Effect of thermal processing and storage on digestibility of starch in whole wheat grains

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ABSTRACT

Gelatinisation and retrogradation of starch in wheat flour systems and whole wheat grains were studied using differential scanning calorimetry (DSC) and the impact of these events on starch digestibility was investigated. Retrogradation of starch was studied with partially and fully cooked (boiled at 100°C for 12 min and 32 min, respectively) wheat grains that were subjected to storage at 22°C for 48 h. Stored samples had lower digestibility values when compared to the freshly cooked counterparts. The effect of moisture on retrogradation was studied with fully cooked wheat grains that were dried to a range of moisture contents (14.6 to 35.9%, wwb) and stored at 20°C for 24 h. The retrogradation enthalpy increased with increasing moisture content. The possibility of estimating the degree of retrogradation in fully cooked grains (32 min cooking) was investigated using a wheat flour-water system. The retrogradation enthalpy of the fully cooked grains was slightly higher than the wheat flour-water system (at a moisture content of 49%, wwb) during the course of storage at 22°C.

Keywords: whole wheat grains; gelatinisation; retrogradation; starch digestibility; moisture content

INTRODUCTION

Whole wheat grains are usually subjected to heat treatments in the presence of water before consumption. This produces an edible product, increases the nutritive value of the grain and generates desirable flavour and texture. At various stages of whole grain processing the product may contain uncooked, gelatinised and retrograded starch with different degrees of digestibility due to the structural changes that take place during heat-moisture treatment. This work aims to investigate the gelatinisation and retrogradation of starch during the cooking and storage of the whole wheat grains using differential scanning calorimetry (DSC) and their effect on starch digestibility. The study is comprised of three parts and it investigates: (1) the effect of different degree of cooking and storage on retrogradation and digestibility of starch in whole wheat grains (2) the effect of moisture on the retrogradation of starch and starch digestibility in fully cooked grains (3) the possibility of estimating retrogradation enthalpies of whole wheat grains using a wheat flour system.

MATERIALS & METHODS

Wheat grains and flour

The wheat grains used in the study were UK grown (Hertfordshire, Essex and East Anglia Regions) soft grains (Consort wheat) and their size varied between 5.5 – 8.0 mm in length and 3.5 – 4.0 mm in width. The average moisture content (wwb) of the wheat grains was between 11 – 12%. The wheat flour was obtained by milling the whole wheat grains (uncooked) in a Cyclotec 1093 mill with a mesh size of 1 mm. The flour had an initial moisture content of 12.5 ± 0.1 % (wwb).

Cooking of the wheat grains: The whole wheat grains were placed in boiling water in a beaker and cooked on a hot plate with gentle stirring. The grains were cooked at 100°C for 12 and 32 min. Cooked grains were treated with liquid nitrogen and stored at -23°C, when required. Before the analysis, frozen grains were thawed at ambient temperature for approximately 15 min.

Flour of the cooked grains: The cooked wheat grains (12 and 32 min cooking) were milled in a Cyclotec 1093 mill with a mesh size of 1 mm. The samples were treated with liquid nitrogen to ease the milling process.

Moisture content determination

Moisture content of the wheat samples was determined by oven drying (Gallenkamp Hotbox Oven) at 105ºC for 24 h.

Differential scanning calorimetry

A PC-driven differential scanning calorimeter (Perkin-Elmer DSC-7, Beaconsfield, UK) was used for the measurements. The instrument was calibrated for temperature and enthalpy with Indium (Tonset = 156.6ºC and ΔH = 28.45 J/ g) and Cyclohexane (Tonset = 6.7ºC). Dry air was used as a purging agent over the head. Cooling was achieved with an intra-cooler allowing temperatures down to -60ºC. Two types of pans were used depending on the type of the experiment, aluminium pans with a volume of 20 µl, or stainless steel pans with a volume of 40 µl. Sample pans were allowed to equilibrate overnight at room temperature unless otherwise stated. All DSC scans were carried out with a heating rate of 10 °C/min. The preparation of samples for analyses for different sections is given below:

<u>Retrogradation in cooked wheat grains</u>

Flour of 12 and 32 min cooked wheat grains were used. Freshly cooked samples (3 - 5 mg) were placed in the DSC pans and analysed immediately. The same pans were scanned the second time after storage at 22ºC for 48 h. DSC conditions were: Aluminium pans, 5-100ºC, sample / water ratio: 1 / 3, number of replicates: 3.

<u>Effect of moisture content on retrogradation</u>

The flour of the fully cooked (32 min cooking) wheat grains was dried to various moisture contents (14.6, 19.4, 22.6, 28.6 and 35.9%, wwb). 3 - 5 mg sample was placed in the DSC pans. DSC conditions were: Aluminium pans, 5-100ºC, sample / water ratio: 1 / 3, number of replicates: 3.

<u>Studying retrogradation: whole grains versus wheat-flour water mixture</u>

Water was added to wheat flour (untreated) in the DSC pans to generate a moisture content of 49%, wwb and this sample was heated in the DSC to ensure cooking. Both wheat flour-water mixture and the flour of the 32 min cooked wheat grains were stored in DSC pans during storage. Approximately 8 mg of sample was used. DSC conditions were: Stainless steel pans, 5-100ºC, sample / water ratio: 1 / 3, number of replicates: 5.

Starch digestibility assay

12 ml distilled water, 18 ml 0.1 M citrate phosphate buffer (pH 6.9, containing 6 mM NaCl) and pancreatic α- amylase (EC.3.2.1.1, A6255, Sigma Chemical Co., St. Louis, MO, USA) were added to approximately 100 mg of the flour of the cooked (cooked for 12 and 32 min) and untreated wheat grains. The amount of enzyme corresponded to 45.4 units α- amylase per ml starch suspension. The mixture was placed on a roller shaker (Denley), which was housed in an incubator at 37°C. Duplicate aliquots were taken at suitable intervals (15 and 60 min) and centrifuged at 3000xg for 2 min. The supernatants were collected and the amount of total sugar was determined by phenol-sulphuric acid method. The development of yellow colour was measured spectrophotometrically. A standard curve was drawn with glucose solutions at different concentrations (0-1000 µg/ml). The digestibility of the sample was expressed as a percentage of mg total sugar / mg sample.

Statistical analysis

All results were expressed as mean ± standard deviation. F-test and Student's t test were applied to determine significant differences. A value of ($P<0.05$) was considered significant. Correlation analysis was conducted to estimate the relationship between enthalpy of retrogradation and starch digestibility values.

RESULTS & DISCUSSION

The effect of different degree of cooking and storage

This part of the study looked at the effect of cooking on gelatinisation and retrogradation of starch in whole wheat grains. The flour of partially cooked grains (cooked for 12 min, with a visible uncooked section at the centre) and fully cooked grains (cooked for 32 min, with no visible uncooked section) were analysed by DSC and starch digestibility assay. The samples were analysed immediately in the DSC to prevent retrogradation. The DSC endotherms of both samples registered flat lines (figure not shown). This suggested that the uncooked starch in 12 min cooked grains did not gelatinise during the DSC run. However, the results of the digestibility analysis implied that 12 min cooked grains retained some uncooked starch as they exhibited lower digestibility values (Table 1). The grains had a digestibility value of 57.6% (dwb) after 12 min cooking and this value increased to 66.8 % after 32 min cooking. The effect of cooking on starch digestibility was significant (P<0.05). The digestibility results in the current study suggested a difference in the degree of gelatinisation with the partially cooked (cooked for 12 min) and fully cooked (cooked for 32 min) grains but the results from DSC were inconclusive as an endotherm due to uncooked starch was not observed with the 12 min cooked grains.

Storage at 22ºC for 48 h led to a significant decrease in the digestibility values of 12 min and 32 min cooked grains (50.9 and 60.1%, respectively) (P<0.05). Stored samples displayed DSC endotherms with a peak temperature range of approximately 45-60ºC (Table 1), indicating the occurrence of the retrogradation of amylopectin. The retrogradation enthalpies of 12 min and 32 min cooked samples were 2.6 ± 0.3 and 1.5 ± 0.1 J / g dry sample, respectively. The 32 min cooked grains had higher moisture content and greater degree of digestibility when compared to the 12 min cooked grains. This strongly suggested that more gelatinisation occurred in the 32 min cooked grains. Therefore, one would expect 32 min cooked grains to have a higher enthalpy of retrogradation value. However, the DSC results contradicted this and they implied that more retrogradation occurred in the 12 min cooked grains.

The enthalpy of retrogradation value for the 12 min cooked grains were similar to the value obtained with cooked grains after storage at 20ºC for 48 h (2.5 J / g dry sample) [1]. It should be noted that the cooking time of the grains was not specified in that study. Amylose retrogradation was not thought to contribute to the changes in the starch digestibility values within the time scale of storage as it occurs more rapidly (in the scale of several hours) than amylopectin retrogradation (which is usually a slower process that takes days) [2].

Retrogradation led to lower values of starch digestibility in the cooked (12 and 32 min cooking) and stored whole wheat grains and this was in accordance with the previous studies [3-5]. This decrease in digestibility was significant (P<0.05).

Table 1. Results obtained from DSC and digestibility analysis using partially and fully cooked wheat grains

Sample	**Moisture content, % (wwb)**	**Tonset (ºC)**	**Tpeak (ºC)**	**Tend (ºC)**	**Retrogradation enthalpy (J/g dry sample)**	**% Digestibility (mg total sugar / mg dry sample*100) (15 min hydrolysis)**
12	45.2±0.1	na	na	na	na	57.6±1.5^{a}
32	55.5±0.3	na	na	na	na	66.8±5.1^{b}
12S	45.3±0.2	45.2 ± 0.4	50.8 ± 0.4	56.0 ± 0.6	2.6 ± 0.3	50.9±0.2^{c}
32S	55.8±0.5	45.9 ± 0.2	52.8 ± 0.3	60.4 ± 2.6	1.5 ± 0.1	60.1±2.6^{d}
Uncooked grains	12.5±0.1	_	_	_	_	16.5±1.3^{e}

Results are mean ± standard deviation (n=3 for DSC analysis and n=6 for digestibility).
12: 12 min cooked sample; 32:32 min cooked sample. Suffix "S" represents the samples stored at 22°C for 48 h.
Values followed by different letters differ significantly from one another (P<0.05).

The effect of moisture content on retrogradation

This part of the study investigated the effect of moisture content on the degree of retrogradation in the whole wheat grains. Different from the previous part of the study, only fully cooked grains (32 min cooking) were used (in order to eliminate any interference from uncooked starch that can be present in partially cooked grains). Fully cooked grains were oven-dried to different moisture contents (ranging between 14.6 and 35.9% wwb) and then stored at approximately 20°C for 24 h. These moisture contents corresponded approximately to values ranging between 37.1 and 59.2% starch on wet weight basis.

All samples revealed an endothermic peak positioned between 45.7 and 64.0°C with an average peak temperature of 52.3°C (Table 2). This was the melting endotherm of retrograded starch (amylopectin) and the area of the endotherm was expressed as "the enthalpy of retrogradation" since the grains did not contain uncooked starch. The sample that had the lowest moisture content (14.6%, wwb) had the lowest retrogradation enthalpy (0.8 J/g dry sample) and the values increased with increasing moisture content. This increase was significant ($P<0.05$). Highest enthalpy values were obtained with the samples containing 28.6 and 35.9% moisture (4.1 ± 0.4 and 4.2 ± 0.4, respectively).

The sample with the lowest retrogradation enthalpy had the highest starch digestibility (47.4 %), as expected. The change in the digestibility values at different moisture contents during storage at 20°C for 24 h was significant ($P<0.05$). The correlation analysis between the retrogradation enthalpy and starch digestibility values revealed a correlation coefficient of $R = 0.80$.

Retrogradation increases with increasing water content up to 45-50% and then it decreases with further increase in the water content due to excess dilution [6-8]. It was possible to monitor the effect of moisture content on the rate of retrogradation with the fully cooked wheat grains that were stored at 20°C for 24 h and the results were in line with the previous studies.

Table 2. Data obtained from the DSC and digestibility analysis (fully cooked grains)

Moisture (%,wwb)	**Tonset (ºC)**	**Tpeak (ºC)**	**Tend (ºC)**	**Retrogradation enthalpy (J/g dry sample)**	**% Digestibility (mg total sugar/ mg dry sample*100) (15 min hydrolysis)**
14.6	47.0 ± 0.3	52.9 ± 0.1	62.5 ± 1.5	0.8 ± 0.2	47.4 ± 3.2
19.4	46.6 ± 0.7	52.2 ± 0.5	61.2 ± 4.5	2.5 ± 0.3	36.1 ± 3.1
22.6	45.7 ± 0.4	51.6 ± 0.2	64.0 ± 0.4	3.4 ± 0.2	34.7 ± 5.0
28.6	45.8 ± 0.3	52.3 ± 0.3	62.7 ± 0.2	4.1 ± 0.4	33.1 ± 4.7
35.9	45.9 ± 0.6	52.7 ± 0.2	63.8 ± 0.2	4.2 ± 0.4	36.2 ± 1.6

Results are expressed as mean ± standard deviation (n = 3).

Studying retrogradation: whole grains versus wheat flour-water system

The final part of the study looked at the possibility of studying starch retrogradation in whole wheat grains using a wheat flour system. For this purpose, the retrogradation enthalpies of the wheat flour and whole wheat grains (cooked) were measured by DSC during storage. Wheat flour at 49% moisture was cooked in the DSC instrument and then stored at 22ºC for 36 h. The flour of the fully cooked (32 min cooking) grains (containing approximately 56 % water) was subjected to storage at 22˚C for up to 24 h. Both samples were stored in the DSC pans during storage. The retrogradation enthalpies of the wheat flour-water system were fitted to the Avrami model and a good fit was obtained (Figure 1). The enthalpy values rose steadily for up to 12 h and then they continued to increase at a much slower rate. Apart from one enthalpy value, the retrogradation enthalpies of the cooked wheat grains were slightly higher than the values for the wheat flour-water system. These results suggested that the wheat flour-water system could be used to estimate the rate of retrogradation in the whole wheat grains.

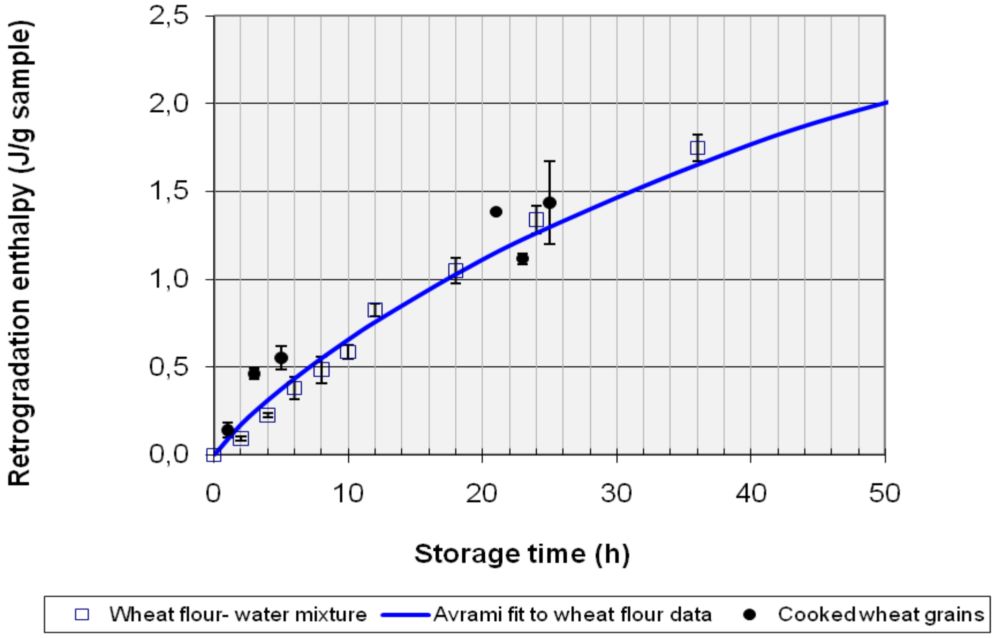

Figure 1. Retrogradation enthalpies of the wheat flour-water system and fully cooked grains. Error bars indicate ±1 standard deviation.

CONCLUSION

Starch digestibility can be used to accompany various physical methods for the investigation of structural changes in starch. In this study, starch digestibility assay was used in conjunction with DSC. The digestibility analysis helped elucidating some of the changes, especially when some of the DSC analyses were giving inconclusive results due to a possible interference from uncooked starch.

The results of the present study indicated that processing (heat moisture treatment) and storage altered the digestibility of starch by influencing its gelatinisation and retrogradation. During the processing of the cereals and other starchy foods, the conditions can be altered to favour various types of starch (gelatinised, retrograded or native starch) to modify the nutritional quality of the final product (such as a product designed for diabetics that contain slowly digestible starch and/or resistant starch). This is of great importance to food industry as it offers the possibility of manufacturing foods and foodstuffs with different starch digestibility.

REFERENCES

[1] Jankowski, T., Rha, C.K., 1986. Retrogradation of starch in cooked wheat. Starch 38, 6-9.
[2] Miles, M., Morris, V., Orford, P., Ring, S., 1985. The roles of amylose and amylopectin in the gelation and retrogradation of starch. Carbohydrate Research 135, 271-281.
[3] Botham, R.L., Morris, V.J., Noel, T.R., Ring, S.G., 1996. A study on the *in vivo* digestibility of retrograded starch. Carbohydrate Polymers 29, 347-352.
[4] Cui, R., Oates, C., 1997. The effect of retrogradation on enzyme susceptibility of sago starch. Carbohydrate Polymers 32, 65-72.
[5] Eerlingen, R.C., Jacobs, H., Delcour, J.A., 1994. Enyme-resistant starch.V. Effect of retrogradation of waxy maize starch on enzyme susceptibility. Cereal Chemistry 71, 351-355.
[6] Gudmundsson, M., 1994. Retrogradation of starch and the role of its components. Thermochimica Acta 246, 329-341.
[7] Longton, J., LeGrys, G., 1981. Differential scanning calorimetry studies on the crystallinity of ageing wheat starch gels. Starch/Staerke 33, 410-414.
[8] Zeleznak, K., Hoseney, R., 1986. The role of water in the retrogradation of wheat starch gels and bread crumb. Cereal Chemistry 63, 407-411.