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Effects of Retrogradation on the Nutritional and Starch Characteristics of Some Foods

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Abstract: The changes that starch undergoes during retrogradation are major determinants of its functional properties for food processing, during digestion and in industrial applications. These properties determine the quality, acceptability, nutritional value and shelf-life of the finished food products. This research was carried out to evaluate the effects of retrogradation on different nutritional components of six selected starchy foods. Fresh tubers of white Yam (*D. rolundata*), Cassava (*Manihot utillsima*), Potato (*Solanum tuberosum*), Cocoyam (*Colocasia esculenta*), Three-leaved Yam (*D. domenturum*) and Aeriel Yam (*Discorea bulbifera*) were obtained from Ebonyi State and used for this work. The foods were mashed and modified by retrogradation. Proximate analysis and functional properties were carried on both retrograded and fresh (control) starch samples. Result of proximate analysis showed significant increase ($p<0.05$) for moisture content, dry matter and bulk density for retrograded samples against the controls. However, the reverse was the case for mineral ash, crude fibre, crude protein, swelling index and water absorption capacity. The results also showed that sample F (Yam) had the higher gelatinization temperature of 86°C, followed by Potato and Arial Yam (84ºC), Three-leaved Yam (80ºC), Cocoyam (72ºC) and then Cassava (69ºC).

Key words: Retrogradation · Gelatinization · Starch · Nutrients · Amylose · Amylopectin

INTRODUCTION

Gelatinization and retrogradation are the key functional properties of starch that determine the quality and nutritional value of starchy foods. Tubers are important sources of carbohydrates as an energy source and are used as staple foods in tropical and sub-tropical countries [1]. These products have nutritionally beneficial components, such as a resistant starch and mucilage. Resistant starch has been attributed with a slow digestion in the lower parts of the human gastrointestinal tract which results in the slow liberation and absorption of glucose and aids in the reduction of the risk of obesity, diabetes and other related diseases [2].

Starch gelatinization is a process of breaking down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water [3]. This irreversibly dissolves the starch granule in water. Water acts as a plasticizer. Three main processes happen to the starch granule: granule swelling, crystal or double helical melting and amylose leaching.

During heating, water is first absorbed in the amorphous space of starch, which leads to a swelling phenomenon. Water then enters via amorphous regions the tightly bound areas of double helical structures of amylopectin. At ambient temperatures these crystalline regions do not allow water to enter. Heat causes such regions to become diffuse, the amylose chains begin to dissolve, to separate into an amorphous form and the number and size of crystalline regions decreases. Under the microscope in polarized light starch loses its birefringence and its extinction cross. Penetration of water thus increases the randomness in the starch granule structure and causes swelling; eventually amylose molecules leach into the surrounding water and the granule structure disintegrates [4]

The gelatinization temperature of starch depends upon plant type and the amount of water present, pH, types and concentration of salt, sugar, fat and protein in the recipe, as well as starch derivatisation technology

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used. Some types of unmodified native starches start swelling at 55 °C, other types at 85 °C. The gelatinization temperature of modified starch depends on, for example, the degree of cross-linking, acid treatment, or acetylation. Gel temperature can also be modified by genetic manipulation of starch synthase genes. Gelatinization temperature also depends on the amount of damaged starch granules; these will swell faster. High amylose starches require more energy to break up bonds to gelatinize into starch molecules. Gelatinization improves the availability of starch for amylase hydrolysis. So gelatinization of starch is used constantly in cooking to make the starch digestible or to thicken/bind water in roux, sauce, or soup [5].

When starch is heated in the presence of water (gelatinized) and subsequently cooled, the disrupted amylose and amylopectin chains can gradually reassociate into a different ordered structure in a process termed retrogradation [6, 7]. Starch retrogradation is usually accompanied by a series of physical changes such as increased viscosity and turbidity of pastes, gel formation, exudation of water and increased degree of crystallinity with the appearance of B-type crystalline polymorphs foods [8].

Retrogradation occurs not only in amylose fraction but also amylopectin from gelatinized granules. Association of linear amylose molecules takes place quickly at the first stage of retrogradation, while slow increase in starch gel rigidity is attributed to amylopectin crystallization. This process is also faster at low temperature. Significant acceleration may be obtained by repeated cycles of freezing and thawing of starch gel [9, 10]. It was found that cereal starches are more prone to retrogradation than potato and in the cases of bimodal distribution small granules are less susceptible to this process than large granules and non-fractionated starch [11].

The changes that starch undergoes during retrogradation are major determinants of its functional properties for food processing, during digestion and in industrial applications. These properties determine the quality, acceptability, nutritional value and shelf-life of the finished food products. This research was therefore, aimed at evaluating the effects of retrogradation on different nutritional components of six selected starchy foods.

MATERIALS AND METHODS

Fresh tubers of white Yam (*D. rolundata*), Cassava (*Manihot utillsima*), Potato (*Solanum tuberosum*), Cocoyam (*Colocasia esculenta*), Three-leaved Yam (*D. domenturum)* and Aeriel Yam (*Discorea bulbifera*) were obtained from a commercial market in Ebonyi State. Wholesome portions of each of the samples were peeled, washed and separately ground into mash with a blender. The cassava was allowed to ferment for three days before usage. The mashed samples were modified (gelatinized and retrograded) after which it was sundried to drain the water content. The dried samples were grinded with a manual laboratory-type grinder. The flour samples produced were stored in nylon bags and some were used for chemical analysis.

Proximate analyses for protein, fat, carbohydrate, moisture, ash, ether extract and dietary fibre content were done using the AOAC (1995) standard. The glycemic carbohydrate was obtained by subtracting the dietary fibre content from the total carbohydrate i.e “indigestible” carbohydrate content (dietary fibre) from the total the carbohydrate content to yield the digestible carbohydrate content (dietary carbohydrate) which is in line with the FAO/UN (1998).

Proximate and Functional Property Analysis Method:
Moisture Content: Moisture content was determined by the gravimetric method described by [12]. Grams of the dried mash of the sample was weighed into a weighed moisture can. The can and its content were dried in the oven at 150ºC for 3 hours in the first instance. It was cooled in desiccators and reweighed. The weight was recorded while the sample was retained in oven for further drying. The drying, cooling and weighing was continued repeatedly until a constant weight was obtained. The moisture content was calculated as shown below,

$$\% \text{ moisture} = \frac{W2 - W3X}{W2 - W1} \frac{100}{1}$$

where
W1 = Weight of empty moisture can,
W2 = Weight of can before drying,
W3 = weight of can + sample after drying to a constant weight.

Determination of Total Ash: This was done using the incineration gravimetric method (AOAC, 1996). A measured weight (5g) of sample was put in a previous weighed porcelain crucible. The sample in crucible was put in a muffle furnace and set at 550°C and allowed to burn for 2-3 hours (until the sample become a grey ash). The sample in crucible was carefully removed from the

furnace and cooled in a desiccator. It was reweighed by difference, the weight of ash was obtained and in percentage. It was given by the formula,

$$\% \text{ Ash} = \frac{W2 - W1}{Wo} X \frac{100}{1}$$

where
W1 = weight of crucible,
W2 = weight of empty crucible + Ash,
Wo = weight of sample used

Determination of Protein: This was determined by Kjeldahl digestion method described by James (1995). The total nitrogen was determined and multiplied with the factor 6.25 to obtain the protein. About 0.5g of the sample was mixed with 10mls of concentrated sulphuric acid, in a Kjeldahl digestion flask. A table of selenium catalyst was added to it and the mixture was digested under a fume cupboard until a clear solution was obtained. The acid and other reagent were digested but without sample from the blank control.

All the digests were carefully transferred to a 100ml volume flask using distilled water and made up to a mark in the flask. A 100ml portion of each digest was mixed with equal volume of 45% NaOH solution in Kjeldahl distilling unit. The mixture was distilled and the distillate collected into 10ml of 4% boric acid solution containing three (3 drops mixed indicator cresol green and methyl red). A total of 50ml distillate was obtained and titrated against 0.02N H_2SO_4 solution. The end point is from the initial green colour to a deep red point. The nitrogen content was calculated as shown below

$$\%N2 = \{\frac{100}{W} X \frac{N X 14}{100} X \frac{vf}{v\alpha}\}$$

where
W= weight of sample analyzed, N= Concentrated of H2SO4 titrant, *Vf*= total volume of digest, *Vα*=volume of digest distilled, T=Titre value - Blank, %C*p*= %*N x* 6.25

Determination of Fat Content: Fat content of the sample was determined by the continuous solvent extraction method using a soxhlex apparatus. The method was described by Pearson, (1967). 0.5grams of the sample was wrapped in a porous paper (what man No. 1 filter paper). The wrapped sample was in a soxhlex reflux flask containing 200ml of petroleum ether **(b.p. ???)**. The upper end of the reflux was connected to a condenser. By heating the solvent in the flask through electrothermal heater, it vaporizes and condensed into the reflux flask. The wrapped sample was completely immersed in the solvent and remained in contact with it until the flask filled up and siphoned.

Bulk Density: Method of Leloup *et al.* [13] was used. 50g of the sample was weighed into a graduated cylinder and its volume was recorded. After then, the cylinder was tapped constantly against a table (10-15 minutes) until there is a further change in volume.

Calculation:

$$\text{The bulk density (g\ml)} = \frac{\text{weight of sample (g)}}{\text{Volume of sample (ml)}}$$

Oil/water Absorption Capacity: The method of Liu *et al.* [14] modified by Okaka and Porter [15] was used. One g of the sample was weighed into a 15ml centrifuge tube and 10ml (1/s) distilled water or oil was added. The sample was later mixed thoroughly and allowed to stand for 30 minutes at room temperature and centrifuge at 2000-5000 rpm for 30 minutes. This volume of force water or oil (the supernatant) was read directly from the graduated centrifuge tube.

The amount of oil or water absorbed is multiplied by its density and converted to grams. Density of water is 1g/ml that of oil varies depending on the type of oil.

Swelling Index: The swell index was determined using the method of Okezie and Bello 1998. It was determined as the ratio of swollen volume of a unit weight of the sample when left in contact with excess water; 1g of the sample was dispersed into a calibrated 10ml measuring cylinder. 10ml of distilled water was added to the sample and the volume was noted. The cylinder was left to stand undisturbed for 1hr. the volume which the sample then occupied was recorded. The swelling index was determined by as follows;

Swelling index = V2/V1, Where V1= Initial volume occupied by the sample, V2 = Volume occupied by the sample after swelling.

Gelatinization Temperature Determination: About 10% suspension of the sample was prepared in a test tube. The mixture was boiled with continuous stirring. Then the temperature was recorded 30 seconds after gelatinization is visually noticed [15].

Retrogradation Method by Thermal Analysis: When a system is heated, absorption or release of heat or loss of mass usually occurs as a result of phase transitions (such as melting or crystallization) or chemical reactions (for example, chemical decomposition). The realignment of disrupted amylose and amylopectin molecules to form a partially ordered structure and the exudation of water from starch gels was monitored using differential thermal analysis (DTA) technique.

Statistical Analysis: Retrogradation was determined by measuring the mass loss of bound water or the differential temperature of gelatinized and retrograded starch in samples and controls, according to Okezie and Bello [16].

RESULTS AND DISCUSSION

Results of proximate and functional properties for retrograded and unmodified (control) samples analyzed are presented in Tables 1 and 2 below.

Starch retrogradation is mostly taken to be an undesirable process that occurs during the storage of starchy foods. Retardation or inhibition of starch retrogradation is of special interest and a challenge for the food industry and an area where great efforts have been made to study the influencing factors. As discussed subsequently, water content, starch source and storage conditions are all well-known factors that can greatly influence starch retrogradation. The presence of food components such as lipids, carbohydrates, salts, proteins, or peptides have also been shown to play a significant role in retarding the rate of starch retrogradation.

In the processing and storage of foods, water plays a crucial role in starch gelatinization and retrogradation. The effect of water content on starch gelatinization has been reviewed by Wang and Copeland (2013). The rate and extent of starch retrogradation is also largely dependent on water (moisture) content. The effect of moisture content on starch retrogradation is influenced by

Table 1: Proximate Analyses of Samples for Retrogradation Modification

S/NO	MC	DM	ASH	CF	CP
Sample A	9.56±0.02^{b}	90.49±0.01^{b}	2.18±0.01^{d}	0.52±0.02^{c}	3.41±0.02^{c}
Control	9.45±0.02^{c}	90.55±0.02^{b}	2.38±0.03^{c}	0.64±0.001^{b}	2.16±0.01^{d}
Sample B	9.54±0.02^{c}	90.53±0.02^{b}	2.45±0.03^{c}	0.51±0.002^{c}	2.88±0.01^{d}
Control	9.50±0.02^{b}	90.45±0.01^{b}	2.91±0.03^{ab}	0.55±0.001^{c}	3.77±0.01^{c}
Sample C	9.94±0.3^{ab}	90.56±0.01^{b}	2.75±0.01^{b}	0.53±0.02^{c}	5.16±0.01^{b}
Control	9.78±0.3^{b}	91.06±0.02^{a}	2.93±0.01^{ab}	0.64±0.001^{b}	5.88±0.02^{b}
Sample D	10.78±0.2^{a}	90.23±0.02^{b}	2.71±0.01^{b}	0.60±0.001^{bc}	5.79±0.02^{b}
Control	9.51±0.01^{c}	89.23±0.02^{c}	3.15±0.02^{a}	0.82±0.01^{a}	7.64±0.01^{a}
Sample E	8.46±0.02^{d}	91.56±0.01^{a}	1.88±0.01^{e}	0.44±0.001^{d}	0.43±0.001^{e}
Control	8.25±0.01^{d}	91.75±0.02^{a}	2.37±0.03^{c}	0.65±0.002^{b}	0.58±0.02^{e}
Sample F	9.71±0.2^{b}	90.35±0.01^{b}	2.24±0.03^{d}	0.42±0.001^{d}	2.15±0.001^{d}
Control	8.82±0.02^{cd}	91.2±0.01^{a}	2.8±0.01^{b}	0.45±0.01^{d}	3.17±0.02^{c}

Average of three determinations, means with different alphabets are considered to be significantly different at P<0.05.

Note that Sample A = Three Leaved Yam, Sample B = Ariel Yam, Sample C = Potato,

Sample D = Cocoyam, Sample E = Cassava, Sample F = Yam. The table values were expressed as Mean ± SD. The results were analyzed for statistical significance using One Way Anova, SPSS 20.8 version.

Also, MC= Moisture Contents, DM= Dry Matter, CF= Crude Fibre, CP= Crude Protein

CHO= Carbohydrate, WAC= Water Absorption Capacity, S_I = Swelling index,

OAC= Oil Absorption Capacity, BD = Bulk Density, GT= Gelatinization Temperature.

Table 2: Functional Properties of Samples for Retrogradation Modification

S/NO	WAC (g/g)	S1	OAC (g/g)	BD (g/ml)	GT (°C)
Sample A	4.24±0.02^{b}	2.89±0.01^{c}	3.16±0.01^{a}	0.52±0.002^{bc}	80±0
Control	4.62±0.01^{b}	3.92±0.01^{b}	2.58±0.01^{b}	0.52±0.002^{bc}	75±0
Sample B	3.12±0.01^{c}	4.53±0.12^{a}	1.27±0.01^{c}	0.59±0.002^{bc}	84±0
Control	4.42±0.01^{b}	4.74±0.01^{a}	1.45±0.01^{c}	0.50±0.02^{b}	73±0
Sample C	3.12±0.01^{c}	2.96±0.01^{c}	1.72±0.01^{c}	0.65±0.002^{a}	84±0
Control	4.26±0.02^{b}	3.06±0.01^{b}	2.60±0.01^{b}	0.65±0.002^{a}	70±0
Sample D	2.48±0.01^{d}	2.16±0.1^{c}	2.00±0.01^{bc}	0.51±0.01^{bc}	72±.0
Control	4.85±0.02^{b}	4.00±0.01^{a}	2.65±0.01^{b}	0.46±0.002^{c}	68±0
Sample E	4.56±0.02^{b}	2.88±0.01^{c}	1.52±0.01^{c}	0.67±0.002^{a}	69±0
Control	5.50±0.12^{a}	4.31±0.01^{a}	2.52±0.01^{c}	0.58±0.02^{b}	64±0
Sample F	3.00±0.01^{c}	2.68±0.01^{c}	1.56±0.01^{c}	0.65±0.002^{a}	86±0
Control	5.16±0.01^{a}	3.08±0.01^{b}	3.55±0.02^{a}	0.65±0.002^{a}	76±0

Average of three determinations, means with different alphabets are considered to be significantly different at P<0.05.

amylose content, which can also affect amylopectin crystallization [17]. From the above tabulated result, different samples had different moisture content. However, gelatinized samples gained more moisture, this is because moisturised heat is required to break the glycosidic bonds in amylose and amylopectins [18]. The results showed that different food starch varies moisture content and moisture requirements for gelatinization. Also, from the water absorption capacity, it is clear that retrograded starch did not absorb as much water as the control samples, this is because they already had a built-up moisture which tends to dissipate as amylose and amylopectin chains gradually reassociate into a different ordered structure [19]. Taggart [20], reported that the water absorption capacity depends on the availability of hydrophilic groups and on the capacity of gel formation of the macromolecules. The significant decrease ($p<0.05$) in the bulk density is in-line with Tester and Morrison [21]. They reported that as gelatinization proceeded, the volume of extruded products increased and the bulk density decreased.

The extent to which starch retrogradation is inhibited is largely influenced by the type and concentration of carbohydrate. The mechanisms for the inhibition of starch retrogradation are explained in terms of competition for water between starch and the other carbohydrates. Glucose, ribose, fructose, sucrose, maltose and water-soluble maltodextrins all can retard starch retrogradation. Many studies have shown disaccharides to be more effective than monosaccharides as inhibitors of starch retrogradation [22]. Starch source with higher gelatinization temperature tends to delay retrogradation, compared to those of lower gelatinization temperature [23].

The analysed ash content, crude fibre and dry matter affected retrogradation. Sample C (Potato) had higher retrogradation ability compared to Sample E (Cassava), despite the later having a lower gelatinization temperature, against the claim of Wang, Sharp and Copeland [24]. However, this could be as a result of higher ash, crude fibre and dry matter of Potato, compared to Cassava.

Proteins/polypeptides/amino acids are often keycomponents of starchy foods and can play an important role in contributing to their quality and nutrition. Addition of protein to starchy foods has been shown to influence starch retrogradation [25]. This explains why Sample D (Cocoyam) showed higher retrogradation compared to others.

Temperature and length of storage are the major determinants of the extent of starch retrogradation. In general, retrogradation is rapid initially and then slows down. The onset temperature and enthalpy change for the melting of retrograded starch molecules increase with storage time at constant temperature, whereas the conclusion temperature exhibits little change [26]. Starch with higher gelatinization temperature tends to retrograde with ease compared to their counterparts.

The starch granules start to swell rapidly only after the temperature reached the onset of the gelatinization temperature [27]. Swelling index is the rate at which the starch allowed moisture into the amylose and amylopectin interstices. The swelling power of these samples is often related to their protein and starch contents [20]. Higher protein content may cause the starch granules to be embedded within a stiff protein matrix, which subsequently limits the access of the starch to water and restricts the swelling power. In addition to protein content, a higher concentration of phosphorous may increase hydration and swelling power by weakening the extent of bonding within the crystalline domain, Kohyama and Nishinari [11]. Also, amylopectin is primarily responsible for granule swelling, thus higher amylose content would reduce the swelling factor of starch [7]. However, in the current study, there was no apparent correlation between the amylose content and swelling power of the potato sample.

Gelatinization and retrogradation are the key functional properties of starch that determine the quality and nutritional value of starchy foods. Although many methods are used to characterize starch retrogradation during storage, it is clear that no single method can give a complete picture of the process. Starch retrogradation is affected by many factors including water content, storage temperature, storage time and additives in the system. Food additives can greatly alter the rate and extent of starch retrogradation by competition for water with starch or by interfering with their association of starch chains. Food additives that change the water activity in starch-water systems may be a key to controlling retrogradation of starch.

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