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Effect of Source and Processing on Maize Grain Quality and Nutritional Value for Broiler Chickens 1. Heat Treatment and Physicochemical Properties

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ABSTRACT

The aim of the present study was to evaluate the nutritional quality of traditional sun-dried maize grain, subjected to further heat treatment. The maize grains were obtained from three different locations, namely Moree in NSW, Emerald in QLD and Darling-Downs on the NSW-QLD border, Australia. Once received, one batch (sun-dried) was assessed without further drying. The other two batches from each source were dried artificially using a forced draught-oven at 105°C for 30 min or 24 h. The morphological structures (starch granules) of grains were changed due to heat-treatment for 30 min at 105°C and far-reaching structural changes occurred when samples were heated for 24 h. Proximate analysis of maize revealed that the DM (897.3-967.4 g kg^{-1}), CP (92.1-108.7 g kg^{-1}), EE (49.0-57.5 g kg^{-1}), phytate-P (1.2-2.4 g kg^{-1}) and ash (14.1-19.7 g kg^{-1}) contents of samples varied due to source and heating period. The starch, amylose, available amino acid and mineral contents increased with increasing heating duration. Variable anomeric proton peaks (^{1}H-NMR) were found with an increase in the oven drying period in all maize samples. *In vitro* digestibility of DM, starch and CP was improved due to heat-treatment at 105°C for 30 min 24 h. It may be concluded that the chemical composition, ultra-structural characteristics and energy values of maize samples varied by source and were changed through heating of low-moisture maize over varying time periods. These changes could impact on the nutritive value of the grains and animal performance.

Key words: Grain source, drying, physicochemical characteristics, dietary quality

INTRODUCTION

Maize (*Zea mays*) is one of the most important cereal grains used for poultry feeding all around the world. It is also generally believed that maize has a relatively high and consistent nutritional value for broiler chicks but recent data indicate that the energy value of maize and nutrients available can vary and in the case of energy level may be greater than 1.1 MJ kg^{-1} (Song *et al*., 2004). There are many suggested causes for this variation, such as overall chemical composition, starch content, amylose: Amylopectin ratio, starch structure, the embedding of starch with a matrix of lipid, protein or carbohydrate, presence of amylase inhibitors and phytate concentration (Cowieson, 2005).

However, the viscous Non-Starch Polysaccharides (NSP) present in most temperate cereals such as wheat, triticale and barley are relatively low in maize (Bedford, 1995; Smith and Annison, 1996). In spite of this advantage, the nutritive value of maize is known to fluctuate widely, due to the environmental conditions during growth, harvest and post-harvest processing, especially drying temperature and storage (Collins *et al*., 1998).

Starch from grain is the main energy source for poultry and the ratio between amylose and amylopectin plays a significant role in the digestibility of maize starch. Noy and Sklan (1995) opined that about 150 g kg^{-1} of maize starch remains undigested up to the terminal ileum and is assumed to be resistant to digestive enzymes. This is known as Resistant Starch (RS). It escapes digestion in the small intestine and has dietary fibre-like properties. There is potential for use of exogenous enzymes to digest resistant starch and increase overall digestibility of maize as is practiced with wheat and other temperate cereals (Bedford, 1995). Resistant starch of grain is also challenging for chickens and Brown (1996) reported that an RS3 type is created during feed processing and storage. Retrograded starch (RS3) results from the processing of starch at a high temperature, followed by storage at a low temperature over a long period of time. The starch in high-moisture grain also anneals after heat processing.

Grains may be subjected to heating, for example during drying in the field, pelleting or long storage in air-tight silos. The extent to which these processes occur during routine production and processing of maize has not been well documented. Furthermore, drying of LM maize for a prolonged period of time may also increase resistant starch content. The quality of diets with over-heated grains is low, tending to negatively affect broiler performance (Panigrahi *et al*., 1996). There is a need to assess the response of maize grain to high temperature treatment. In this study, such response is examined in maize from different sources when heat-treated at a constant temperature over different time periods.

MATERIALS AND METHODS

Maize sample collection and drying: Three commercial maize samples were obtained from three different locations, namely Moree in New South Wales (NSW), Emerald in Queensland (QLD) and Darling Downs on the NSW-QLD border, Australia. The maize sample had been sun-dried to a Dry Matter (DM) content of 877.0, 882.0 and 883.0 g kg^{-1}, respectively for Moree, Emerald and Darling Downs. Once received the samples were split into three batches for each source. One batch (sun-dried) was assessed without further drying. The other two batches in each source were dried artificially using a forced draught oven at 105°C for 30 min or 24 h, respectively. These heating times were selected after preliminary assessment at 30 min, 4.5, 10, 12 and 24 h. After the drying process, the warm samples were placed in paper bags and cooled at room temperature overnight. Upon cooling, the samples were kept in sealed air-tight bags prior to grinding.

Proximate analyses: For chemical analyses, representative grain samples were ground by hammer mill, to pass through a 1 mm sieve and then stored in a sealed glass bottle at 4°C until they were analysed. All analyses of grain samples were carried out in duplicate and the results are expressed on dry matter basis. The proximate analyses were done according to the methods of the Association of Official Analytical Chemists (AOAC, 2002).

The nitrogen content of the samples was determined according to the Dumas combustion technique following the method described by Sweeney (1989) using a LECO® FP-2000 automatic nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA). Nitrogen freed by combustion at high

temperature in pure oxygen was measured by thermal conductivity detection and converted to equivalent Crude Protein (CP) using a factor of 6.25. The Ether Extract (EE) was determined indirectly by the Soxhlet Method (AOAC, 2002). Around 2 g of finely ground sample was weighed into pre-weighed dry paper thimbles and extracted for 24 h with chloroform using a Soxhlet apparatus. Thimbles with samples were allowed to drain and then dried at 105°C for 24 h. The EE was calculated as percent of the loss of weight and expressed as a proportion of dried sample weight. The gross energy (GE) contents of maize samples were determined using an IKA 7-WERKE bomb calorimeter (C7000, GmbH and Co. KG., Staufen, Germany). The GE value of maize samples was obtained as MJ kg^{-1} directly from the digital system of the calorimeter.

For ash determination, approximately 4 g dried ground maize grain samples were weighed into pre-weighed silica crucibles. The samples were ignited in a preheated Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK) at 600°C for three hours. The Furnace temperature was set at 350°C initially and then raised to the target temperature after 1 h. The crude ash content was calculated using the following equation and expressed as:

$$\text{Ash\%} = \frac{\text{Ashed sample weight}}{\text{Dried sample weight}} \times 100$$

The phytate-P content of grain samples was determined following a sensitive method for the rapid determination of phytate-P in cereals and cereal products as previously described (Haug and Lantzsch, 1983).

Starch content and composition: The starch and resistant starch contents of the maize samples were determined using the Megazyme total starch and resistant starch kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on the method developed by McCleary *et al.* (1994). The amylose/amylopectin contents were determined with a Megazyme amylose/amylopectin assay kit (Megazyme International Ireland, Bray Business Park, Bray, Ireland) using the selective quantitative precipitation reaction of con-canavalin A (Con A) for amylopectin (Gibson *et al.*, 1997) and by the colorimetric method of iodine binding for amylose (Chrastil, 1987). The ratios of these components were then related to one another.

Non-starch polysaccharides (NSP): Non-starch polysaccharides were measured according to the methods of Englyst and Hudson (1993) and Theander and Westerlund (1993). This involved gas chromatography (VARIAN, CP -3800, USA) of alditol acetate derivatives of monosaccharides. The levels of polysaccharides were calculated from the level of the component sugars using polymerization factors, 0.88, for pentoses (ribose, xylose and arabinose), 0.9 for hexoses (mannose, galactose and glucose), 0.89 for deoxysugars (fructose and ribose) and 0.91 for rhamnose.

Amino acid composition: Concentrations of amino acids were determined using pre-column derivatization amino acid analysis with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) followed by separation of the derivatives and quantification by reversed phase High Performance Liquid Chromatography (HPLC) according to Cohen and Michaud (1993) and Cohen (2001).

Isolation of soluble NSP and nuclear magnetic resonance (NMR) analysis: Ground maize samples were washed twice with 200 mL n-hexane. After air-drying the samples, starch and protein

were enzymatically removed by incubation with 200 mL distilled water in a water bath (100°C, 30 min) in the presence of a heat stable α-amylase (Termamyl 120 L, Type L, Novozymes A/S, Denmark; 0.25 mL, 95°C, 30 min) followed by incubation with 0.5 mL amyloglucosidase (AMG, 300 L, Brewq, Novozymes A/S, Denmark) at 55°C for 16 h. The mixture was cooled and centrifuged at high speed (7500 xg for 30 min). The clear supernatant was then incubated overnight followed by adjustment with two volumes of ethanol to 66% (v/v) and the precipitate was collected, blow-dried under nitrogen and then kept in a cool room at 5°C until NMR analysis. The isolated NSP (10 mg) from maize grains was dissolved in 2 mL D_2O (99.9 atom% D) and freeze-dried. This step was repeated three times to remove interference by exchangeable protons. All spectra were acquired on a Bruker Avance 300 NMR spectrometer using a 5 mm inverse ^1H BB porbhead with a Z-gradient. ^1H-NMR spectra were acquired at 88°C in D_2O (100 atom% D) solution at a concentration of 20 mg mL^{-1}. Chemical shifts are expressed relative to an external acetone standard (2.2 ppm).

Mineral analysis: Minerals were analyzed by Inductively Coupled Plasma (ICP) method (Vista MPX-radial) following the protocol of Anderson and Henderson (1986). The Sealed Chamber Digest (SCD) method was also used for P, S, K, Na, Mg and trace elements.

***In vitro* digestibility:** The *in vitro* digestibility of DM, starch and CP was determined by the method of Babinsky *et al.* (1990) with slight modifications. Fifty mL centrifuge tubes were used instated of 100 mL tubes and reagent/chemical volumes were halved appropriately. The digestibility of DM, starch and CP was calculated by relating the weight of dried residue to weight of original samples:

$$\text{DM digestibility (\%)} = \frac{\text{Weight of maize sample-Weight of dried residue}}{\text{Weight of maize sample}} \times 100$$

Starch and CP digestibility was similarly calculated but the concentrations of starch or CP were substituted into this equation.

Apparent Metabolizable Energy (AME) was estimated according to the equation:

$$\text{AME (kcal kg}^{-1}) = 53+38\times(\%\text{CP}+2.25\times\%\text{EE}+1.1\times\%\text{Starch}+\%\text{Sugar})$$

where CP is crude protein and EE is ether extract (Carpenter and Clegg, 1956). The values were converted to MJ kg^{-1} using a conversion factor of 239 kcal to 1 MJ.

***In vitro* viscosity:** The *in vitro* viscosity (in centipoise, cPs = 1/100 dyne second per cm^2) of grain samples was measured on thawed supernatants with a Brookfield DVIII (Brookfield Model DV-III Rheometer, speed range 0-250 RPM, 0.1 RPM increment; viscosity accuracy "1.0% of full scale range for a specific spindle running at a specific speed) rheometer at 25EC with a cPs 40 spindle at 100 rpm according to the method described by Bedford and Classen (1993). The samples did not exhibit shear thinning at these shear rates. The samples were carefully processed to overcome the effect of viscosity of supernatant due to freezing and thawing of samples.

Electron microscopy: The whole maize grain was scoured around the edges, then frozen in liquid nitrogen to fracture. They were then mounted on 12 mm aluminum stubs using double-sided

adhesive tape and gold-coated using a Polaron (E 5100 sputter coater). The structure of the starch granules of the samples was observed using an electron microscope (JSM-5800LV scanning microscope JEOL; installed 1995 at University of New England, Armidale campus, NSW Australia). All micrographs were taken at a magnification of H 500.

Statistical analysis: The data were subjected to sample one test using SPSS Statistics version 17.0.0 (SPSS, 2009) for average and standard error followed by calculation of the coefficient of variation (CV).

RESULTS

The morphological structural changes of maize grains: The electron micrographs of maize samples from different sources found are shown in Fig. 1. The starch granules of the samples from different sources appeared morphologically similar to one another in the case of sun-dried grains, although granules of samples from Moree were loosely packed with a lot of protein matrix. After 30 min of heating, the shapes of starch granules changed and all sources of grains became shrunken and distorted. The surfaces of the starch granules were rougher, irregular and elongated. Following 24 h of heating the granules were found to be closely packed, irregular and rough in

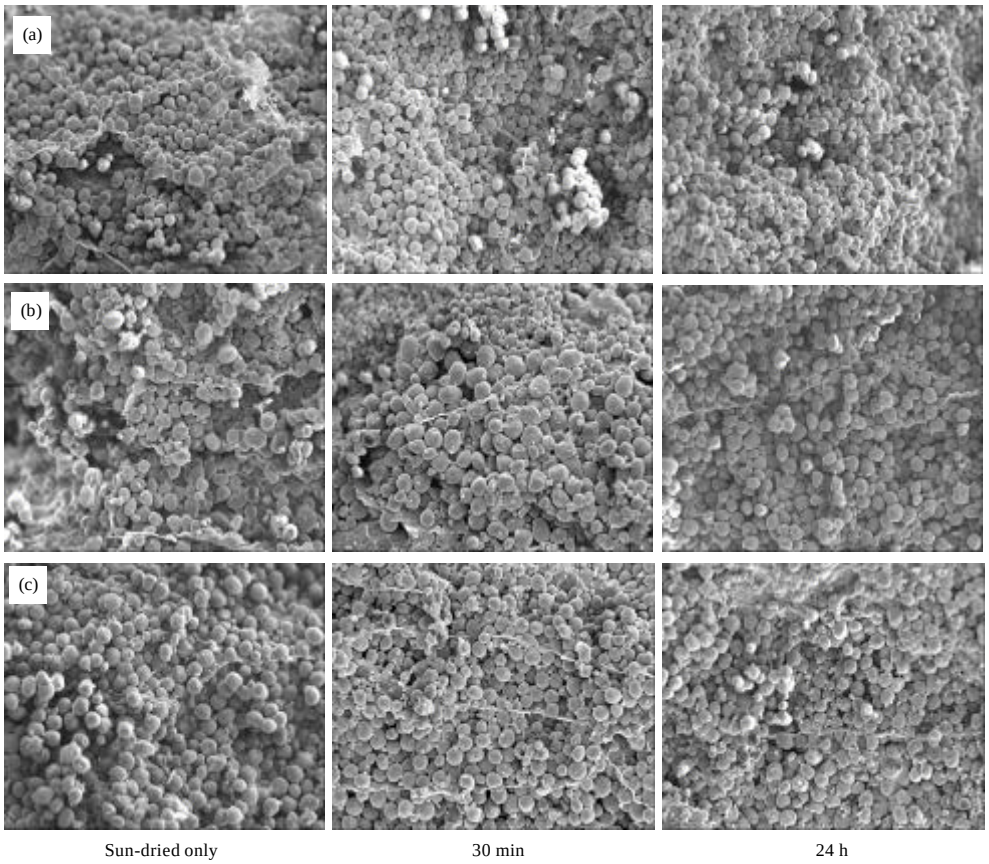

Fig. 1: Electron micrographs (x500) of (a) Moree (top panel), (b) Emerald (middle panel) and (c) Downs (bottom panel) maize as sun-dried only or artificially heat treated at 105°C for varying durations, 30:30 min heating period and 24:24 h heating period

shape compared to the sun-dried samples. In Emerald, the starch granules were bigger and more flattened than the sundried granules and this was observed after 30 min heating, whereas, after 24 h heating, they were more tightly packed with protein matrix.

The electron microscope images for the Downs maize revealed that the starch granules were squeezed and deformed and had more protein matrix than the sun-dried samples. After 24 h of heat treatment, the starch granules were more constricted and overlapped each other. Another characteristic feature was that the granules were tightly packed in contrast to the sun-dried maize. Most protein bodies are located outside the starch granules and were enveloped by the starch granules as well. This was observed in all the maize sources after 30 min of heating.

Proximate composition: The proximate composition of different sources of maize with or without heat treatment is shown in Table 1. Mean values of DM and GE were increased and varied between 877.0, 960.2 g kg^{-1}, 18.6 and 18.9 MJ kg^{-1}, respectively with an increase in drying period for Moree. However, EE, phytate-P and ME were decreased from 65.9-44.9, 2.5-1.9 and 16.5-16.0 MJ kg^{-1}, respectively under the same conditions. There was no clear trend in changes in the CP and ash contents as samples were heated.

The DM content of the Emerald samples varied between 882.8 and 963.9 g kg^{-1}, increasing as samples were heat-treated. The CP, EE and phytate-P decreased and varied between 93.1, 92.7, 67.1, 49.6, 2.2 and 1.5 g kg^{-1}, respectively under 24 h heat treatment. Heat treatment had no effect on ash, GE and ME contents of the Emerald maize.

In the Downs maize DM, CP, EE, phytate-P and ash content but not GE and ME were affected by heat treatment. The DM, CP and ash contents increased and varied between 883.4, 967.4, 101.2, 102.1, 14.0 and 14.8 g kg^{-1}, respectively with an increase in drying period at 105°C. In terms of CV values, EE, phytate-P and ash were found to be the most variable components.

Starch content and composition: The effects of heat treatment on starch content and composition of different sources of maize are presented in Table 2. In the Moree sample, the total starch content slightly increased with an increase in heating duration at 105°C compared to the sun-dried samples, while the total starch content decreased for the Emerald and Downs maize.

Table 1: Proximate composition (g kg^{-1} DM) of maize of different sources sun-dried only or artificially heat treated at 105°C for varying durations

Treatments		DM	CP	EE	Phytate P	Ash	GE	ME
Sources	Heating period	(g kg^{-1})	(g kg^{-1} DM)	(g kg^{-1} DM)	(g kg^{-1} DM)	(g kg^{-1} DM)	(MJ kg^{-1} DM)	(MJ kg^{-1} DM)
Moree	Sun-dried only	877.0	108.3	65.9	2.5	19.4	18.6	16.5
	30 min	897.6	107.2	49.0	2.4	15.8	18.7	16.0
	24 h	960.2	108.7	44.9	1.9	19.7	18.9	16.3
Emerald	Sun-dried only	882.8	93.1	67.1	2.2	15.2	18.8	16.4
	30 min	898.7	92.1	53.2	1.9	14.7	18.7	16.1
	24 h	963.9	92.7	49.6	1.5	15.5	18.8	16.4
Downs	Sun-dried only	883.4	101.2	59.4	1.8	14.0	18.9	16.2
	30 min	902.3	101.5	57.5	1.7	14.1	18.9	16.3
	24 h	967.4	102.1	52.0	1.2	14.8	18.9	16.3
CV		000.04	000.07	00.14	0.22	00.14	00.01	00.01

DM: Dry matter, 30:30 min heating period, 24:24 h heating period, CV: Coefficient of variation

Table 2: Total starch, digestible starch and resistant starch content (g kg^{-1} DM) of maize of different sources sun-dried only or artificially heat treated at 105°C for varying durations

Treatments				
Sources	Heating period	Total starch	Digestible starch	Resistant starch
Moree	Sun-dried only	722.2	706.8	23.0
	30 min	724.3	700.5	23.8
	24 h	729.8	680.9	41.3
Emerald	Sun-dried only	739.6	715.6	14.9
	30 min	731.1	709.2	21.9
	24 h	730.5	689.3	50.3
Downs	Sun-dried only	725.2	705.8	16.6
	30 min	718.6	693.4	25.1
	24 h	722.4	683.7	41.5
CV		0.01	0.02	0.43

DM: Dry matter, 30:30 min heating period, 24:24 h heating period, CV: Coefficient of variation

Table 3: Amylose and amylopectin contents (g kg^{-1} DM) of maize of different sources sun-dried only or artificially heat treated at 105°C for varying durations

Treatments			
Sources	Heating period	Amylose content	Amylopectin content
Moree	Sun-dried only	197.8	524.2
	30 min	205.3	518.7
	24 h	254.3	475.5
Emerald	Sun-dried only	193.6	546.0
	30 min	211.1	520.0
	24 h	219.1	511.4
Downs	Sun-dried only	196.7	528.3
	30 min	214.2	504.4
	24 h	224.8	497.6
CV		0.09	0.04

DM: Dry matter, 30:30 min heating period, 24:24 h heating period, CV: Coefficient of variation

However, in all three sources of maize the total starch content did not change considerably after 30 min of heating. The Emerald maize was higher in total starch content than the other two sources.

The amount of digestible starch was slightly decreased by heat treatment of maize grains at 105°C over different durations, irrespective of the source of maize. The trend from 30 min to 24 h of heating at 105°C was more or less the same in all sources of maize. The resistant starch content increased sharply with increase in heating period at 105°C for all sources of maize. The trend was similar in the case of Emerald and Downs maize in contrast to Moree maize. In terms of CV values, resistant starch found to be the most variable component.

Amylose and amylopectin contents: The amylose content increased with increasing heating duration, the highest amylose content being in the Moree samples heated over 24 h (Table 3). The same level of amylose content was found in sun-dried only samples for all sources of maize.

Table 4: Concentration of non-starch polysaccharides (g kg^{-1} DM) of maize of different sources as sun-dried only or artificially heat treated at 105°C for varying durations

Treatments				
Sources	Heating period	Total NSP content	Soluble NSP content	Insoluble NSP content
Moree	Sun-dried only	57.5	1.9	55.5
	30 min	53.1	1.8	51.4
	24 h	52.5	1.6	50.9
Emerald	Sun-dried only	60.5	1.9	58.6
	30 min	56.5	1.8	54.7
	24 h	56.7	1.6	55.2
Downs	Sun-dried only	64.6	1.5	63.2
	30 min	61.2	1.6	59.6
	24 h	60.7	1.7	59.0
CV		0.07	0.10	0.07

DM: Dry matter, 30:30 min heating period, 24:24 h heating period, NSP: Non-starch polysaccharide, CV: Coefficient of variation

Table 5: Available free sugar contents (g kg^{-1} DM) of maize of different sources sun-dried only or artificially heat treated at 105°C for varying durations

Treatments							
Sources	Heating period	Arabinose	Xylose	Mannose	Galactose	Glucose	Total
Moree	Sun-dried only	0.5	0.5	1.1	1.9	12.5	16.5
	30 min	0.3	0.3	1.0	0.7	12.6	14.9
	24 h	0.3	0.2	0.8	0.6	12.8	14.8
Emerald	Sun-dried only	0.3	0.1	1.1	0.9	14.7	17.2
	30 min	0.2	0.1	1.0	0.8	14.2	16.4
	24 h	0.3	0.9	1.0	0.7	13.5	16.5
Downs	Sun-dried only	0.3	0.2	1.0	1.0	14.7	17.3
	30 min	0.3	0.1	1.0	0.9	13.6	16.0
	24 h	0.3	0.1	0.8	0.8	13.9	15.9
CV		0.18	0.94	0.11	0.43	0.06	0.05

DM: Dry matter, 30:30 min heating period, 24:24 h heating period, CV: Coefficient of variation

Dissimilar results were observed for amylopectin contents. The amylopectin content decreased with increase in heating period at 105°C for all sources of maize. The maize from Emerald and Downs had the highest amylopectin value of three sources.

NSP and free sugar contents: The total NSP content decreased after 30 min of heating, but increased following 24 h of heating (Table 4). The maize from Emerald and Downs had the highest total NSP content of the three. Moree and Emerald maize had a similar soluble NSP content, but the trend shown by Downs was different to the other two sources.

In the Downs maize, the soluble NSP content increased with increase in heating duration. Moree and Emerald were the highest soluble NSP content. The levels of insoluble NSP increased with increase in duration of heating at the same temperature for all sources. Downs maize had the highest insoluble NSP content.

For maize from Moree the arabinose, xylose, mannose and galactose contents decreased with an increase in heating period while the glucose content increased (Table 5). In the case of Emerald

Table 6: Amino acid contents (g kg^{-1} DM) of maize of different sources sun-dried only or artificially heat treated at 105°C for varying durations

Treatments									
Sources	Heating period	His	Thr	Ala	Lys	Met	Ile	Leu	Pal
Moree	Sun-dried only	2.6	3.2	7.1	2.5	1.3	3.4	12.1	4.7
	30 min	2.8	3.3	7.4	2.8	1.5	3.5	12.3	4.9
	24 h	2.9	3.6	8.1	2.8	1.7	3.9	13.8	5.4
Emerald	Sun-dried only	2.2	2.8	6.2	2.3	1.2	2.9	10.2	4.1
	30 min	2.3	2.9	6.5	2.4	1.2	3.1	10.9	4.3
	24 h	2.5	3.0	6.8	2.6	1.3	3.3	11.2	4.5
Downs	Sun-dried only	2.6	3.2	6.8	2.8	1.4	3.3	11.2	4.7
	30 min	2.7	3.3	7.0	2.9	1.4	3.4	11.6	4.9
	24 h	2.8	3.5	7.4	3.0	1.6	3.6	12.0	5.0
CV		0.09	0.08	0.08	0.09	0.12	0.08	0.09	0.08

DM: Dry matter, 30:30 min heating period, 24:24 h heating period, His: Histidine, Thr: Threonine, Ala: Alanine, Lys: Lysine, Met: Methionine, Ile: Isoleucine, Leu: Leucine, Pal: Phenylalanine, CV: Coefficient of variation

and Downs, there was little effect of heating on arabinose, xylose and mannose contents. However, galactose and glucose contents decreased with an increase in oven drying duration at the same temperature. Xylose, galactose, arabinose and mannose were the most variable components of these free sugars.

Amino acid contents: The amino acid composition of the maize batches differed by source, as shown in Table 6. The amino acid content increased with an increase in the oven drying period in all samples. In addition, the amino acid contents increased considerably after 24 h oven drying period compared to the sun-dried only samples. Moree and Down had the highest histidine, threonine, alanine, lysine, methionine, isoleucine, leucine and phenylalanine. The Emerald maize was lower in amino acid contents than other sources.

Nuclear magnetic resonance (^{1}H-NMR) shifts: The anomeric regions of the ^{1}H-NMR of the three isolates from each source of maize are shown in Fig. 2. A variable anomeric peak was observed for each treatment and the protons from the neutral sugars were resonating (4.6-5.2 ppm). In Moree maize, the anomeric proton peak of, for instance, a-glucose, isomaltose and β-glucose increased gradually with the duration of oven drying at 105°C compared with the sun-dried only sample.

An unknown peak was observed next to the β-glucose with the sun-dried only Moree and Emerald samples. In the Emerald maize, very consistent peaks of anomeric protons were observed, increasing gradually with an increase in heating duration of samples. However, an unknown peak was also found just next to the β-glucose peaks with the 30 min and 24 h samples of Emerald grains. Dissimilar anomeric proton peaks were observed in the maize obtained from Downs. For example, the highest peaks of those protons were found in the grain samples heated for 30 min.

Mineral contents: The mineral contents of the Downs sample had different results from the other two sources (Table 7). The values of Ca, Mg, P, Fe, Cu, Mn and Zn increased with increase in heating time compared to the control. On the other hand, the Na value decreased. The values of Mg, P and Fe increased and other mineral contents were reduced after 24 h of heating when compared to sun-dried only maize.

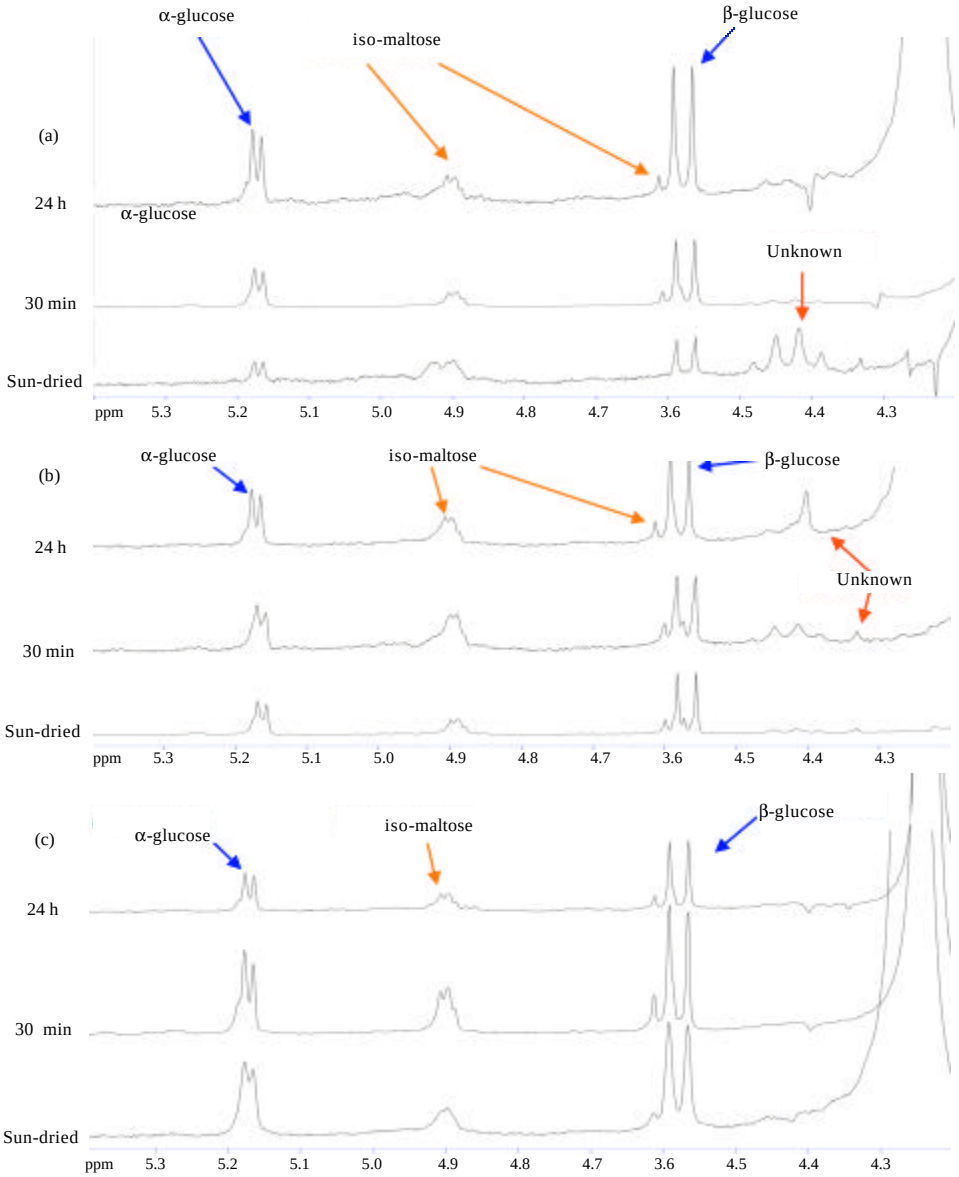

Fig. 2: Region of ^{1}H-NMR spectrum showing anomeric protons of the three maize samples (a) Moree, (b) Emerald and (c) Downs with sun-dried only or artificially heat treated at 105°C for varying durations

***In vitro* digestibility:** The *in-vitro* digestibility of DM, starch and protein of Moree batches varied between 49.5, 53.8, 56.7, 58.8, 43.4 and 46.1, respectively (Table 8). The digestibility of DM, protein and starch was improved by heat treatment of the samples. A similar pattern was observed in *in vitro* DM and starch digestibility of Emerald maize batches except protein digestibility and these values varied between 49.4 and 52.1%, 55.1 and 57.4%, respectively. *In vitro* protein digestibility was not affected by heat treatment of Emerald maize. Similar trends were observed for these variables in the Downs maize at the same drying temperature, but the range was narrower than in the other two sources of maize. *In-vitro* protein and starch digestibility was more variable than that of DM.

Table 7: Concentration (DM basis) of macro and trace minerals of maize of different sources sun-dried only or artificially heat treated at 105°C for varying durations

Treatments		Conc. (g kg^{-1})			Conc. (mg kg^{-1})				
Sources	Heating period	Ca	Mg	P	Fe	Cu	Mn	Zn	Na
Moree	Sun-dried only	0.14	1.4	3.6	35.5	2.1	8.8	17.7	119.5
	30 min	0.09	1.5	3.7	20.1	1.5	8.2	16.1	74.0
	24 h	0.08	1.4	3.4	30.8	1.4	7.6	16.2	73.0
Emerald	Sun-dried only	0.11	1.3	2.9	13.0	1.2	6.1	12.7	110.6
	30 min	0.11	1.2	2.9	12.7	1.7	5.9	11.9	82.2
	24 h	0.05	1.2	3.1	12.3	1.4	5.9	11.6	71.9
Downs	Sun-dried only	0.08	0.9	2.5	16.9	2.3	7.0	20.3	81.2
	30 min	0.09	1.1	2.9	20.1	2.3	7.8	22.4	78.7
	24 h	0.08	1.1	2.7	25.0	2.9	7.8	23.5	72.1
CV		0.29	0.15	0.14	0.39	0.30	0.15	0.26	0.21

DM: Dry matter, 30:30 min heating period, 24:24 h heating period, CV: Coefficient of variation

Table 8: *In vitro* Dry Matter (DM), starch and CP digestibility and viscosity of maize batches sun-dried only or artificially heat treated at 105°C for varying durations

Treatments		Digestibility (%)			
Sources	Heating period	DM	Starch	Protein	Viscosity[1]
Moree	Sun-dried only	49.5	56.7	43.4	0.93
	30 min	52.1	57.1	47.4	0.92
	24 h	53.8	58.8	46.1	0.90
Emerald	Sun-dried only	49.4	55.1	55.9	0.94
	30 min	50.6	56.4	56.2	0.91
	24 h	52.1	57.4	55.2	0.90
Downs	Sun-dried only	52.8	53.6	44.8	0.91
	30 min	53.2	54.1	52.9	0.89
	24 h	53.8	55.2	58.9	0.89
CV		0.03	0.06	0.11	0.02

[1]cPs (centipoise): 1/100 dyne second per centimeter squared, DM: Dry matter, CP: Crude protein, 30:30 min heating period, 24:24 h heating period, CV: Coefficient of variation

***In vitro* viscosity:** The *in vitro* viscosity decreased and varied between 0.90 and 0.93 cPs for Moree batches (Table 8). A similar pattern was observed in Emerald samples and the value varied between 0.90 and 0.94 cPs. The viscosity of the Downs's maize was less variable with change in drying period with values between 0.89 and 0.91 cPs.

DISCUSSION

Morphological structure of maize grains: It is clear from the current study that maize starch granules, protein bodies and matrices were affected by artificial heating compared to sun-dried only sample. Most of the starch granules became distorted, with some overlap and they were tightly packed, after heat treatment. It is speculated that differences in size and shape of starch granules within each variety as well as between sources was possibly due to loss of moisture and overheating. The size of the starch granule is an important factor in determining the energy value of starch, with smaller granules having a relatively larger surface area and so a greater potential

for hydrolysis by endogenous amylase (Carre, 2004). During heat processing, the starch gelatinizes to an extent that is dependent on granule size, moisture content, amylose: Amylopectin ratio (Klucinec and Thompson, 1999; Tester *et al*., 2004). It was postulated by Peplinski *et al*. (1994) that most changes in the physical properties of kernels became obvious only in maize dried at above 40°C, as is the case in this study.

Proximate composition: Additional drying of maize grains with relatively low moisture contents resulted in a sharp decline in moisture content and a simultaneous increase in the concentrations of the solid components, especially protein and ash. The concentration of nutrients such as EE slightly decreased but GE and ME were only minimally increased as a consequence of the heating treatment. Similar results were found by Debora *et al*. (2004) who mentioned that the CP, EE, Crude Fibre (CF), Ca, P and energy (AME and AMEn) contents of maize-based diets were increased by drying temperature, when the maize grain sample were dried at 120°C . Peplinski *et al*. (1994) showed only minor change in chemical composition of maize dried from 25 to 100°C. Dissimilar results were found by Velu *et al*. (2006) who showed that there was no change in protein and starch contents of maize samples due to drying. This may be due to use of lower temperature during the process.

Reduced levels of EE and phytate-P in particular, in the Downs maize are in reverse to the increase in the DM content of grains with increase in drying temperature. These findings agree with those of Weller *et al*. (1988) who reported a reduction in protein configuration and contents as maize drying temperature increased, especially at high harvest moisture contents (>28%). In another study, however, Iji *et al*. (2004) reported an increase in CP content due to artificial heating. These differences in CP content may be due to differences in variety and moisture content at harvest. Pontoppidan *et al*. (2007) reported that the effects of thermal treatment of various cereals and oilseed meals on phytate concentrations and the composition of the phytate (lower esters etc). They found that though phytate (IP6) is somewhat thermostable, it is partially destroyed by heat. For example, they found that the total insoluble phytate-P concentration in raw wheat was 2.56 g kg^{-1} DM and after pelleting (90°C) reduced to 2.38 g kg^{-1} DM, a reduction of approximately 7%. Further, extrusion at 130-EC resulted in a reduction in total insoluble phytate-P concentrations of 15%. However, oilseeds do not respond in this way, perhaps because they have already been heated. Nevertheless, there is evidence in the literature that heating at least for wheat, does partially destroys IP6 and resulted in an increased concentration of lower esters. As plant phytases are destroyed fairly at 90°C, it is likely that phytate is partially heat labile and these reductions are not enzymatic. The vulnerability may be due to the location of phytate in the plant material i.e., in the aleurone with wheat or in germ as in maize. Perhaps it has something to do also with the nature of the phytin salt and/or association with other compounds such as starch or protein. In this study it is possible that the reductions are >real, especially as the heating was done over 24 h rather than the few minutes in the Pontoppidan study. However, it may also be that the reduction in phytate concentrations is due to extraction problems or possibly the result of endogenous phytase activity (plant or fungal contamination of the grain).

In this study, oven drying also resulted in an increase in the amino acid contents of the maize grains and these findings agree with those of Iji *et al*. (2004). The benefit for high temperature drying of maize is severe stress cracking resulting in increased accessibility of the protein matrix

to enzymes (Eckhoff and Tso, 1991). It may also result in change in the protein configuration and an increase in the concentrations of most essential amino acids. This may have some implication for feed formulation as grains tend to be classified with the same nutrient compositions regardless of their moisture content at time of use.

Starch composition: Discernible variations were found in starch contents due to heating for different durations as well as for different sources of maize, in this study. A higher drying temperature over an extended duration, increased the starch, resistant starch and amylose content of samples. Although, resistant starch is formed during seed development, its proportion may increase during feed processing, especially drying (Brown, 1996) and storage. While most maize is sun-dried *in situ*, wet weather may necessitate the use of artificial drying techniques. Artificial drying results in the annealing of starch, while long periods of sun-drying have been known to cause stack-burn, a defect used to downgrade the quality of the crop.

In this study, amylose content increased while amylopectin content decreased with an increase in the heating period. These changes in structure associated with heating also alter the subsequent digestibility of the starch. It was noted that resistant starch content increased with an increase in the heating duration and this observation supports those of Berry (1986), Russell *et al.* (1989) and Iji *et al.* (2004) who observed an increased RS content due to heat treatment of maize grain at 100°C under low moisture condition. It has been reported that RS content of total starch increased from 200.0-650.0 g kg^{-1} and physicochemical properties changed due to heat treatment (Itoh *et al.*, 1997). The decline in the content of amylopectin suggests deterioration in grain quality since amylopectin is more quickly digested than amylose and affects the digestion of nutrients other than carbohydrates.

NSP concentrations: In the current study, both soluble and insoluble NSP contents decreased in Moree and Emerald, respectively. However, the soluble NSP content only increased in Downs as a result of heat treatment of maize grain. Soluble NSP is responsible for the reduction in value of diets based on temperate cereals but these are generally low in maize and other tropical cereals (Choct and Annison, 1992). This may be responsible for the low viscosity of the *in vitro* maize sample in this research. The above difference in results could be due to the difference in the sources or duration of the oven drying.

Nuclear magnetic resonance (^{1}H-NMR): In the present study, it is clear that the nuclear magnetic resonance shifts were dependent on artificial drying of low moisture maize grains over varying time periods. The changes in structure of the samples through heat treatment were further confirmed by ^{1}H-NMR analysis. Assignment of the major peaks was made by reference to data from the literature (Annison *et al.*, 1992; Cheetham *et al.*, 1993). The poorly resolved resonance of both NMR spectra makes interpretation difficult. Each ^{1}H-NMR peak increased sharply with an increase in the heating period of the grains except for the Downs source. This is probably due to the higher content of glucose and maltose in artificially dried maize.

Mineral contents: In the current study, it is obvious that the mineral contents of maize varied from one source to other and were changed through artificial drying of low moisture maize grains over varying time periods. For example, Ca, Cu and Mn contents were the most affected in the Moree samples while Ca and Zn were most altered in Emerald. The Fe and Zn contents of the Downs samples were most altered. Sodium content was greatly altered in all 3 samples.

***In vitro* digestibility:** In the present study, the *in vitro* digestion of DM, starch and protein increased considerably with an increase in the duration of heating at 105°C. This may be due to the decreased amount of RS, as well as positive changes in the protein configuration of samples. These findings are supported by those of Nir *et al.* (1994) who reported a 4.5 fold increase in *in vitro* digestion of maize starch by amylase after heat treatment. The decrease in the content of resistant starch suggests an increase in grain quality, since resistant starch is unresponsive to animal enzymes (Noy and Sklan, 1995).

CONCLUSION

It can be concluded that the chemical composition, ultra-structural characteristics and energy values of maize samples varied from one source to the other and were changed through heating of low-moisture maize grains over varying time periods. In particular, there was increase in the concentration of protein, amino acids, total starch, resistant starch and total NSP due to heating. An improvement in *in vitro* nutrient digestibility was also observed in artificially dried maize in contrast to sun-dried only maize. Conversely there was a reduction in the concentrations of phytate-P, digestible starch, amylopectin and soluble NSP.

There was a change in ultra-structure of grain such as shape of starch granules and protein matrix of samples due to heat treatments. These changes could impact on the nutritive value of the grain and animal performance if diet formulations are only based on generalized compositional values. For these reasons, it is recommended that diet formulation for broiler chickens takes into consideration the source of maize grain and moisture content at use. Such changes could occur in the field and may be necessary to consider when using maize grain. There is more scope to do further study about the quality and nutritional value of maize grain when dried artificially due to unpleasant climate condition. The impacts of these changes on animal response are further investigated in this series of study.

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