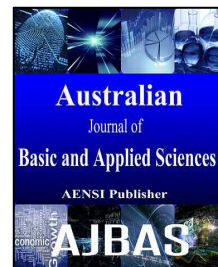




AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com



Physicochemical Properties and Morphological Characteristics of Composite Flour Added with Cornlettes (*Zea Mays*) for Functional Food Ingredient

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ARTICLE INFO

Article history:

Received 3 March 2016

Accepted 2 May 2016

published 26 May 2016

Keywords:

Cornlettes, swelling power, thermal profile, amylose content, morphological characterization

ABSTRACT

Background: Cornlettes (young corn) is rarely used in maximizing its usage as well as to accommodate the demands in functional food development. **Objective:** This study aimed to investigate physicochemical properties and morphological characteristics of composite flour containing cornlettes powder (CP) which partially replacing wheat flour. Composite flour was prepared by using CP mixed with wheat flour (WF) with the proportion of 10:90, 20:80, 30:70 and 100:0 (CP: WF). **Results:** Amylose contents significantly increased when the percentage of cornlettes powder in composite flours is increased. The highest amylose content was found in 100% CP sample (4.40g/20g) while the lowest amylose content was found in control sample (0.072g/20g). The percentage of swelling power in composite flour added with CP was significantly higher than control and there is significant difference in the setback viscosity of the composite flour starch samples. Composite flour shows uniformed wheat starch granules which clustered with irregular shape of cornlettes starch granule. **Conclusion:** Addition of CP to partially replace wheat flour to form composite flour has resulted in improvement of swelling power and amylose composition while slightly altering the starches morphological structures. Composite flour with higher proportions of CP (30:70) can be used to partially replace wheat flour in order to meet the demands of food production.

INTRODUCTION

Presently, plant-based food products become major food source since ages, thus the production of it is crucial. Food manufactures need to come out with another options of underutilized plant species in order to accommodate the demands. Underutilized crops are often indigenous ancient crop species which are still used at some level within the local, national or even international communities, but have the potential to contribute further to the mixture of food sources than usual (Mayes *et al.*, 2011). The term underutilized refers to categories of wild and cultivated plants species whose potential has not been fully realized (Padulosi and Hoeschle-Zeledon, 2004). Underutilized crops usually are domestic plant species that have been used before as foods, but have been neglected over the times.

Cornlettes (*Zea Mays*) or young corn is a type of underutilized crop which is harvested in the early stage. During harvesting, cornlettes are small, immature, silk either do not appear or just appear and fertilisation not occurred (Hooda and Kawatra, 2013). There are different varieties of cornlettes in the market, results from natural hybrids and mutation which cause a very large numbers of cultivars (Adejumo *et al.*, 2013). Properties

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To Cite This Article: Wan Rosli W.I. and Nurul Aliim Z. A., Physicochemical Properties and Morphological Characteristics of Composite Flour Added with Cornlettes (*Zea Mays*) for Functional Food Ingredient. *Aust. J. Basic & Appl. Sci.*, 10(11): 300-306, 2016

of each variety differentiate type of cornlettes, especially physical appearance, texture, and structure – function of the starch. Cornlettes can be eaten either raw or used as ingredients in food preparation as pickled, soup and others. As cornlettes taste delicious and very nutritious, people tend to add them in their prepared foods. Moreover, cornlettes is popular at both local and international levels even though their nutritive values have not been known clearly. The nutritive values of cornlettes are comparable to several high-priced vegetables like cauliflowers, cabbage; french beans, spinach, lady finger, brinjal, radish and potato (Hooda and Kawatra, 2013).

Cornlettes contains high concentration of total dietary fibre (TDF) where dried young cornlettes has been recorded to contain 30.4g/100g (Wan Rosli and Che Anis, 2012). Corn or maize contains high amount of starch, by far as the largest starch source dominantly found in wheat, tapioca, potato and rice. Plants store glucose as the polysaccharide starch. Starch consists of two polysaccharide molecules, amylose and amylopectin (Brown, 2011) where natural starches are mixture of amylose (10-20%) and amylopectin (80-90%). The ratio of amylose and amylopectin affects the physicochemical properties of starch (Sasaki *et al.*, 2000).

Starch will undergo a transition process when heated in excess water where the semi-crystalline structure in granules transforms into amorphous form known as gelatinization. Besides that, structural properties of starch especially amylose and amylopectin content is affected by both processes. Besides, the branch chain length of amylopectin is related to affect starch crystallization, gelatinization and pasting properties (Uarrota *et al.*, 2013). Starch gelatinization and pasting properties also can be affected by the particles size, damaged starch granules as well as molecular structure. Furthermore, gelatinized starch tends to re-associate in an ordered crystalline structure during storage, which is termed as retrogradation (Zhou *et al.*, 2010).

This study aimed to investigate physicochemical properties and morphological characteristics of composite flour containing cornlettes powder (CP) which partially replaced wheat flour as an alternative health food ingredient.

MATERIALS AND METHODS

Plant material:

Cornlettes were purchased from Siti Khadijah's wet market, Kota Bharu district of Kelantan, Malaysia. The cornlettes was then placed in refrigerator with the husk intact to conserve ear moisture and preserve their quality. All-purpose wheat flour was purchased from local supermarket in Kota Bharu district, Kelantan, Malaysia.

Sample preparation:

The cornlettes ears were detached and separate from it silk, tassel and husk. The cornlettes were then washed using distilled water. Then, the sliced cornlettes were air dried for one day at room temperature and two days in an oven (Memmert GmbH & Co. KG, Germany) at 55°C until it turns brownish in colour. Dried young corn was ground using electric grinder (National MX – 895M) to obtain fine cornlettes powder. Cornlettes powder (CP) was sifted through 125 microns mesh – sieve (Endecotts Ltd. England) to acquire uniformed fine powder. Finally, cornlettes powder was kept in screw cap bottle (Scoot Duran) and stored in refrigerator at 4°C. Wheat flour (WF) was substituted with CP with the ratios of 0:100 (control), 10:90, 20:80, and 30:70 (CP:WF w/w).

Determination of composite flour thermal profiles:

The thermal properties of the samples were analyzed using Perkin-Elmer DSC-7 (Norwalk, CT, USA) equipped with an intracooler I and Thermal Analysis Controller TAC 7/DX. An empty pan is used as the reference and calorimeter was calibrated with indium (melting point = 156.6°C, $\Delta H = 28.5\text{J/g}$). The operation of DSC analyzer was run under nitrogen gas atmosphere (30 mL/min). Starch samples thermal transitions define as, (1) onset temperature (T_o), (2) peak of gelatinization temperature (T_p), (3) conclusion temperature (T_c) and (4) enthalpy gelatinization (ΔH_{gel}) where enthalpies were calculated on a starch dry weight basis. Three mg of CF starch (dry basis) was weighed in an Al pan. Distilled water was added into the starch subsequently using 1:2 ratio (starch: water, w/w). The weight of the sample was controlled continuously until the desired moisture content was attained. The pan was sealed hermitically and was allowed it to stabilize at 4°C for overnight to reach equilibrium before heating in the calorimeter. The amount of water was adjusted accordingly in order to achieve starch – water suspension. Then the sample pan was heated at a rate of 10°C/min from 20–95°C. Thermal transitions and gelatinization temperature range was recorded.

Determination of composite flour swelling power and amylose content:

Swelling power is a measurement of the hydration capacity of starch and is expressed as the weight of centrifuged swollen granules. The swelling power of samples was determined based on a modified method of Fu (2008). First, 30g of samples was mixed with 1.5ml distilled water in pre - weighed 2 mL polyethylene micro centrifuge tubes with lids. It was then mixed again at room temperature for 30 minutes on a rotating mixer at 30 rpm. Then it was centrifuged at 3000 rpm for 10 minutes, flipped and mixed again on the rotating mixer for several minutes until completely mix. It was then placed in a constant temperature (95°C) thermomixer at 1400

rpm. After 30 minutes, the tubes were placed in a water bath (20°C) for 10 min and then centrifuged at 10000 rpm for 10 minutes. The supernatant was removed using an aspirator and centrifuged at 10000 rpm for 10 seconds to remove the residual water attach to the inner wall of the tubes. The residual supernatant was removed by an aspirator and the tube with the sediment gel was weighed. The flour swelling power was calculated as the weight of the sediment gel divided by the original dry weight of the flour sample.

Determination of amylose contents of starches was analyzed using the method of Adejumo *et al.* (2013). Twenty grams of sample was mixed with 10 ml of 0.5N KOH. Sample was transferred into 100 ml of volumetric flask and was diluted with distilled water until marked area. 10 ml of solution aliquot pipette was used to transfer solutions into 50 ml volumetric flask and 5 ml of 0.1N HCl and 0.5 iodine reagent was added. It was diluted until 50 ml and spectrometer with the absorbance at 625 nm was used. Amylose content of samples was then determined and recorded.

Morphological characterization:

Scanning Electron Microscope (SEM) was used to characterize the morphology of starch granules after extraction processes. Starch of composite flour was mounted on circular Al stubs with double-sided sticky tape. Samples were evenly distributed on the surface of the tape. Samples with 12 nm gold, was examined and photograph at an accelerating voltage of 5 KV with a magnification of x100, x300 and x500 was viewed under SEM (Quanta FEG 450, FEI Electron Microscopy).

Data analysis:

Data obtain was analyzed using the ANOVA procedure in IBM SPSS 22.0 (USA) software. Results are expressed as mean \pm standard deviation. All measurements were carried out in triplicate (n=3). Significant level establishes at $P \leq 0.05$.

RESULTS AND DISCUSSION

Thermal profiles of composite flour:

Table 1 shows the results of DSC analysis where the transition temperatures (T_o , T_p , and T_c), range (T_c - T_o), enthalpies of gelatinization (ΔH_{gel}) and peak height indices (PHI) of starches differed significantly ($P < 0.05$). The substitution of cornlettes added in the composite flour decreased the T_o and T_p and increased the ΔH_{gel} . The gelatinization of starches was initiated at 84 to 109°C and most of the peak temperature fell in the range of 108 to 134°C. The results show that starch from sample 10:90 had the highest gelatinization temperature which indicated that it had a higher level of crystallinity; while starch from sample 100:0 had the lowest gelatinization temperature which could be due to the lowest degree of crystallinity of its granules (Wang *et al.*, 2011). Starch from sample 100:0 shows the highest heat of gelatinization with the ΔH_{gel} value (1462.80 J/g), followed by 70:30 (S3) starch (1121.72 J/g) while it was lower for wheat starch 100:0 (153.95 J/g). The 10:90 starch samples appeared to have the lowest ΔH_{gel} value (483.99 J/g) among composite flour. The increasing of ΔH_{gel} value indicates the increasing amount of amylose content of the starches (Iouchi, 1984).

The presence of amylose lowers the melting temperature of crystalline regions and energy for starting gelatinization (Sasaki *et al.*, 2000). To initiate melting in the absence of amylose rich amorphous regions, more energy is needed (Krueger *et al.*, 1987). This correlation is proportional to the amount of amylose content in starch, as higher amylose content has more amorphous region and less crystalline, lowering gelatinization temperature and endothermic enthalpy. Wide temperature range implied a large amount of crystals with varied stability (Sasaki *et al.*, 2000). Furthermore, starch granule crystallites which required less energy to melt would melt first (Obanni and Bemiller, 1997). Cornlettes powder with higher amylose content would start to melt first and wheat flour melted successively in mixed samples, which results in lowering gelatinization onset and peak temperature of composite flour.

Swelling power and amylose content of composite flour:

The amylose content of different levels of cornlettes powder incorporated with wheat flour was increased proportionally when the concentration of cornlettes powder increased (Table 2). The result shows that amylose content of all samples was ranged between 3.40 to 4.40% compared to control (0.01%). In addition, CP (100:0) had the highest amylose content (4.40%) which significantly higher ($p < 0.05$) than control (0.01%). Starch polymers, amylose and amylopectin molecules have been widely used in both food and pharmaceutical industries many years back.

Higher amylose content in composite flour added with cornlettes might be explained by richer amylose content (4.40%) in cornlettes compared to wheat flour (0.01%). Amylose contents in plant-based food sources are different based on the sources. Plant based products contains high amylose such as corn which contains 70%, regular corn contains 28%, wheat and sago contains 26%, arrowroot contains 21%, potatoes contains 20%, sweet potatoes contains 18% and cassava contains about 17% of amylose (Obanni and Bemiller, 1997). Furthermore,

plant-based products are likely to have most amylose content and other types of resistant starch that helps in limiting spikes in blood sugar levels (Corn Refiners Association 2006). Starches with a high percentage of amylose are difficult to gelatinize because of the extra energy needed to hydrate and disintegrate the firmly-bonded, crystalline aggregates of amylose (Bird *et al.*, 2007). In addition, higher amylose content will slow down starch digestion and thus resulted in delaying the glucose release in small intestine. Therefore, foods which contain high percentage of amylose and amylopectin had low glycemic index (GI) value.

Table 1: Thermal properties of composite flour added with cornlettes.

Sample	Conclusion temperature (°C, T _c)	Peak temperature (°C, T _p)	Onset temperature (°C, T _o)	Enthalpy (ΔH) J/g
0:100 (Control)	135.60±0.72 ^a	117.19±1.00 ^a	108.58±0.44 ^a	153.95±8.19 ^d
10:90	141.83±21.37 ^a	128.28±14.63 ^a	107.42±34.24 ^a	483.99±342.91 ^{cd}
20:80	132.53±2.90 ^a	117.64±1.05 ^a	84.64±1.11 ^a	853.95±87.00 ^{bc}
30:70	142.80±21.14 ^a	134.33±21.06 ^a	98.70±23.14 ^a	1121.72±156.96 ^{ab}
100:0	128.69±3.67 ^a	108.70±19.49 ^a	106.19±20.93 ^a	1462.80±74.22 ^a

Mean ± SD values followed by the same letter in each column are not significantly different (P < 0.05)

Amylose contents of starches influence the degree of gelatinization. High amylose content is slowing the process of gelatinization and at the same time slowing the process of starch breaking down into glucose thus reducing GI levels. Starchy foods with high amylose levels are associated with lower blood glucose levels and slower emptying of the human gastrointestinal tract as it significantly affects starch digestion in the gastrointestinal tract (Denardin *et al.*, 2012). Hence, composite flour with ratio of 30:70 (WF:CP) and 0:100 (WF:CF) that contains high amylose are suitable for the production of foods products aiming for people whose suffers with diabetic complications.

In addition, the swelling power of composite flour added with cornlettes varies from 4.76 to 13.21%; where the percentage of swelling power in composite flour added with cornlettes were significantly higher than control (P<0.05). Swelling power of composite flour starch was increased when 10% of cornlettes was added in wheat flour (4.76%), while when 20% of cornlettes is added the swelling power is decreased to 3.45%; and in composite flour with 30% cornlettes powder shows an increased in swelling power (4.15%). Composite flour added with cornlettes has higher starch swelling power than wheat flour. In addition, swelling power of cornlettes powder was significantly higher (13.21%) than wheat flour (3.06%).

Swelling power indicates the water holding capacity of starch (Kaur *et al.*, 2011). Swelling power of starch has been found to depend on the water binding capacity of starch molecule by hydrogen bonding (Uarrotta *et al.*, 2013). Starch swelling behaviours is the property of their amylopectin content, where amylose acts as diluent and inhibitor of swelling especially in the presence of lipids which can form insoluble complexes with amylose during swelling (Ashogbon and Akintayo, 2012). Swelling power can be influenced by the temperature, where swelling power is increased when temperature is increased. During the analysis, starch is heated in 60°C water bath for at least 30 minutes before measured. Amylose plays a part in limiting initial swelling because swelling proceeds more rapidly after amylose has been moved.

High swelling power results shown by cornlettes powder might be an indicative of weak bonding forces within the granules which make it less compact when compared to other levels of composite flour and wheat flour granules. In addition, amylose content and starch granular properties differences may also have affected the swelling power (Singh *et al.*, 2003). The concentration of starch swelling fraction is reduced in the present of high amylose content thus decrease the gelatinization viscosity (Ikegwu, 2009). Since the cornlettes powder swelled much more than wheat flour and composite flour, swelling may be caused by the occurrence of both amylose and amylopectin in the cornlettes starch. Amylose is a good gelation agent while amylopectin functions as prominent thickening agent. Gelation and thickening properties exhibited by both amylose and amylopectin may explain the reason why cornlettes starch shows the most potent swelling power as compared to wheat starch.

Table 2: Amylose content and swelling power of composite flour added with cornlettes.

	Composite flour Content (g/100g WF:CP)				
	0:100 (Control)	10:90	20:80	30:70	100:0
Amylose (%)	0.01±0.0 ^d	3.40±0.05 ^c	3.37±0.01 ^c	4.01±0.02 ^b	4.40±0.62 ^a
Swelling power (%)	3.06±1.18 ^b	4.76±0.35 ^b	3.45±0.88 ^b	4.13±1.53 ^b	13.21±1.35 ^a

^{a-b} Mean values having different superscript within column are significantly different (P < 0.05).

WF: Wheat flour, CP: cornlettes powder

Morphological characterization:

Starch granules of 100:0 (Control), 90:10, 80:20, 70:30 and 0:100 composite flours are shown in Figure 1. Cornlettes starch granules seemed to be broken and present in clustered shapes. This may be due to the denaturation or destruction of cornlettes granules during extraction of CP (Figure 1D). The distortion of granule

integrity by shearing forces was the main reason of continuous leakage of the soluble starch material during its extraction (Blaszczaka *et al.*, 2003). Cornlettes granules are strongly hydrophilic and it takes in more water during extraction process. Starch granules exhibit hydrophilic properties and strong inter-molecular association via hydrogen bonding formed by the hydroxyl groups on the granule surface (Lu *et al.*, 2009). The hydrophilic characteristics of starch granules cause an internal interaction and morphology of starch which will be readily changed by water molecules. Extraction process of composite flour only destructed cornlettes starch granules in the composite flour without changing the shape of wheat starch granules. Cornlettes starch granules dispersed after taken in water during extraction process and forms aggregated granules.

Wheat starch granules are bigger than cornlettes starch granules, and the number of cornlettes granules is increased when the levels on cornlettes in composite flour is increased. It can be seen that, the clusters of cornlettes granules is getting bigger as the percentage of cornlettes in composite flour increased. Furthermore, wheat flour starch granules also interacted to binds with cornlettes starch granules. As cornlettes contains high amylose content than wheat, it causes an interaction between both types of granules and binds to each other. Figure 1A shows wheat starch granules after extraction process using alkaline method. There is a present of wheat starch granules with similar shape and size. Meanwhile, in Figure 1B, 1C and 1D, there were wheat starch granules and cornlettes starch granules with different shapes and sizes. Wheat starch had bigger granular size and longer in diameter than cornlettes starch granules. The interaction between wheat and cornlettes starch granules can be seen in Figure 1B, 1C and 1D).

There are two dominant types of starch granules present which were wheat flour granules and cornlettes starch granules. Wheat flour starch granules are typically small and spherical in shape (Mandala and Bayas, 2004). Meanwhile, the shape of maize starch granules are ranging from small to large and oval to polyhedral and angular (Singh *et al.*, 2003). Besides that, cornlettes showed the presence of smallest size granules with mean diameter of 6.33 μm and also with small oval shape granules (Sandhu and Singh, 2007). However, other corn types showed different shapes and length of starch granules. As an example, popcorn medium grain fraction starch had the largest granules with mean diameter of 13.64 μm and Dent corn bold grain starch had a higher mean diameter. The difference in granular morphology may be attributed to the biological origin, biochemistry of the amyloplast and physiology of the plant (Sandhu and Singh, 2007).

Conclusion:

Swelling power of composite flour added with cornlettes is increased when percentage of CP increased. The increase level of CP was significantly increased the peak viscosity, hot paste viscosity, break down viscosity and cold paste viscosity of composite flour. The thermal properties of 30:70 composite flour added with cornlettes shows higher values as compared to wheat flour. Wheat starch granules show uniformed in shape and size, while the structure of cornlettes starch granule was in clustered form. Starch granules is believed to be broken during the starch extraction process thus resulted in formation of cluster. However, both wheat and cornlettes starch still attached to each other in composite flour samples. In brief, cornlettes can be used to partially replaced wheat flour in food products as it contains high amylose content and their pasting properties and temperature allowing it to be used in designing low GI food products. Furthermore, it also can maximize the usage of underutilized vegetables in the future.

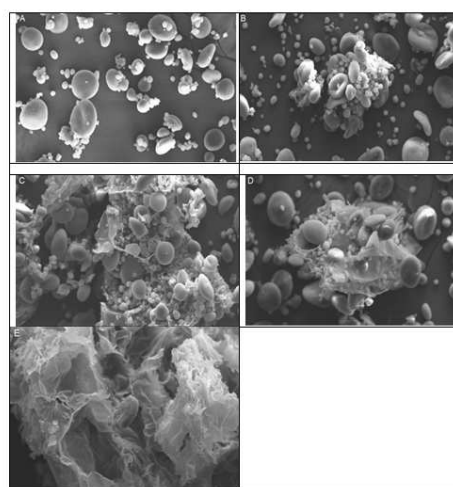


Fig. 1: Scanning electron micrographs of composite flour added with cornlettes (Wheat Flour: Cornlettes Flour where A=100:0, B=90:10, C=80:20, D=70:30 and E=0:100) viewed under 300 X magnification.

ACKNOWLEDGEMENT

The author would like to thank School of Health Sciences, (USM) and FRGS Research Grant (203.PPSK.6171190, Ministry of Higher Education of Malaysia) for the research undertaking. A special thanks to Nordiana Abu Bakar, Roziyani Hashim and Azita Nasir for the kind help in this research.

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