0.5 mm. Each sample was done in triplicate.

Molecular structure of corn flour

The change in the chemical and molecular structure of different corn flours was recorded by scanning the flour pellet using FTIR (Bruker, Vertex-70). One milligram of sample was mixed with 100 mg KBr and then pressed it to make tablets and spectra was recorded from the range of 400–4000 cm\(^{-1}\).

Rheological characterization of dough

Rheological properties were measured by using rheometer (Anton Paar, Modulus compact rheometer MCR-302). Frequency sweep test was performed by adopting the following parameters, parallel plates (50 mm), a gap (1 mm), temperature 25°C. Samples were placed on the plate and excessive material were wiped off through spatula. Silicone oil is added to the sample to avoid the evaporation and 10 min rest was given to equilibrate the stresses. First of all, the linear viscoelastic region was defined through a strain sweep test. Storage (G’), loss modulus (G’”) and tangent delta (tan) were determined at constant shear strain and frequency range 0.1–10 Hz.

Statistical analysis

The data obtained for each parameter was subjected to statistical analysis by using Statistical Package Origin-Pro 8.5. The experiment was performed under completely randomized design (CRD) and standard deviation and the analysis of variance was applied to determine the level of significance followed by LSD.

Results and discussion

Proximate composition

The results (Table 1) showed that moisture contents varied from 10.40% to 10.77% while the highest protein content was found in Jade 3 variety and lowest in Zhengdan 958 variety. The fat content was
founded in the range of 3.9% to 4.20%. Zhengdan 958 variety contains a significantly higher amount of fat followed by Jade 3 and Xianyu 335, respectively. Crude fiber content varied from 3.11% to 3.28%. The highest fiber content was found in Jade 3 variety. The nitrogen-free extract was observed higher in Zhengdan 958 variety and lowest in Jade 3 variety as shown in Table 1.

The proximate composition is slightly different in all the varieties. This variation may be attributed to many reasons including environmental factors, agronomic practices, genetic variation, contamination, storage conditions, and others. The literature reported by Chel Guerrero et al. showed similar results as ash 2.04%, crude fiber 2.96%, crude protein 9.8%, crude fat 2.04% while Sule Enyisi et al., reported 10% moisture and similar chemical composition of corn flour. Our findings are in agreement with the report of previous studies.\(^{15,16}\)

**Near-infrared spectroscopy**

The results regarding the chemical profile of corn through NIR are presented in Table 2. The high content of moisture, protein, and fat has been found in Jade 3 followed by Xianyu 335 and Zhengdan 958, respectively. A comparatively lower level of starch is present in Xianyu 335 while a higher level is present in Zhengdan 958. Test weight of corn grains was ranged from 775.5 to 786.8. Tan Qimei and Zhou Jie,\(^1\) used NIR to analyze corn with the same parameters and find the same results as determined in the current study. Zhu Su-wen et al.,\(^{17}\) used NIR technique to test the starch content of corn and concluded that the NIR practice has great precision with the ability to replace conventional techniques. Chemical composition is slightly different in all the varieties. This variation may be due to environmental factors, agronomic practices, genetic variation, accuracy, contamination, storage conditions, and others.

**Quantification of minerals in corn flour**

Significant variations were found in mineral contents among all the corn varieties (Table 3). Mg and P were found to be in higher amount ranged from 32.22% to 29.54% and 27.55% to 24.38%, respectively. Ca and Fe was present in small amount while potassium percentage ranged between 11.95% and 13.58%. A higher amount of mineral contents has been found in corn flour which is in agreement with the findings of Ikram et al.\(^{18}\) and Oshodi et al.\(^{19}\) They concluded that in corn flour, a large amount of phosphorus, magnesium, and potassium are present while Ca and Fe are present in low concentration. However, the observed variations in mineral contents among different varieties may be due to the change in agronomic practices, genetic variation, environmental factors or soil composition.

**Pasting properties of flour**

The RVA parameter of different corn flour is shown in Table 4. Figure 1 shows the typical RVA curves for the different corn flour varieties. A higher peak viscosity was exhibited in Jade 3 followed by Xianyu 335 and Zhengdan 958. The pasting properties of the corn flour are closely related to the starch pasting properties and peak viscosity mainly related to the swelling of starch granules. Higher peak viscosity attributed to the higher swelling of granules and also related to the higher phosphorus and lower amylose content.\(^{20,21}\) Additionally, trough, breakdown and set back viscosities increased

| Variety     | MC (%)     | Ash(%)     | Crude Fat (%) | Crude Protein (%) | Crude Fiber (%) | NFE (%)     |
|-------------|------------|------------|---------------|-------------------|-----------------|-------------|
| Xianyu 335  | 10.55 ± 0.09\(ab\) | 2.11 ± 0.01\(a\) | 3.92 ± 0.2\(b\) | 9.78\(ab\) ± 0.02 | 3.11 ± 0.01\(b\) | 70.50 ± 0.2\(ab\) |
| Zhengdan 958| 10.40 ± 0.06 \(b\) | 1.96 ± 0.02\(b\) | 4.20 ± 0.2\(a\) | 9.13\(b\) ± 0.02 | 3.17 ± 0.02\(ab\) | 71.11 ± 0.2\(a\) |
| Jade 3      | 10.77 ± 0.1\(a\)  | 2.00 ± 0.02\(ab\) | 4.00 ± 0.1\(ab\) | 9.94\(a\) ± 0.01 | 3.28 ± 0.01\(a\) | 69.92 ± 0.1\(b\) |

MC: Moisture content NFE: Nitrogen free extract
superscript alphabets was mean different letters are represent significant difference from each other
| Variety    | MC (%)   | Protein (%) | Fat (%)   | Starch (%) | Protein DM (%) | Fat DM (%)  | Starch DM (%) | Test weight |
|------------|----------|-------------|-----------|------------|----------------|-------------|---------------|-------------|
| Xianyu 335 | 12.75 ± 0.06\textsuperscript{b} | 9.41 ± 0.02\textsuperscript{b} | 2.47 ± 0.01\textsuperscript{b} | 66.36 ± 0.1\textsuperscript{b} | 9.49\textsuperscript{ab} ± 0.03 | 3.38 ± 0.06\textsuperscript{ab} | 74.86 ± 0.06\textsuperscript{b} | 779.3 ± 0.1\textsuperscript{b} |
| Zhengdán 958 | 11.59 ± 0.05\textsuperscript{c} | 8.30 ± 0.06\textsuperscript{c} | 3.22 ± 0.02\textsuperscript{ab} | 68.35 ± 0.09\textsuperscript{a} | 9.21\textsuperscript{b} ± 0.01 | 3.66 ± 0.02\textsuperscript{b} | 75.69 ± 0.04\textsuperscript{a} | 775.5 ± 0.1\textsuperscript{c} |
| Jade 3     | 13.79 ± 0.07\textsuperscript{a} | 10.45 ± 0.02\textsuperscript{a} | 3.80 ± 0.02\textsuperscript{a} | 66.35 ± 0.06\textsuperscript{b} | 10.52\textsuperscript{a} ± 0.03 | 4.40 ± 0.04\textsuperscript{a} | 74.82 ± 0.01\textsuperscript{b} | 786.8 ± 0.1\textsuperscript{a} |

MC: Moisture content, DM: Dry matter
which may reflect the retrogradation tendency that inhibited the increase in viscosity upon cooling.\textsuperscript{[22]} Final viscosity of corn flour (Jade 3, Xianyu 335 and zhengdan 958) showed the value of 2797, 3071, and 2485, respectively. It was reported that an increase in final viscosity might be due to the aggregation of amylose molecules.\textsuperscript{[23]}

Table 3. Mineral contents in corn flour.

| Treatment  | Ca (%)       | Mg (%)       | Fe (%)       | K (%)       | Na (%)       | P (%)       |
|------------|--------------|--------------|--------------|-------------|--------------|-------------|
| Xianyu 335 | 0.24 ± 0.01<sup>a</sup> | 30.29 ± 0.85<sup>ab</sup> | 0.28 ± 0.02<sup>b</sup> | 12.60 ± 0.73<sup>ab</sup> | 2.30 ± 0.28<sup>b</sup> | 25.98 ± 0.81<sup>ab</sup> |
| Zhengdan958| 0.18 ± 0.02<sup>a</sup>  | 29.54 ± 1.36<sup>b</sup>  | 0.22 ± 0.01<sup>b</sup>  | 11.95 ± 0.60<sup>b</sup>  | 1.89 ± 0.18<sup>b</sup>  | 24.38 ± 0.73<sup>b</sup>  |
| Jade 3     | 0.2 ± 0.01<sup>a</sup>   | 32.22 ± 1.49<sup>b</sup>  | 0.32 ± 0.01<sup>c</sup>  | 13.58 ± 0.89<sup>b</sup>  | 3.70 ± 0.66<sup>a</sup>  | 27.55 ± 0.60<sup>a</sup>  |

CA: Calcium Mg: Magnesium Fe: Iron K: Potassium Na: Sodium P: Phosphorus

Table 4. RVA parameters are the mean of three values.

| Treatment    | Peak Viscosity (RVU) | Trough Viscosity (RVU) | Breakdown Viscosity (RVU) | Final Viscosity (RVU) | Setback Viscosity (RVU) | Peak temperature (°C) | Pasting Temperature (°C) |
|--------------|----------------------|------------------------|---------------------------|-----------------------|------------------------|------------------------|--------------------------|
| Xianyu 335   | 1119 ± 25<sup>b</sup> | 1066 ± 14<sup>a</sup> | 53 ± 4<sup>c</sup> | 3071 ± 29<sup>a</sup> | 2005 ± 19<sup>a</sup> | 5.06 ± 0.3<sup>b</sup> | 84.85 ± 2.65<sup>a</sup> |
| Zhengdan 958 | 948 ± 18<sup>c</sup> | 796 ± 21<sup>c</sup> | 152 ± 3<sup>b</sup> | 2485 ± 21<sup>c</sup> | 1689 ± 13<sup>c</sup> | 5.6 ± 0.2<sup>a</sup> | 78.2 ± 2.20<sup>a</sup>  |
| Jade 3       | 1168 ± 28<sup>a</sup> | 940 ± 15<sup>b</sup> | 228 ± 8<sup>a</sup> | 2797 ± 17<sup>b</sup> | 1857 ± 18<sup>b</sup> | 5.06 ± 0.2<sup>b</sup> | 82.4 ± 2.99<sup>a</sup>  |

Figure 1. RVA curves of maize varieties.

significantly in different corn varieties. Which may reflect the retrogradation tendency that inhibited the increase in viscosity upon cooling.\textsuperscript{[22]} Final viscosity of corn flour (Jade 3, Xianyu 335 and zhengdan 958) showed the value of 2797, 3071, and 2485, respectively. It was reported that an increase in final viscosity might be due to the aggregation of amylose molecules.\textsuperscript{[23]}

**Micromorphology of corn**

Pictorial micrographs of different corn varieties were taken by SEM at certain magnifications as shown in Figure 2. Micromorphology of corn flour can be well examined by SEM as it provides a direct observation to explain the potential properties at a microscopic level. Corn flour granules were oval or round with smooth edges with a slight difference in size and shape of the granules among different varieties. Zhengdan 958 and xianyu 335 showed round shape with soft edges while jade 3 had an oval or polygonal shape with more intense bonding. Smaller particles have a better water holding capacity and rheological properties. In previous studies, similar results were observed.\textsuperscript{[24]} Abida et al.\textsuperscript{[25]} also reported oval to polyhedral granules and found similar results as obtained in the current study. The morphological variations of corn flour may be due to the
differences in composition, structure, physiology of the plant, biological origin and its biochemistry which are crucial components to regulate the morphology, granule size, length, and shape.\textsuperscript{[26]}

**Textural analysis of corn flour**

The textural properties of corn flour dough were determined by the textural analyzer and shown in Table 5. There was a remarkable difference in all the doughs in terms of their textural properties. Jade 3 presented higher hardness (97.09 g) followed by Zhengdan 958 (72.25 g) and xianyu 335 (60.04 g) respectively. Hardness and firmness mainly caused by retrogradation, syneresis and amylopectin loss. Moreover, hardness tends to have long amylopectin chains and high amylose content. Adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience showed the same pattern among all the varieties. Qin Yang et al.\textsuperscript{[27]} studied the textural properties of corn flour and observed that corn flour possesses more hardness (75\%) than fermented flour. Lower hardness results in much better rheology and proper dough structure.

**Molecular structure of corn flour**

The FTIR spectra showed similar profiles of different corn flours as presented in Figure 3 A and 3B. There were multiple discernible absorbencies in the fingerprint region at 930, 1021, 1085 and

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**Table 5. Textural properties of different varieties of corn flour.**

| Treatment     | Hardness (g) ± | Adhesiveness (g.sec) ± | Springiness (mm) ± | Cohesiveness ± | Gumminess (g) ± | Chewiness ± | Resilience ± |
|---------------|----------------|------------------------|--------------------|----------------|----------------|------------|-------------|
| Xianyu 335    | 60.04 ± 4\textsuperscript{a} | −15.11 ± 0.2\textsuperscript{c} | 0.92 ± 0.01\textsuperscript{b} | 0.77 ± 0.02\textsuperscript{a} | 27.03 ± 1.31\textsuperscript{c} | 26.70 ± 0.80\textsuperscript{c} | 0.09 ± 0.02\textsuperscript{a} |
| zhengdan 958  | 72.25 ± 4\textsuperscript{b} | −24.71 ± 0.5\textsuperscript{a} | 0.98 ± 0.02\textsuperscript{a} | 0.68 ± 0.01\textsuperscript{ab} | 31.65 ± 2.39\textsuperscript{b} | 30.69 ± 2.10\textsuperscript{b} | 0.17 ± 0.02\textsuperscript{a} |
| Jade 3        | 97.09 ± 6\textsuperscript{a} | −18.51 ± 0.2\textsuperscript{b} | 0.94 ± 0.02\textsuperscript{ab} | 0.49 ± 0.03\textsuperscript{b} | 36.90 ± 1.34\textsuperscript{a} | 45.03 ± 1.70\textsuperscript{a} | 0.13 ± 0.01\textsuperscript{a} |

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\textsuperscript{a,b,c} Different superscripts indicate significant difference (P < 0.05) among the varieties.
1168 cm$^{-1}$, which represented the C-O bond stretching.\cite{28} The mid-infrared range represents the water content in the product, the water peaks were observed at 1637 cm$^{-1}$ and a broad spectrum ranged from 3000 to 3700 also attributed to moisture content. The absorption spectra accounts on the base of H and OH functional group, and the broad spectrum can also be attributed to the vibration of O-H groups. The band at 1548 and 1660 reflects the presence of protein in the sample which can be quantified through the amide I and amide II group. Amide I represent the C = O of the peptide group while amide II represents primarily NH bending and secondly the CN stretch. The strong absorbance at 1600 attributed to amide II and NH bending while 1550 represents amide I showed that flour is rich in protein.\cite{29} The absorption spectra ranged from 1600 to 1700 and 1550 to 1570 represents CH bond and credited to the amount of fat in the sample. Strong peaks have been found in the same region resulted due to the fat present in the flour sample. Starch is the major constituent of corn flour while the major starch components are amylose and amylopectin. The peaks below 800, fingerprint region and the broad spectrum from 3000 to 3700 (OH) represent starch. The current study is well sustained by the earlier studies of Yan Gao et al.\cite{4,5,30}

**Rheological characterization of dough**

Rheological behavior of the flour was the key and vital factor to develop a better and high-quality product.\cite{31} Quality and process-ability of dough ingredients have been well studied through frequency sweep tests. The variations of loss, storage modulus, and tan delta are shown in Figure 4. Storage modulus $G'$ varied between the range of 2000–40000, which was a typical range of cereals flour rheology.\cite{7} The storage modulus and loss modulus increased with the increase in frequency while storage modulus is always higher than loss modulus, concluded that dough had more elastic behavior and the less time to relax at a higher frequency. Xianyu 335 dough exhibited higher storage and loss modulus followed by Jade 3 and Zhengdan 958 whereas tan delta of Zhengdan 958 was slightly lower but Xianyu 335 and Jade 3 have shown the same results. The protein and lipids mostly starch-bound act as surfactants involved in mixing of the dough. The available water content in flour was not enough for the formation of dough, thereby lack of lubricating agent for interaction resulted in higher storage and loss modulus while lowering the tan delta.\cite{32}

Conventional methods (proximate analysis) are expensive, time-consuming and cause sample destruction. On the other hand, spectroscopy is a fast, reliable, cheap, and accurate tool to visualize chemical composition. FTIR is one of the most important and precise tool which demands no external calibration, less sample preparation, more speed for analyzing different tests especially
Rheological measurement through farinograph, mixograph, and amylograph took more time, labor, and sample preparation while rheometer is easy, quick, and authentic for the estimation of viscoelastic characteristics of different samples. The RVA has been widely used and is well known for assessing the pasting properties of flour or starch in heating cooling cycles and provide many useful indicators for the quality assessment of final products and raw materials.[33]

**Conclusion**

The present study clearly explored a significant variation in the nutritional profile of different corn varieties. On the basis of results, it is concluded that analytical techniques (FTIR, NIR) are more reliable than standard methods (proximate analysis) to analyze physicochemical mapping and molecular structure. Jade 3 is considered to have a high amount of phosphorus, more water absorbing capacity, higher peak viscosity, hardness, higher protein, fat and intense molecular structure. Further studies would be needed to assess the modification of corn flour in other food systems, but so far, sufficient information was extracted from this work.

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