PJN

ISSN 1680-5194

Pakistan Journal of Nutrition

ANSInet

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

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Pakistan Journal of Nutrition 15 (4): 312-318, 2016
ISSN 1680-5194

Effect of Different Modification Methods on Gelatinization Properties and Amylose Content of Kithul (*Caryota urens*) Flour

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Abstract: Detailed studies have been carried out on Kithul (Caryota urens) native and modified flour as two pre-gelatinized treatments (PGI and PGII), acid modified (AC) and dextrinized modification (DX) flour treatments to compare gelatinization properties and amylose content. Among all treatments, PG-II (modified with excess water at 75°C heat treatment) showed significant changes with higher gelatinization temperatures (T_O = 74.02°C and T_P = 80.51°C) and lower Conclusion temperature (T_C = 90.04°C), gelatinization enthalpy (5.78 J/g) and amylose content (25.36). This study provides evidence that PGII is the most suitable modification treatment for native Kithul flour for introducing new flour source with an applicable form to meet specific needs with a view to effort expand application within the food industry.

Key words: Kithul, (*Caryota urens*), modified flour, gelatinization properties, amylose content, hydrothermal treatment

INTRODUCTION

Starch is an essential ingredient not only for food industry but also in the pharmaceutical, textile (Shiihii *et al*., 2011) Cosmetics, plastics, adhesives and paper industries (Kolawole *et al*., 2013). Being very versatile raw material in lots of fields, it is a very demanding polysaccharide which has growing demand day by day (Kayode *et al*., 2005). Most commercial manufacturers overlook the Physico-chemical properties of comparative starches for their specific applications. However, there is some limitation to use starch with specific properties to obtain the designed product such as dissolution in water (Ertugay and Kotancialar, 1998). The starch modification is the solution to overcome these limitations, altering the starch structure, which facilitates to meet the requirements of the manufacturer, by realizing the designed products.

By dominating hydrogen bond of above components in a controllable manner such as physical alteration, chemical degradation, enzymatic modification or genetic transformation (Leach *et al*., 1959) or a combination of them (Singh *et al*., 2006) starch modification is generally achieved. Pre-gelatinization (PG), Heat-Moisture treatment (HMT) and Annealing (ANN) as form of physical modifications involves alteration within the starch granules (Ashogbon and Akintayo, 2014) which have more attention of industry since it is chemical free and fairly convenient than other methods (Majzoobi *et al*., 2011) since water and heat only involve (Gunaratne, 2006); with time for physical treatments; it has received more attention in food industry.

Acid modification of starch realized by treatment of starch below its gelatinization temperature in aqueous acid suspension (Wurzburg, 1986). Enzymatic modifications comprise the exposure of starch suspensions to a number of enzymes primarily, including hydrolyzing enzymes that tend to produce highly functional derivatives (Neelam *et al*., 2012). Dextrination means to partial de-polymerization which is achieved through hydrolysis and recombination in a branched manner (Manu *et al*., 2013).

Pre-gelatinization refers the complete gelatinization process by destructing the granular structure which leads to complete fragmentation and to the absence of optical birefringence (Ashogbon and Akintayo, 2014). This treatment affects on significant changes of both physicochemical and functional properties of starch (Lai, 2001).

Other two hydrothermal treatments (HMT, ANN) occur physical modification without destroying the starch granular structure (Stute, 1992). The similarities of these two modifications are both conducted below the gelatinization temperature (Tp) or starch melting point (Lim *et al*., 2001; Waduge *et al*., 2006), but above the glass transition (Tg) temperature of the starch (Ashogbon and Akintayo, 2014). The difference is the hydrothermal process of ANN is used with the presence of excess moisture (above 40% w/w) while HMT refers restricted moisture content (<33%w/w) during the heat treatment.

Amylose and amylopectin are the main distinct polymers (Hoover *et al*., 1991) which are responsible for the starch properties (Charles *et al*., 2005) by varying the ratio between amylose and amylopectin based on botanical source and different starches exhibit various properties. Amylose has high potential to retrograde and form tough

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gels and strong films while amylopectin produces soft gels and weak films (Perez and Bertoft, 2010).

Gelatinization is a very important behaviour of both amylose and amylopectin (Charles *et al*., 2005). This is the process which causes to make considerable changes in both physical and chemical nature of the starch granules (Majzoobi *et al*., 2011). While the starch is given the heat presence of enough water crystalline organization of the granules cause to decompose to form amorphous region (Beninca *et al*., 2008). This irreversible (Majzoobi *et al*., 2011) molecule disordering could observe as endothermic phenomenon using Differential scanning colorimetry (DSC) (Beninca *et al*., 2008). Retrogradation, the association of gelatinized starch granules in ordered manner (Charles *et al*., 2005) which effect on quality, acceptabilities and nutritional properties of the starch-based foods (Biliaderis, 1991). During the gelatinization which causes the significant changes in the starch granule by rearranging of intra and intra molecular hydrogen bonding between moisture and starch molecule (Majzoobi *et al*., 2011). This irreversible swelling (Hug-Iten *et al*., 2001) loss of the crystalline order and leaching of both amylose and amylopectin to solubility (Sakonidou *et al*., 2003). These series of incidents are very critical in the product development not only in food industries but also another usage where the starch applicable (Neelam *et al*., 2012). To identify the gelatinization properties differential scanning Colourimetry (DSC) can be used as an energy absorbing process (Freitas *et al*., 2004). The most important temperature, from the DSC measurements, are onset (T_O) which refer the initial stage. Peak temperature (TP) which begin the gelatinization or crystal melting and final temperature called conclusion temperature (T_C) (Coral *et al*., 2009). ΔH basically refers the loss of double helical order and it's a measurement about overall crystallinity of amylopectin (Morris, 1990; Cooke and Gidley, 1992). The area between the baseline of the thermograph represents the gelatinization enthalpy (ΔH) (Coral *et al*., 2009).

However, existing few prominent starch sources could not fulfill high demand for industrial purposes (Shiihii *et al*., 2011). Therefore, it is needed to be discovered newer, commercially viable starch sources. The aim of the present study is to determine the gelatinization properties and deviation of amylose content of modified Kithul (*Caryota urens*) flour with four different modification methods which are represented both by physical and chemical modification vs native flour, to find out the suitability as a new modified flour source for industrial use.

MATERIALS AND METHODS

Flour sample collection and modification: Freshly prepared Kithul flour (RW) samples were used for the modifications given in sections 2.2.1-2.2.3 and all samples were sifted through a 355 µm sieve before further analysis.

Pre-gelatinization modifications of kithul flour: Pre-gelatinized Modification-I (PG-I) was done with a slight modification of the method described by Knight (1969). A 1:1 flour solution (100 g flour for 100 ml deionized water) was incubated at 70°C (PG-I) and 75°C (PG-II) for 5 min. Gelatinized flour was dried in a hot air dryer at 40°C till moisture level dropped to 10 to 15% (Knight, 1969). Raw Kithul flour was used as the control (Knight, 1969).

Acidic modification (AC): 50 ml of 0.1 M HCl solution was added to a mixture of 100 g of flour and 50 ml of deionized water and mixed for 30 min. Then pH was adjusted to 7 with 1M NaOH. Neutralized flour was dried at room temperature (30°C) following the washing and filtering through watchman (No:1) filter paper (Caglarirmak and Cakmakli, 1993).

Dextrinized modification (DX): 75 g of flour mixed thoroughly with 60 ml of 0.1M HCl. Then the mixture was dried at 50°C for 24 h (Until moisture level dropped to 5%). The dried flour was again dissolved in 75 ml deionized water and pH was adjusted to 7 by using 1M NaOH. Then flour was dried in the hot air dryer at 40°C till moisture level dropped to 10 to 15% (Method of Caglarirmak and Cakmakli (1993) with slight modifications).

Analysis of the flour properties

Determination of amylose content: Amylose content of Kithul flour was determined in three steps using simple iodine colorimetric method as described by Juliano (1971) with slight modifications (Juliano, 1971).

Differential scanning calorimetry (DSC): Thermograms for Kithul flour from raw and modified were taken by DSC (Model DSC TA instrument Q 200, USA). Flour was weighed onto the aluminium DSC pan and distilled water was added with a micro syringe for 50% (w/w) mixture. Pan was sealed and allowed to stand for 1hr at room temperature. The scanning temperature range and heating rates were 30-140 and 5°C/min, respectively, using the empty pan as the reference. The onset temperature (T_O), peak temperature (T_P), conclusion temperature (T_C) and gelatinization enthalpy (ΔH) were recorded.

Statistical analysis: Results were analyzed using one-way analysis of Variance (ANOVA) at 0.05 probability level with MINITAB software package (version 17 for Windows).

RESULTS AND DISCUSSION

The effect of the different modification methods on the amylose content and gelatinization properties of Kithul flour (Table 1) revealed that each type of modification influence on the flour in a contradictory manner, Amylose content of the native Kithul flour (RW = 28.34) has increased after Acid modification (AC = 29.46) and dextrination (DX = 29.44) was observed. Similar observation has been reported by Wurzburg (1986) as during the acid modification amylose contain increases due to acid preferable hydrolyses of amylopectin (Wurzburg, 1986) and this theory being applicable for used acid based dextrination process, due to acid hydrolysis as the main step of the modification (Kulp and Ponte, 2002; Effah-Manu *et al*., 2013).

Pre-gelatinized starch samples were showed different observations. PG-I which was prepared below the onset temperature (T_O) of the Kithul flour (68°C) indicated little bit with higher Amylose contents (28.88) than native flour while PG-II which applied above the T_O, but below the peak temperature (T_P = 78°C) showed significantly low Amylose content (25.36) among all treatments. This observation is aligned with the result of Hoover and Vasanthan (1994) and Lorenz and Kulp (1982) as decrements of apparent Amylose content in heat moisture treated starches (Wheat and Potato) due to additional interaction among lipid and Amylose chain. Some observation has reported cereal starches by occurring Amylose-lipid complex formation during the HMT (Lim *et al*., 2001; Hoover and Vasanthan, 1994; Hoover and Manuel, 1996; Hoover *et al*., 1993). The Same scientist found that Amylose content and Starch chain length are very significant to determine the physical properties of the starchy product.

However an error of the relevant used method (blue value method) to determine the Amylose content was reported by Kasemsuwan *et al*. (1995). According to his finding, long branch chain of amylopectin also can bind with Iodine to form a helical complex which leads to overestimating the Amylose content (Kasemsuwan *et al*., 1995). In this study, it was assumed that same condition has applied to all treatments to avoid this overestimation. Because of most available and common method among the researchers for determining the Amylose content, the blue value method, was used for this study.

Gelatinization temperatures ranged from 68.50 to 74.02°C for onset temperature (T_O); from 78.64 to 80.51°C for peak temperature (T_P) and from 90.04 to 92.79°C for conclusion temperature (T_C). Acid-thinned flour treatment was not presented any significant difference with the native flour sample for T_O, T_P and T_C and similar results was reported by Karaoglu *et al*. (2006) for acid thinned wheat and Corn starch for T_P. However according to that researchers T_O and T_C and ΔH were lower than native flour. Although gelatinization process depends on some factors such as the botanical source of starch as well as the process applied before gelatinization amylose/amylopectin content of starch (Mistry *et al*., 1993).

T_O (70.30°C) of dextrinized treatment was higher than native four sample while T_P (78.85°C) was similar and T_C was (90.65°C) lower than native flour. There was no reported data for comparison with this situation.

Pre-gelatinized flour treatment showed interesting results as PG-I low T_O, T_P and T_C but high ΔH than PG-II. Because of the treated temperature of PG-II, it was showed a significant change in the starch granules which has highest T_O, T_P and T_C and lowest ΔH among all treatments. This result is compared with a number of researchers which had based on hydrothermal treatments. Starch chain interaction within the amorphous region during the hydrothermal treatments may influence on destabilization on the crystalline region which leads to increase the gelatinization temperature (T_P). Generally higher gelatinization temperature means that most energy necessary to initiate the gelatinization process (Liu and Thompson, 1998; McPherson and Jane, 1999).

By considering both results of gelatinization properties and Amylose content among all treatments, a significant modification was showed by hydrothermal treatment named PG-II. Actually it is a combination of two physical modification methods. The flour was applied heat below the gelatinization temperature (T_P) but above the onset temperature (T_O) with the excess water. According to the many types of research who had conducted studies based on annealing process used considerable time period as long hours with heat treatment as 24 h (Kayode *et al*., 2005), 2 to 72 h (Waduge *et al*., 2006). However in this study the time used for applying the heat treatment was 5 min. Some researchers have mentioned annealing is going to lower the onset temperature (T_O) (Neelam *et al*., 2012; Waduge *et al*., 2006). While some scientists refer the required temperature for the treatment below the melting point/peak temperature (T_P) (Kayode *et al*., 2005; Waduge *et al*., 2006; Jacobs *et al*., 1995).

According to the hydrothermal modification which has conducted by Knight (1965) with the presence of excess water (1:1 w/w) Corn and Wheat starches incubated for 5 minutes at 63°C called, pre-gelatinization process. Therefor PG-I modification was used heating temperature (65°C) just below the onset (To = 68°C). Nevertheless which was not enough to present significant change than the gelatinization parameters as well as amylose content of native Kithul flour (Table 1) which are the indications of modification of starch granule. Therefore, PG-II process was used with a slight modification of the process as applied temperature (75°C) which was above the onset (T_O) since below the peak temperature (T_P =78°C) of native Kithul flour. Figure 1 clearly shows the significant changes of gelatinization properties of PG-II flour treatment. Some scientists reported that amylopectin branch chain length and arrangement attributes the gelatinizing properties (Jane *et al*., 1999), further gelatinization temperature incensement indicates the increasing branch chain

Table 1: Amylose content and DSC transition parameters of modified and native flour samples

		DSC results			
Sample name	Amylose content	T_0 (°C)	T_P (°C)	T_C (°C)	ΔH (J/g)
RW	28.34±0.47[b]	68.69±0.24[d]	78.73±0.29[c]	92.79±0.24[a]	13.65±0.21[d]
PG-I	28.88±0.80[ab]	69.93±0.09[c]	79.54±0.19[b]	90.77±0.25[b]	14.38±0.19[c]
PG-II	25.36±0.88[c]	74.02±0.18[a]	80.51±0.41[a]	90.04±0.23[c]	5.78±0.28[e]
AC	29.46±0.60[a]	68.50±0.33[d]	78.64±0.38[c]	92.55±0.24[a]	15.31±0.24[b]
DX	29.44±0.22[a]	70.30±0.18[b]	78.85±0.17[c]	90.65±0.26[b]	17.75 ±0.25[a]

Gelatinization Temperature, T0: Initial, TP: Peak, Tc: Conclution, ΔH: Gelatinization enthalpy

Table 2: Correlation coefficient (r) between gelatinization parameters and amylose content of samples

Parameter	T_0 (°C)	T_P (°C)	T_C (°C)	ΔH (J/g)
T_P (°C)	0.864*			
T_C (°C)	-0.809*	-0.689*		
ΔH (J/g)	-0.804*	-0.796*	0.399*	
Amylose (%)	-0.812*	-0.793*	0.448*	0.906*

*Correlation is significant at 0.05 level

length. Based on this observation it is clear that flour treatment of PG-II which was presented higher gelatinization temperature than native flour has modified with increased branch length after hydrothermal treatment. PG-II treatment has applied temperature somewhat lower than T_P which was allowing measurable molecular rearrangement and more organized structure (Blanshard, 1987) it is attributed by high gelatinization temperature of PG-II.

The result of PG-II both determination of Amylose content and DSC parameters represented more similarities with other ANN and HMT modified starch results from different researchers. According to the Hoover and Vasanthan (1994) who were reported incensement of gelatinization temperature and decrement of apparent amylose content comparable with the result of PG-II treated flour treatment. The presence of amylose-lipid complex is evident by blue value studies which used to determine the amylose content. Due to amylose-lipid complex the iodine binding point has reduced as compare with lipid-extracted starch (Morrison, 1995), as blue value method refers the absorbance of starch portion which is complexed with iodine (Kaur and Singh, 2000).

According to the study of Ratnayake *et al.* (2012) the DSC thermogram which represented the phase transition is unique to the botanical source of the starch, because, it depends on that granular structure, arrangement of polymers, chain length etc (Ratnayake and Jackson, 2007; Wootton and Bamunuarachchi, 1979;, Buck and Walker, 1988. Based on these conditions this study provides suitable modification method for Kithul flour which is extracted from the bark of the tree. Hence, it is very practical that some deviations of the modification than tuber and cereal starches. Sago (*Metroxylonsagu*) starch which is extracted from bark also has little bit higher gelatinization temperature (ranged 69.4 to 70.1°C) than corn and pea like cereals while it's lower than sweet potato, Tania and yam (Mohamed *et al.*, 2008).

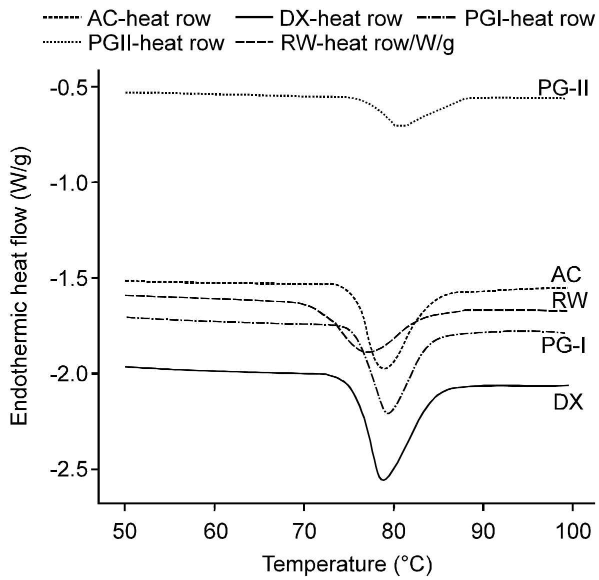

Fig. 1: Differential Scanning colorimetry thermograms of Native and modified Kithul flour

The relationship between gelatinization parameters and amylose content is according to the Table 2. The correlation between onset (T_O) with the peak (T_P) and conclusion (T_C) temperatures were 0.864 and -0.809, respectively. Onset temperature and gelatinization enthalpy presented negative, a strong correlation (-0.804) while T_O was inversely correlated with amylose content (-0.812), which evident that linear polymer of the starch is easy to initiate the gelatinization process. However relationship between ΔH and Amylose content represented strong positive correlation (0.909) which has reported by earlier studies (Park *et al.*, 2007; Hermansson and Svegmark, 1996; Varavinit *et al.*, 2003; Tester and Morrison, 1990). According to the postulation of Park *et al.* (2007) high non-waxy (high amylose) rice starch cohabited the hydration of the amorphous phase. In this study the outcome clearly point out the onset and peak temperatures are negatively correlate with amylose content which means large amorphous region is caused to initiate gelatinization but during the melting correlation become positive (0.448) as that amylose is coming out and make a network around the starch granule by restricting the swelling (Hermansson and Svegmark, 1996) hence waxy starches more likely to swell than non-waxy starches (Varavinit *et al.*, 2003;

Tester and Morrison, 1990). These all findings gave a comprehensive idea on strong positive correlation (0.906) among amylose content and gelatinization enthalpy where more energy require for completing the gelatinization.

Conclusion and further study: Native Kithul (*Caryota urens*) flour was subjected to three main modifications as two pre-gelatinized treatments (PGI and PGII), acid modified (AC) and dextrinized modification (DX). PG-II produced with somewhat higher temperature which is very closer but not above the gelatinization temperature of native (78°C) is the most suitable modification treatment for Kithul flour based on the evidence of this study as significant incensement of gelatinization temperatures and reduction of amylose content which cause to re-order the granular structure.
However, these results provide a clear idea that DX, PG and AC flour treatments can be used as a deliberate working tool to operate the Kithul flour to meet specific needs with a view to effort expand application within the food industry.

ACKNOWLEDGEMENTS
The corresponding author thanks her dearest parents Mr & Mrs Wijesinghe and Janitha Ambagaspitiya for being the spirit of her education. The authors thank the University of Sri Jayewardenepura, Sri Lanka for providing the financial assistance (Grant No: ASP/06/RE/SCI/2012/04) for this study.

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