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Variability of kernel morphological and flour physicochemical properties in tropical dent corn hybrids

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Abstract. *Humam BK, Suwarno WB, Azrai M, Makkulawu AT, Efendi R. 2023. Variability of kernel morphological and flour physicochemical properties in tropical dent corn hybrids. Biodiversitas 24: 1600-1609.* Corn is an important crop worldwide for food, feed, and industry. The aims of this study were to study kernel morphological and corn flour physicochemical properties and hybrids selection according to nutritional values for food industries. Plant materials were 15 test hybrids and four check varieties, namely RK457, RK57, BISI18, and NASA29. Kernel morphological and corn flour physicochemical variations are influenced by kernel type. All dent hybrids had 40/0 mesh corn flour yielded significantly higher compared to NASA29. °Dent scores were negatively correlated to 40/0 mesh corn flour yields. The total and soluble carbohydrates of dent corn flour hybrids are significantly higher compared to semi-dent and semi-flint corn flour hybrids. According to corn flour nutritional values in polygon GT biplot analysis, dent hybrids had relatively high starch, total carbohydrate, and soluble carbohydrate contents. Semi-dent hybrids had relatively high moisture, crude protein, and total ash contents. Semi-flint hybrid had relatively high crude fat and crude fiber contents. Cluster-2 was dominated by dent hybrids according to the fuzzy clustering method and selected as potential hybrids. MSM53 was the female parent of dent hybrids in cluster-2. Potential hybrids could be evaluated further by corn flour functional properties analysis.

Keywords: Dent corn flour hybrids, fuzzy clustering, GT biplot analysis, potential hybrids, starch content

INTRODUCTION

Corn is an important crop as a carbohydrate source which is useful for food, feed, and industries. There are seven kernel types according to variation of their endosperm characteristics; dent, flint, flour, sweet, pop, pod, and waxy types. The dent corn has flinty sides and a soft core and contains a high amount of soft starch (Awata et al. 2019). Yellow dent corns are primarily used as feed whereas white dent corns are preferred for food (Milind and Isha 2013). The demand for corn hybrid varieties increases every year. Unfortunately, dent hybrid varieties are limited in the Indonesian market due to the domination of semi-flint and semi-dent hybrids. The endosperm is the largest part about 80-85% of the kernel and varies among varieties depending on the amount of photosynthate taken by endosperm during development stages (Ognakossan et al. 2018; Awata et al. 2019). Xu et al. (2019) clearly explain the effect of different endosperm ratios on kernel morphological characteristics according to kernel types. The variations of kernel dimensions and physical properties based on floating and bio-speckle tests are explained briefly by Weber et al. (2014). The combination of the milling evaluation factor and kernel density resulted in the best predictive ability for dry-milled product yields (Blandino et al. 2012). The variations in corn flour nutritional values are caused by different endosperm types (Alvarez-Iglesias et al. 2021). The tropical dent corn hybrids probably will be more useful for food industries.

Generally, 100 g kernel consisted of 364 kcal, 80.8 g carbohydrate, 6.2 g protein, 1.74 g crude fat, 271 mg total mineral, and 7.05 mg total vitamin, etc. (USDA 2020). Corn flours are made from dry milling for separating pericarp, endosperm, germ and are continued by reducing the size (Duensing et al. 2003). Degermination can reduce the fat content of corn grits and kernel endosperms, aimed at repairing corn flour's functional properties (Yulianto et al. 2013). Starch is the most abundant reserve carbohydrate consisting of amylose and amylopectin are two macromolecule components and had different starch granules characteristics between corn types (Sandhu et al. 2003). Starch metabolism and starch granule size, number, and morphology are key factors that influence the starch content in the kernel (Wang et al. 2015). Previous studies commonly point out the effect of different kernel types on corn flour nutritional values, but still less information on how to select according to their superiority (Singh et al. 2008; Oladapo et al. 2017; Alvarez-Iglesias et al. 2021). Germinated corn flour from local varieties is suitable for food development in food industries (Bello and Udo 2018).

'Binthe Kiki' is the local corn variety from Gorontalo which is resistant to plant hopper, rich in carbohydrate and protein contents, and low in fat content (Ahmad et al. 2021).

GT biplot can be used for visualization of the association between traits and test genotypes (Yan 2014). A recent approach for multitrait genotype selection combines GT biplot and yield to become a genotype by yield*trait biplot, and this method is useful to obtain information about yield relative to other breeding targets (Yan and Fregeau-Reid 2018). The GT biplot analysis has been applied to other crops such as rice (Sharifi and Ebadi 2016), sorghum (Mukondwa et al. 2020), and corn (Orhun 2020; Shojaei et al. 2020). Unfortunately, there has been limited study into how GT biplot analysis is used to identify potential hybrids based on corn flour nutritional values by plant breeding approaches. The application of fuzzy clustering and unmanned aerial vehicles' remote sensing images allows better identification of genetic differences at different stages of crop development and is useful in maize breeding (Han et al. 2019). This study aimed to obtain information on kernel morphological and flour physico-chemical characters, identify superior hybrids based on corn flour nutritional values by GT biplot analysis, and select potential hybrids for food industries by the fuzzy clustering method.

MATERIALS AND METHODS

Plant materials and study location

This study used 15 test hybrids derived from single crossing six inbred lines (BCY, ERC24, G102612D, GLT226, MSM53, P2) in a half diallel mating design and four check varieties (RK457, RK57, BISI18, NASA29) that had a variation of kernel types. The trial was conducted in Ponorogo from August until December 2021, and the kernel samples were taken. Corn milling was conducted at Pilot Plant SEAFAST of IPB University in February 2022. Proximate analysis of corn flour was conducted at the Biotechnology Center Laboratory of IPB University and starch content analysis was conducted at the Animal Sciences Biotechnology Laboratory of Hasanuddin University in March 2022.

Morphological characteristics of the corn kernel

Dent scores (°Dent) were observed on 10 ears per experimental design according to their frequency, with criteria as follows: (1 and 2) dent; (3) semi-dent; (4) semi-flint; (5) flint. Kernel thickness was measured on the thickest part of the kernel by a digital micrometer screw. The weight of 1000-kernel (g) was measured after drying by converting to the moisture content at 15%. Kernel density (g mL^{-1}) was measured based on kernel weight at 15% moisture content divided by the volume of 100 mL measuring cup. The weight of 100 mL measuring cup has been measured previously.

Fraction of corn flour

Cleaned kernels of 500 g per experimental unit were milled by full-fat dry milling method using two reduction stages. The material recovered was passed through a 20 mesh sieve in a hammer mill. The fraction that passed through a 20 mesh sieve was further sieved through a 40 mesh sieve. The fraction retained on a 20 mesh sieve was designated as +20 mesh and that passed through on 20 mesh was designated as 20/0 mesh. The fraction that passed through 20 mesh and retained on 40 mesh was designated as 20/40 mesh, while the fraction that passed through 40 mesh was designated as 40/0 mesh. The percentage of each fraction was calculated by dividing the weight of fractions by 500 g kernel weight and was multiplied by 100%.

Nutritional values of corn flour

A sample of 50 g of corn flour was tested for proximate content using Indonesian National Standard SNI-01-2891-1992 (BSN 1992) and AOAC analysis methods (AOAC 1995), with two observations per experimental unit. Moisture and total ash contents were analyzed by the gravimetry method (BSN 1992). Crude protein content was analyzed by the semi-micro Kjeldahl method (%N x 6.25) (AOAC 1995). Crude fat content was analyzed by the Soxhlet method (BSN 1992). Crude fiber content was analyzed followed by ashing due to corn generally having crude fiber content of more than 1% (AOAC 1995). Total carbohydrate content was calculated by difference method with the formula: 100% - (moisture content + total ash content + crude protein content + crude fat content). Soluble carbohydrate content represented digestible carbohydrates and was calculated by the difference method with the formula: Total carbohydrate content – crude fiber content. Starch content was analyzed by the Luff Schoorl method to obtain glucose content and then was multiplied by 0.9 as a correction factor for calculated starch content (Asquieri et al. 2019).

Data analysis

The data reported are the average of three replications for kernels morphological and corn flour fraction components properties whereas for corn flour nutrition values reported are the average of two replications, and for starch content only used one replication. This experiment used a randomized complete block design (RCBD). Analysis of variance followed by the least significant difference test at 0.05 level was performed using the STAR software (bbi.irri.org).

Genotype by trait for corn flour nutritional values was visualized using a "which-won-what" biplot using the GEA-R software (CIMMYT). Cluster analysis used Euclidean distance, and the optimum number of clusters was determined by the fuzzy clustering method using the PBSTAT-CL 2.1.1 software (www.pbstat.com). The difference in nutritional content among clusters was analyzed using a t-test for two independent samples. The t-test and Pearson correlation were performed using Minitab software (State Colege, PA).

RESULTS AND DISCUSSION

Morphological characteristics of the corn kernel

The °dent scores varied among hybrids from 2.2 to 4.1 with NASA29 being the highest with semi-flint type. G01, G04, G05, G06, G07, G08, G10, G11, and G13 hybrids had dent type. The dent on the top side of the kernel was formed about 90-95 DAP (days after planting) depending on hybrids. The region of peripheral endosperm and lateral middle endosperm in dent and flint kernel were vitreous endosperms whereas the region of central endosperm and dorsal middle endosperm were floury endosperms (Xu et al. 2019). G12 and G14 had W100Kn significantly higher compared to RK457, RK57, and BISI18. NASA29 had the heaviest W1000Kn, significantly higher than the grand mean. Dent-type corn has a vitreous per floury (V/F) endosperms ratio lower compared to semi-dent and flint which influences the hardness and quality of the kernel (Blandino et al. 2012; Weber et al. 2014). Corn variations according to kernel types are caused by the pattern of endosperm proportion (Gwirtz and Garcia-Casal 2013).

The DKn of G03, G09, G12, and G15 was significantly higher compared to BISI18. RK457 and NASA29 varieties were the semi-flint type with DKn significantly higher compared to the grand mean. Commercial dent corn has a 56% flotation index, indicating the lower proportion of V/F endosperms will reduce density (Weber et al. 2014). G03 and G14 had a TKn significantly higher compared to RK457 and NASA29. The grand means of TKn for dent, semi-dent, and semi-flint hybrids were 4.04 mm, 4.49 mm, and 4,22 mm, respectively. The dark orange color is caused by a higher amount of total carotenoid content in the kernel (Chandler et al. 2013). Color is a vital preference trait and must be attractive to food consumers (Abriana et al. 2020).

Fractions of corn flour

The obtained data showed that the +20 mesh fraction of G1, G4, G5, G7, and G10 were significantly lower compared to RK457, RK57, and NASA29. The dent hybrids had ranged data of +20 mesh fractions from 37.33% to 47.47% and lower than semi-flint type. NASA29 had a +20 mesh fraction significantly higher compared to all dent hybrids. The characteristic of the V/F endosperms ratio influences total grits yields and is useful as one of the milling evaluation factors (Blandino et al. 2012). G1, G4, G5, G7, and G10 hybrids had 20/0 mesh fraction was significantly higher compared to RK457, RK57, and NASA29. The kernel with lower TKn was mainly possessed by dent hybrids and tended to result in a higher 20/0 mesh fraction yield. This result indicated that kernel types affected kernel milling ability and the dent hybrids were more efficient for dry milling, probably due to the lower V/F endosperms ratio. Crude corn flour is influenced by various physical and chemical characteristics of the kernel, the main factor is kernel hardness (Blandino et al. 2010). The kernel hardness depends on moisture content and is negatively correlated to milling time (Klajk et al. 2020).

Table 1. Variations among corn hybrids on kernel morphological and corn flour fraction characteristics

Hybrids		°Dent scores		W1000Kn (g)	DKn (g ml^{-1})	TKn (mm)	Fraction components (%)			
							+20 mesh	20/0 mesh	20/40 mesh	40/0 mesh
G01	BCY x G102612D	2.2	D	277.97c	1.273	4.10	40.36pqs	59.64abd	33.03	26.61abd
G02	GLT226 x G102612D	2.9	SD	279.19c	1.271	4.43	46.01s	53.99d	28.93r	25.06ad
G03	GLT226 x ERC24	3.0	SD	288.17c	1.292c	4.82ad	44.25ps	55.75ad	30.13r	25.62ad
G04	P2 x G102612D	2.4	D	255.43	1.268	4.05	37.65pqs	62.35abd	33.17	29.18abcd
G05	MSM53 x BCY	2.4	D	291.28c	1.263	4.08	40.61pqs	59.39abd	31.62r	27.77abcd
G06	MSM53 x P2	2.5	D	267.18	1.273	3.85	47.47s	52.53d	29.65r	22.89d
G07	MSM53 x ERC24	2.3	D	271.24	1.271	3.93	42.40pqs	57.60ab	31.84	25.76abd
G08	P2 x BCY	2.4	D	276.16c	1.272	3.96	45.33s	54.67d	29.68r	24.99ad
G09	P2 x ERC24	3.0	SD	258.82	1.290c	4.22	48.80s	51.20d	27.54r	23.66d
G10	ERC24 x G102612D	2.2	D	245.77	1.257	4.01	37.33pqs	62.67abd	34.22	28.45abcd
G11	MSM53 x G102612D	2.4	D	286.34c	1.269	4.39	46.81s	53.19d	30.00r	23.18d
G12	GLT226 x BCY	2.9	SD	308.30abc	1.295c	4.17	48.67s	51.33d	28.52r	22.76d
G13	ERC24 x BCY	2.5	D	251.50	1.277	4.03	45.54s	54.46d	30.46r	24.00d
G14	GLT226 x MSM53	2.9	SD	312.77abc	1.279	4.86ad	51.96s	48.04d	28.31r	19.73
G15	GLT226 x P2	2.7	SD	292.91c	1.299c	4.69a	53.58s	46.42d	25.33pqr	21.09
G16	RK457	3.7	SF	285.46	1.308	4.15	48.46	51.54	28.96	22.58
G17	RK57	3.1	SD	278.27	1.282	4.37	46.66	53.34	28.95	24.39
G18	BISI18	2.8	SD	250.95	1.270	4.36	40.65	59.35	34.48	24.87
G19	NASA29	4.1	SF	303.98	1.323	4.29	60.51	39.49	20.54	18.96
Grand mean		-		277.98	1.281	4.25	45.95	54.03	29.76	24.29
LSD 0.05		-		21.04	0.019	0.51	4.06	4.06	2.75	2.16
Hybrid F-value		-		7.17**	6.220**	2.68**	16.05**	16.05**	11.34**	12.92**
Coefficient of variation (%)		-		4.57	0.900	7.27	5.34	4.53	5.58	5.38

Note: **: Significant at p<0.01; a,b,c,d). Significantly higher compared to RK457, RK57, BISI18, NASA29 check varieties, respectively based on LSD 0.05; p,q,r,s). Significantly lower compared to RK457, RK57, BISI18, NASA29 check varieties, respectively based on LSD 0.05; D,SD,SF) Dent, semi-dent, and semi-flint types respectively; W1000Kn: 1000-kernels weight on 15% moisture content; DKn: Kernel density; TKn: Kernel thickness

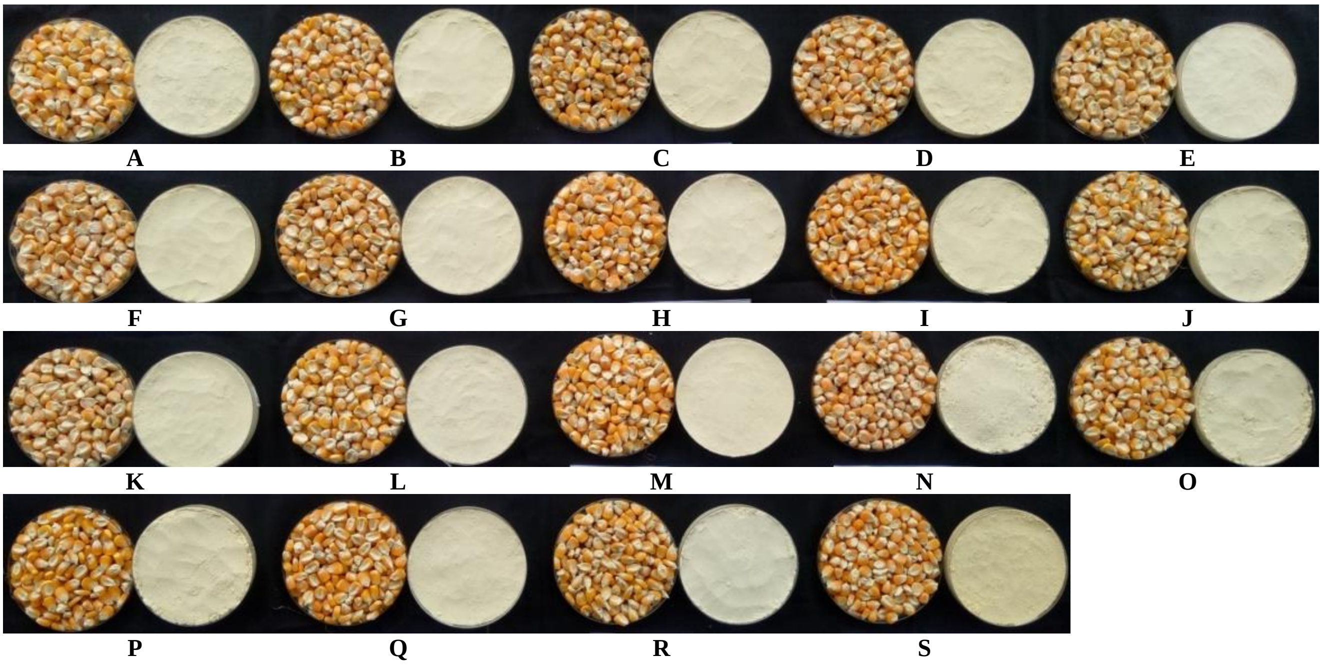

Figure 1. Kernels and 40/0 mesh flour performance of 19 hybrids. A. G01, B. G02, C. G03, D. G04, E. G05, F. G06, G. G07, H. G08, I. G09, J. G10, K. G11, L. G12, M. G13, N. G14, O. G15, P. G16, Q. G17, R. G18, S. G19

The 20/40 mesh fraction was defined as pericarp yields and G15 was significantly lower compared to RK457, RK57, and BISI18. BISI18 had the highest pericarp yields and was significantly higher compared to the grand mean. All hybrids had a pericarp yields percentage of more than 50% of 20/0 mesh fraction and became evident that 20/0 mesh fractions had coarse texture. Pericarp yields indicated the cleanliness level of separated pericarps from endosperms (Singh et al. 2008). G04, G05, and G10 had a 40/0 mesh fraction significantly higher compared to all check varieties. Generally, 40/0 mesh corn flour yields of dent hybrids were significantly higher compared to NASA29. Dent type has the highest 40 mesh corn flour yields (Singh et al. 2008). Full-fat dry milling is a non-degerminating process that yields corn flour with a soft texture, strong flavor, and nutrition rich (Duensing et al. 2003). NASA29 had a reddish-yellow of 40/0 mesh corn flour color and was darker compared to other test hybrids. The color of the kernel and corn flour hybrids was dominated by yellow-orange and light golden-yellow colors, respectively (Figure 1).

Correlation between kernel morphology and corn flour fractions

The correlation matrix showed that W1000Kn, DKn, TKn, and °dent scores variables were positively correlated to +20 mesh fractions and negatively correlated to +20 mesh, 20/0 mesh, dan 40/0 mesh fraction components. The obtained data suggested that hybrids with higher values of dent scores tended to have higher total grits yields and lower percentages of corn flour yields. This result probably was affected by the proportion of V/F endosperms. The average of W1000Kn and DKn semi-flint hybrids was higher than dent hybrids and W1000Kn was positively correlated to DKn (r=0.41**). The dent kernel has a central core of floury endosperm extending to the top whereas the flint kernel has a large proportion of vitreous endosperm surrounding the central core (Xu et al. 2019). The softer kernels are more digestible than harder kernels as dry-rolled corn feed (Harrelson et al. 2019).

This experiment suggested the dent hybrids tended to have relatively high percentages of 40/0 mesh corn flour yields. The W1000Kn and DKn were positively correlated with dent scores, indicating more flint-type kernels tended to be heavier. The TKn was positively correlated to W1000Kn (r=0.42**). Proportion V/F endosperms can be used as an indicator of corn milling ability (Blandino et al. 2010). The lighter kernels with smaller volume and size will be easier to break (Klajk et al. 2020). Semi-dent and semi-flint hybrids had superiority in W1000Kn whereas dent hybrids had corn flour yields higher. These results are interesting due to growers in developing countries put focus on kernel quantity yields while milling industries are more interested in kernel quality. It becomes a challenge for corn breeders to match both of them. A significant hybrid x year interaction is found for all kernel characteristics (Harrelson et al. 2019). Correlation between kernel properties depends on genotype, environment, and analysis technique in a laboratory (Nikolic et al. 2020).

Table 2. Pearson's correlation coefficients between kernel morphological variables with corn flour fraction components

Kernels morphological variables	Corn flour fraction components (%)				°Dent scores
	+20 mesh	20/0 mesh	20/40 mesh	40/0 mesh	
W1000Kn	0.60**	-0.60**	-0.55**	-0.58**	0.31*
DKn	0.65*	-0.65*	-0.63*	-0.59*	0.69**
TKn	0.41**	-0.41**	-0.33*	-0.44**	0.15ns
°Dent scores	0.57**	-0.57**	-0.56**	-0.50**	1.00

Note: ns: Not significant; * and **: Significant at p<0.05 and p<0.01, respectively; W1000Kn) 1000-kernels weight on 15% moisture content; DKn: Kernel density; TKn:Kernel thickness

Nutritional values of corn hybrids

The characteristics of the dent, semi-dent, and semi-flint hybrids are provided in Table 1. We used the 40/0 mesh corn flour for nutritional content analysis. From Table 3, there was a variation in nutritional values between kernel types based on their total averages. Moisture, total ash, crude protein, and starch contents were not significantly different between dent corn flour hybrids (D-CF), semi-dent corn flour hybrids (SD-CF), and semi-flint corn flour hybrids (SF-CF). The starch contents of D-CF, SF-CF, and SF-CF were not significantly different (p=0.11). The number of hybrids in each corn type was not the same and caused SF-CF to have a standard error of mean higher in all observed nutritional values. The genetic component of starch content is affected by additive gene action in recombinant inbred line populations and affected by the environment (Wang et al. 2015). Genetic variance contributes more than 70% of the starch content variation (Mansilla et al. 2021).

The crude fat content of D-CF was significantly lower compared to SD-CF and SF-CF. These results indicated the kernel type difference influenced crude fat and this was consistent with Singh et al. (2008) and Alvarez-Iglesias et al. (2021) that corn flour of dent hybrids have lower fat content compared to those with other kernel types. The crude fiber content of D-CF showed 22% significantly lower compared to SF-CF. These results indicated that SF-CF had a higher amount of nutritional content which is not easily digestible. Hemicellulose and neutral detergent fiber of dent corn are significantly lower than semi-flint (Zilic et al. 2011). Total and soluble carbohydrate contents of D-CF were significantly higher compared to SD-CF and SF-CF. Carbohydrates became a major component of corn flour's nutritional value. The D-CF had higher digestible carbohydrates and possibly resulted in more energy. These results could be early consideration for developing food products with corn flour as raw material.

The moisture contents of G03, G06, and G10 were significantly lower compared to RK57. G04 was significantly lower compared to RK57, BISI18, NASA29, and the grand mean (Table 3). Corn flour of all hybrids had low moisture content and could be stored for several months in a suitable storage condition. Moisture content variation can be influenced by the morphology and physical state of the kernel, pericarp thickness, and protein content (Vega-Rojas et al. 2016). The total ash content of G10 was significantly lower compared to RK457, BISI18, and NASA29. G04 and G07 were significantly lower compared to RK457. The total ash contents of yellow and white corn flours are significantly higher compared to wheat flour (Nikolic et al. 2019). Total ash represents mineral content and kernel milling processes can reduce Fe, Mg, Na, and Cu contents (Gwirtz and Garcia-Casal 2013). The obtained data showed crude protein content ranged from 7.48% DW to 11.31% DW with G09 and G15 significantly higher compared to RK457, RK57, and BISI18. The protein matrix is more abundant in vitreous endosperm so that starch granules can be more tightly packed (Xu et al. 2019). Flint corn has a high protein content and is significantly different compared to dent corn (Alvarez-Iglesias et al. 2021).

The crude fat content ranged from 3.97% DW to 9,48% DW with G07 significantly lower compared to all check varieties and the grand mean. NASA29 was the highest and significantly higher compared to the grand mean and all dent hybrids. Low crude fat content became corn flour's requirement besides moisture content for a long time stored for preventing rancid odor and decreasing quality. The variation in fat content was affected by environmental differences (Giupponi et al. 2021). Among dent hybrids, only G13 had similar crude fiber content with NASA 29. Low crude fiber content is useful as one of the food product quality indicators for consumers. Soluble and insoluble dietary fiber can be used for food diet parameters (Prasadi and Joye 2020). Pop-corn flour has the highest crude fiber and is significantly higher compared to yellow and white corn flours (Oladapo et al. 2017).

The total carbohydrate content of all dent corn hybrids was significantly higher compared to NASA29 with G10 significantly higher compared to all check varieties and the grand mean. Raw processing corn flour results in 368 kcal of energy (Prasanthi et al. 2017). The soluble carbohydrate content of G10 was significantly higher compared to all check varieties. The digestibility of dent corn is higher than semi-flint, but lower compared to sweet corn (Zilic et al. 2011). Information on proximate content variation is useful for selecting potential genotypes besides agronomic characteristics. The starch content ranged from 51.28% DW to 71.70% DW and all check varieties were below the grand mean. Only G8 from dent hybrids had starch content lower than RK457 and RK57. The average starch content per total carbohydrate content was 95.45%, indicating starch became a major component of carbohydrates. On the other hand, crude fat content was negatively correlated to starch content (r= -0.48*) but was not correlated to crude protein content. These results indicated crude fat associated with starch, and hybrid with lower crude fat tended to have higher starch content. Low crude fat and high starch contents were dominated by the dent hybrids. Wang et al. (2015) report that a 48.6% variation in starch content can be explained by six quantitative trait loci (QTL). Endosperm-specific transcription factors (*ZmNAC128* and *ZmNAC130*) can reduce starch and protein accumulation in transgenic corn seeds (Zhang et al. 2019).

Table 3. Variations among corn hybrids on proximate and starch contents characteristics of 40/0 mesh corn flour

Hybrids		Proximate and starch contents per 100 g of sample's dry weight basis (% DW)							
		Mst	**TAsh**	**CPrt**	**CFat**	**CFbr**	**TCbh**	**SCbh**	**Sch**
G01	BCY x G102612D	9.13	2.62	9.06	6.58s	2.14s	72.62d	70.48d	68.87
G02	GLT226 x G102612D	9.12	2.64	8.62	5.85ps	2.04s	73.78d	71.74d	69.96
G03	GLT226 x ERC24	8.95q	2.81	9.54	6.91s	2.02s	71.79d	69.78d	69.56
G04	P2 x G102612D	8.29qrs	2.34p	9.15	5.28ps	1.85s	74.94acd	73.09ad	67.40
G05	MSM53 x BCY	9.80	2.67	8.35	5.09ps	1.73s	74.08d	72.36d	71.61
G06	MSM53 x P2	8.99q	2.84	9.41	5.64ps	1.68s	73.13d	71.45d	67.01
G07	MSM53 x ERC24	10.00	2.28p	9.55	3.97pqrs	1.61s	74.21d	72.59d	69.25
G08	P2 x BCY	9.26	2.74	8.30	7.40s	1.89s	72.28d	70.40d	64.51
G09	P2 x ERC24	10.48	2.77	11.11abc	6.58s	1.74s	69.07	67.33	67.18
G10	ERC24 x G102612D	8.76q	2.24prs	8.31	4.65prs	1.61s	76.04abcd	74.43abcd	68.37
G11	MSM53 x G102612D	10.39	2.48	10.05	5.19ps	1.60s	71.90d	70.30d	70.72
G12	GLT226 x BCY	10.16	2.90	8.93	7.13s	2.36	70.88	68.53	69.38
G13	ERC24 x BCY	10.10	2.60	7.48	5.78ps	2.25	74.05d	71.80	71.70
G14	GLT226 x MSM53	9.91	3.12	9.86	6.50s	1.91s	70.61	68.70	71.14
G15	GLT226 x P2	9.40	3.38	11.31abc	8.39	2.46	67.52	65.06	70.85
G16	RK457	9.06	2.84	9.09	7.38	1.98	71.64	69.66	66.96
G17	RK57	10.17	2.36	8.79	5.46	2.00	73.22	71.23	66.82
G18	BISI18	9.80	2.77	8.69	6.69	1.98	72.06	70.07	51.28
G19	NASA29	9.41	2.66	9.77	9.48	2.68	68.69	66.01	51.57
Grand mean		9.53	2.69	9.23	6.31	1.97	72.24	70.26	67.06
LSD 5%		1.11	0.49	1.70	2.03	0.52	2.81	3.03	-
Hybrid F-value		2.67*	2.96*	2.72*	3.80**	2.91*	5.40**	5.44**	-
Coeficient of variation (%)		5.53	8.66	8.76	15.27	12.63	1.85	2.05	-

Note: * and **: Significant at $p<0.05$ and $p<0.01$, respectively; a,b,c,d). Significantly higher compared to RK457, RK57, BISI18, NASA29 check varieties, respectively based on LSD 0.05; p,q,r,s). Significantly lower compared to RK457, RK57, BISI18, NASA29 check varieties, respectively based on LSD 0.05; Mst: Moisture content; TAsh: Total ash content; CPrt: Crude protein content; Cfat: Crude fat content; CFBr: Crude fiber content; TCbh: Total carbohydrate content; SCbh: Soluble carbohydrate content; Sch: Starch content

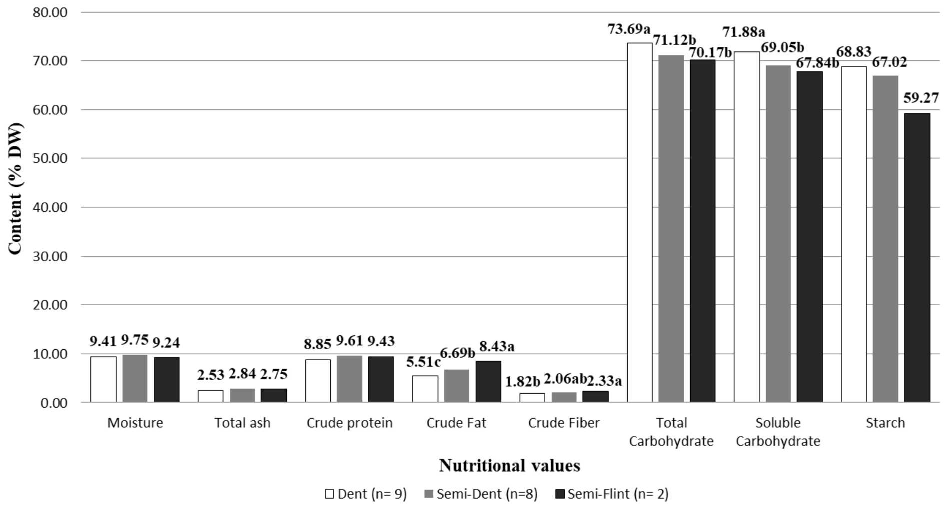

Figure 2. Variation of corn flour nutritional values among different kernel types. Note: Means followed by the same letter are not significantly different based on LSD 0.05

GT-biplot and cluster analysis of 40/0 mesh corn flour nutritional values

The polygon GT biplot explained 73.79% of the total variation and had 5 from 7 sectors that contain nutritional traits (Figure 3A). The obtained data were standardized by subtracting the grand mean and dividing it by the standard error (Scaling=1). The interpretation of the GT biplot is similar to that of the GGE (genotype and genotype by environment) biplot and superior genotypes are located at the vertex of each trait-cluster (Yan and Tinker 2006). G05 and G07 at sector I were not superior because this sector does not contain a nutritional trait. G03 and G06 approximately had closer coordinates to the GT biplot origin coordinate, indicating their nutritional contents were relatively not much different from the grand mean for each nutritional trait. G02, G04, G06, G10, G13, and G17 at sector II had relatively high total carbohydrate and soluble carbohydrate contents and G10 was superior. That sector was dominated by dent hybrids and only G17 was a semi-dent hybrid. Food product potential probably resulted in more calories and energy. Corn flour is a rich source of energy and has appreciable functional properties and is useful for bakery and food products (Oladapo et al. 2017).

Sector IV consisted of G01, G03, G08, G16, G18, and G19 which had relatively high crude fiber and crude fat contents and G19 was superior. That sector was dominated by semi-dent and semi-flint hybrids, only G01 and G08 were dent. Semi-dent and semi-flint hybrids in sector IV contained crude fiber and crude fat contents above the grand mean. Dietary fiber food from cereals has a positive effect on weight management, blood glucose levels, and gastrointestinal, and cardiovascular health (Prasadi and Joye 2020). G12 and G15 at sector V had relatively high total ash and crude protein contents and G15 was superior. G15 had total ash and crude protein 25.65% and 22.53% higher compared to the grand mean, respectively. Protein and lipid contents are the highest in flint hybrids so they are preferred for maize bread (Alvarez-Iglesias et al. 2021). Popcorn flour may be useful for overcoming protein energy malnutrition and can be processed as a weaning food formulation (Oladapo et al. 2017). G09 and G14 at sector VI had relatively high moisture content. The polygon GT biplot analysis was consistent with total ash, crude protein, crude fat, crude fiber, total carbohydrate, and soluble carbohydrate contents in Table 3 where each superior hybrid in the biplot had a significantly higher average compared to the grand mean.

G11 at sector VII had a relatively high starch content. The starch content of G11 was lower than G05, G13, and G14, indicating there was 26.21% another factor that could not be explained by the GT biplot. The baby dent corn starch suitable as a thickening agent for products is stored under frozen conditions (Sandhu et al. 2003). According to polygon GT biplot analysis, the dent hybrids had superiority in starch, total carbohydrate, and soluble carbohydrate contents. The semi-dent hybrids had superiority in crude protein and total ash contents. The semi-flint hybrids had superiority in crude fat and crude fiber contents. Principal component analysis (PCA) biplot for analog rice and kernel nutritional values was previously studied by Sumardiono et al. (2021) and Orhun (2020), respectively, but only explained the relationship among traits. The polygon GT biplot in this study is useful for obtaining information on the potency of each hybrid and the differences in nutritional values of corn flour between kernel types.

The two components resulting from the fuzzy clustering method explained 74.76% of the total variation and showed two clusters (Figure 3B). Cluster 1 (C-1) consisted of 10 hybrids and was dominated by semi-dent and semi-flint types. G01 and G08 in C-1 were dent type and had negative silhouette evaluation scores, indicating G01 and G08 were less convincing for clustered in C-1. Cluster 2 (C-2) was dominated by dent hybrids consisted of G02, G04, G05, G06, G07, G10, G11, G13, and G17 hybrids, only G02 and G17 which semi-dent type. Semi-dent hybrids at C-1 and dent hybrids at C-2 were derived from GLT226 and MSM53 female parents, respectively. G102612D was the male parent and tended to result in dent hybrids, except for G02 due to GLT226 being the female parent. The C-1 and C-2 clusters are adequate to separate kernel types based on corn flour nutritional values. The fuzzy clustering method has major limitations in handling outliers (Malhotra et al. 2014). The fuzzy clustering method could effectively cluster hybrids and simply explain the variation between kernel types. The polygon GT biplot and the fuzzy clustering analysis can be considered besides the analysis of variance for understanding the variation among hybrids.

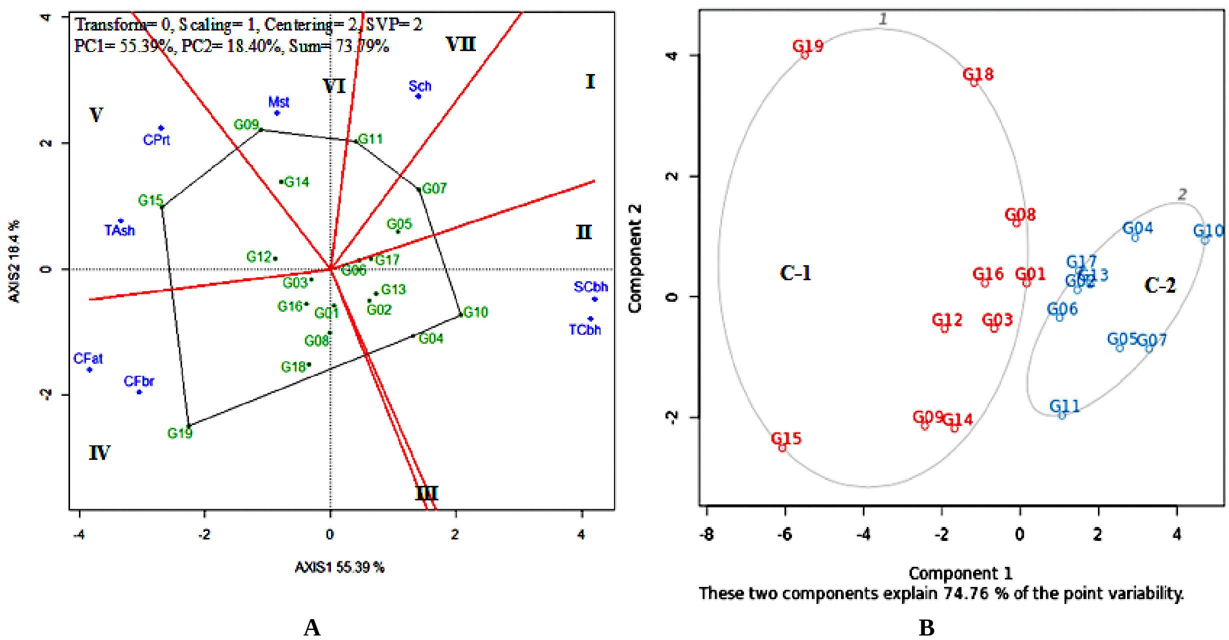

Figure 3. Polygon GT biplot analysis (A) and fuzzy clustering method (B) based on 40/0 mesh corn flour nutritional values

Table 4. Nutritional contents difference among two clusters according to t-test

Cluster	n	Nutritional values % DW (Mean ± Standard deviation)							
		Mst	TAsh	CPrt	CFat	CFbr	TCbh	SCbh	Sch
C-1	10	9.56 ± 0.51	2.86 ± 0.23	9.57 ± 0.99	7.30 ± 0.95	2.12 ± 0.29	70.72 ± 1.73	68.60 ± 1.90	65.13 ± 7.48
C-2	9	9.36 ± 0.74	2.45 ± 0.22	8.95 ± 0.62	5.09 ± 0.58	1.75 ± 0.17	74.15 ± 1.36	72.40 ± 1.41	68.83 ± 1.68
t-value		0.65ns	3.97**	1.64ns	6.18**	3.38**	-3.43**	-4.99**	-1.52ns

Note: ns: Not significant; **: Significantly different at p<0.01; Mst: Moisture content; TAsh: Total ash content; CPrt: Crude protein content; Cfat: Crude fat content; CFBr: Crude fiber content; TCbh: Total carbohydrate content; SCbh: Soluble carbohydrate content; Sch: Starch content

The moisture, crude protein, and starch contents among the two clusters were not significantly different (Table 4). The C-1 had averages of total ash, crude fiber, and crude fat contents significantly higher whereas total carbohydrate and soluble carbohydrate contents were significantly lower compared to C-2. These results are consistent with the polygon GT biplot analysis that sector II was dominated by the C-2 hybrids whereas sectors IV and V were dominated by the C-1 hybrids. The C-2 hybrids were selected as potential hybrids due to having higher soluble carbohydrates, but lower crude fiber and lower crude fat which are good for corn flour's nutritional values. Previous corn flour nutritional contents analysis indicated that dent hybrids resulted in more carbohydrates and better digestibility. Anti-nutritional factors in corn flour must be concerned because they cause oxalate content to be higher on the kernel (Oladapo et al. 2017). Degerminated processing is statistically significant for reducing fat content in corn flour compared to raw processing (Bello and Udo 2018).

Figure 4 shows a 3D scatter plot using three characteristics and the hybrids are separated based on fuzzy clustering results. The grand mean of W1000Kn, 40/0 mesh, and Sch were 277.98 g, 24.29%, and 67.06% DW, respectively. G02, G03, and G05 had scores above the total grand mean for these three characteristics. Unfortunately, G03 and G14 indicated male sterility from field observation and could not be released as a variety. The 1000-kernel weight represented the kernel index and is useful for indicators of endosperm filling for grain yield. G02, G03, G05, G11, G12, G13, G14, G15, G16, G17, and G19 which were dominated by the C-1 hybrids had W1000Kn above the grand mean. The 40/0 mesh corn flour yields represented the dry milling process's ability. G01, G02, G03, G04, G05, G07, G08, G10, G17, and G18 were dominated by the C-2 hybrids had 40/0 mesh above the grand mean. The starch content is useful as a main reference for corn starches products by wet milling processes. G01, G02, G03, G04, G05, G07, G09, G10, G11, G12, G13, G14, and G15 hybrids had Sch above the grand mean.

G10 and G19 hybrids could be a contrasting example of the difference between dent and semi-flint types based on a 3D scatter plot (Figure 4). G10 had the 40/0 mesh and Sch above each of the grand means but the W1000Kn below the grand mean whereas G19 vice versa. G06 with its coordinate closer to the GT biplot origin had scores below the grand mean for these three characteristics. The number of hybrids among two clusters that had starch content above the grand mean was not much different and could strengthen the result in Table 4. The C-2 hybrids were dominated by dent hybrids and tended to have high 40/0 mesh and Sch, but low W1000Kn. The scatter plot visualized the hybrids' variation among the two clusters. On the other hand, the same trend was found in previous analyses about the superiority difference in kernel types in kernel morphological characteristics, dry milling processes ability, and corn flour nutritional values. The tropical dent hybrids had potencies in the kernel dry milling process's ability and corn flour nutritional values whereas semi-dent and semi-flint hybrids in the kernel quantity. These results are interesting for further study with agronomy, milling, and food industries' approach to multitrait selection. Culling levels are essential for maximizing genetic gains (Batista et al. 2021). The V/F endosperms ratio affects the size and roundness of starch granules which influences the product's physicochemical properties (Vega-Rojas et al. 2016; Xu et al. 2019).

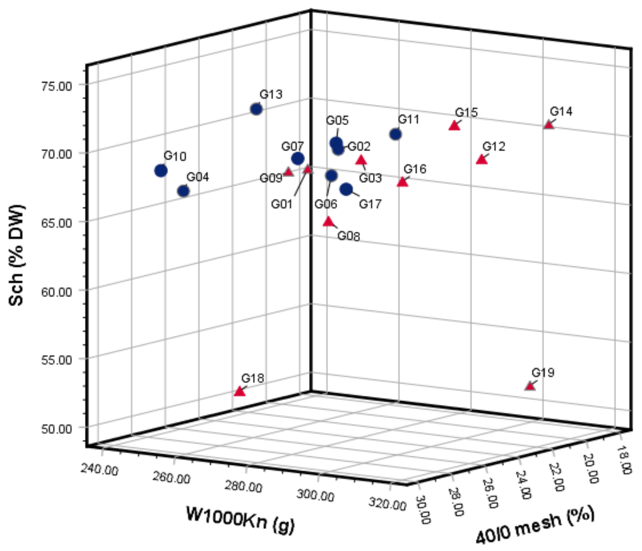

Figure 4. 3D scatter plot based on starch content (Sch), 1000-kernel weight (W1000Kn), and 40/0 mesh corn flour yield percentage (40/0 mesh): red triangle symbols are the C-1 hybrids and blue circle symbols are the C-2 hybrids according to the fuzzy clustering method

The hybrids have their potency so must be wisely considered for selection. The kernel type difference affected dry milling processes and corn flour nutritional values. This study provided information for breeders and millers to release specific varieties according to corn flour physico-chemical properties so that they can develop specific-end-uses products. A forthcoming trend will be to adopt commercial corn varieties which have not only good agronomic performances but also their technological properties (Blandino et al. 2010). Corn flour with 80°C drying is the best result based on the organoleptic evaluation (Abriana et al. 2020). The local corn varieties have the benefits of complementary foods and support food resilience (Bello and Udo 2018; Ahmad et al. 2021). The corn hybrid variety focused on the food industry should have important criteria such as high yielding, resistance to main diseases, and suitability based on milling evaluation factors. There are four policy recommendations for developing corn aimed at food industries and one of those is accelerating the release of tropical dent varieties resistant to aflatoxin (Suarni 2021).

In conclusion, the corn flour physico-chemical variation is influenced by hybrids, especially in the kernel type difference. The polygon GT biplot and the fuzzy clustering can be used as efficient methods for explaining the 40/0 mesh corn flour nutritional values variation and superiorities. The semi-flint hybrids had 1000-kernel weight and kernel density significantly higher compared to the grand mean. All dent hybrids had 40/0 mesh corn flour yields significantly higher compared to NASA29. There was a significant correlation between the morphological kernel characteristics and the fraction components. The crude fat content of D-CF was significantly lower whereas its total and soluble carbohydrate were significantly higher compared to SD-CF and SF-CF. The crude fiber content of D-CF was significantly lower compared to SF-CF. All check varieties had starch content below the grand mean. According to the polygon GT biplot analysis, sectors II and VII were dominated by the dent hybrids whereas sectors IV, V, and VI were dominated by semi-dent and semi-flint hybrids. The C-2 hybrids were selected as potential hybrids for corn flour because it has relatively high total carbohydrate and soluble carbohydrate contents. MSM53 as the female parent resulted in dent hybrids that clustered at C-2. G02, G03, and G05 hybrids had scores above the grand means according to the 3D scatter plot. The potential hybrids could be evaluated further by corn flour's functional properties and agronomic performance analysis for the release of corn hybrid varieties for food industries.

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