The effect of baking and enzymatic treatment on the structural properties of wheat starch

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Abstract
In this study, bread was baked with and without the addition of α-amylase. Starch was extracted from the baked bread and its molecular properties were characterized using 1H NMR and asymmetric flow field-flow fractionation (AF4) connected to multi-angle light scattering (MALS) and other detectors. The approach allows determination of molar mass, root-mean-square radius and apparent density as well as the average degree of branching of amyllopectin. The results show that starch size and structure is affected as a result of the baking process. The effect is larger when α-amylase is added. The changes include both a decrease in molar mass and size as well as an increase in apparent density. Moreover, an increase in average degree of branching and the number of reducing ends H-1(β-r) and H-1(α-r) can be observed.

1. Introduction
Starch is the principal carbohydrate reserve in plants and a major source of energy in the human diet and animal feed as well as having many industrial applications. Starch often contributes to the characteristic properties of foods and is also being added as a functional ingredient in many formulated foods. However, chemical or enzymatic modification of the starch is often necessary in order to improve its functional characteristics.

Starch contains two principal types of polysaccharides, amylose and amyllopectin. The ratio of these two polysaccharides varies according to the botanical origin of the starch and contains about 20–30% of amylose and 70–80% of amyllopectin. Amylose is a mainly linear molecule of (1–4)-α-D-glucopyranosyl units which in some cases can be slightly branched (1-6)-α-linkages (Buléon, Colonna, Planchot, & Ball, 1998; Pérez & Bertoft, 2010). Amylopectin is the other component of starch that is highly branched. It is composed of hundreds of short (1-4)-α-glucan chains, which are interlinked by (1–6)-α-linkages. The molar mass (M) of amylose is approximately 10^5 - 10^6 g/mol (Hassid & McCready, 1943; Hoover, Hughes, Chung, & Liu, 2010) and about 10^7–10^8 g/mol for amyllopectin (Hoover et al., 2010; Perez-Rea, Bergénstahl, & Nilsson, 2015).

In order to improve starch properties and functionality, it is important to understand the relationship between the properties of the initial material and the processing conditions on the properties of starch in a final product. This is, for instance, manifested by the influence of molecular size on viscosity enhancing properties (Rojas, Wahlund, Bergénstahl, & Nilsson, 2008) and adsorption kinetics (Nilsson, Leeman, Wahlund, & Bergénstahl, 2007).

One of the most common food products that contain high amounts of starch is bread. Bread has been, and still is, an important part of the human diet for thousands of years (Goesaert et al., 2005). In the western world, bread is considered to be an essential staple food and a perishable product. Bread is preferably consumed when fresh, but unfortunately, bread freshness is maintained only for a few days (Giménez et al., 2007). Bread loses its freshness during storage that causes alteration in the physical, chemical and organoleptic properties. In particular, bread staling is of concern because it is a complex process which may be difficult to control and causes a number of interactions and changes inside the bread (Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014; Purhagen, Sjöö, & Eliasson, 2011).
Starch retrogradation and moisture redistribution inside the bread are recognized as dominant factors for staling (Gray & Bemiller, 2003). The crumb loses its softness and shows an increase in firmness whereas the crust loses its crispiness and becomes soft and loosely attached to the crumb as a result of moisture migration from the crumb to the crust (Cauvain, 2015) and especially changes in amyllopeptin are thought to be responsible for increase in the bread firmness (Gray & Bemiller, 2003; Schoch & French, 1947). There are several factors that can dramatically affect the shelf-life of bread: other flour components, increased amount of gluten, storage temperature, and processing parameters such as formulation, manufacturing methods among others (Kim & D’Appolonia, 1977; Kulp & Ponte, 1981; Martin, Zeleznak, & Hoseney, 1991).

Nowadays, various additives and processing aids are often utilized in the baking industry to suppress staling and to some extent enhance the shelf-life of bread. Enzymes and emulsifiers are used to reduce the crumb firmness of breads (León, Durán, & Benedetto de Barber, 2002). Various enzymes like α-amylase, lipase and protease are regularly being used in baking to influence dough quality and shelf-life of bread. The impact of enzymes on reducing the staling rate and maintaining the freshness of bread for longer times has been reported in several studies (Fiszman, Salvador, & Varela, 2005; Manningat, Selb, Bassi, Woo, & Lasater, 2009).

Among the enzymes, α-amylase is widely used in breadmaking to reduce staling (Caballero, Gómez, & Rosell, 2007; Kulp & Ponte, 1981; Sanz Penella, Collar, & Haros, 2008). Novamyl 10000 BG, the enzyme that was used in the present study, is a commercial preparation of the maltogenic α-amylase from Bacillus sp. TS-25, formerly Bacillus starchthermophilus (Didrichsen & Christiansen, 1988). Novamyl 10000 BG is thermo-stable and well known for its anti-staling properties. It is an enzyme which catalyzes the hydrolysis of α-1,4-glucosidic linkages in amylose, amyllopeptin and related glucose polymers and the primary activity results in the formation of maltose and longer maltodextrins (Christophersen, Otzen, Noman, Christensen, & Schäfer, 1998). Due to the common use of Novamyl 10000 BG in breadmaking, it is interesting to investigate the effect it has on the physicochemical properties of the starch polymers in the bread. Similarly, it is interesting to investigate the effect of the breadmaking process without added enzyme has on the same properties.

Asymmetrical flow field-flow fractionation (AF4) is a separation method which has, in recent years, shown to be a powerful technique for the analysis of many food macromolecules (Nilsson, 2013) and several applications of AF4 to starch analysis have been reported (Dou, Zhou, Jang, & Lee, 2014; Perez-Rea et al., 2015; Van Bruijnsvoort, Wahlund, Nilsson, & Kok, 2001). AF4 becomes especially powerful when connected to suitable detectors such as multi-angle light scattering (MALS) and differential refractive index (dRI) which enables the determination of molar mass (M), root-mean-square radius (\( r_m \)) and the conformation.

The purpose of this study was to investigate the influence of the baking process on size, molar mass (M), apparent density (\( p_{app} \)), conformation and degree of branching (DB) of wheat starch with and without the addition of α-amylase (Novamyl 10000 BG) using AF4-UV-MALS-dRI and \(^1\)H NMR.

2. Materials and methods

2.1. Bread samples

The experiments were performed on bread samples baked at Lantmännens Schulstad (Lantmännens Schulstad, Copenhagen, Denmark). The bread recipe is given in Table 1. Wheat flour was obtained from Lantmännens Cerealia (Lantmännens Cerealia, Vejle, Denmark), vegetable fat from Dragsbaek A/S (Dragbaek, Thisted, Denmark), yeast from De Danske Gaerfabrikker (De Danske Gaerfabrikker, Grenå, Denmark), sugar from Nordic Sugar (Nordic Sugar, Copenhagen, Denmark), salt from Azelis (Azelis, Lyngby, Denmark), and α-amylase (Novamyl 10000 BG) was obtained from Novozymes (Novozymes, Bagsvaerd, Denmark). The water utilized was tap water.

The bread samples were baked with two different concentrations of the added α-amylase: 65 mg/kg flour and 100 mg/kg flour that are in the range of the dosage recommended by the manufacturer. Reference bread was baked without the addition of α-amylase (Novamyl 10000 BG). The starch was extracted from the bread samples and the wheat flour as described below and used for analysis.

2.2. Baking procedure

All the ingredients were transferred to a dough mixer (Diosa, Osnabrück, Germany). Powdered α-amylase (Novamyl 10000 BG) was first mixed with a small amount of wheat flour in a plastic bag and subsequently added to the dough. The temperature of the wheat flour was 21 °C while the temperature of water during addition was calculated in order to get the final desired dough temperature about 27–29 °C. The dough was kneaded for 2 min at slow speed and 5 min at high speed. The dough was then mixed with the help of a plastic dough scraper from the vessel and the dough temperature was measured at 26 °C. A small amount of wheat flour was spread on the working table to prevent the dough from sticking to the wooden surface. The dough was covered with a thin clean plastic and allowed to rest for about 5–7 min. The dough was divided into 7 loaves each one weighing 670 g. All the loaves were molded manually for a short period approximately 1 min and allowed to rest for 10 min. Loaves were then manually treated to change into a cylindrical shape that fits the baking box with size about 253x108x100 mm. Baking plate greaser (VM Margarine) was sprayed on all baking boxes and lids before keeping the loaves. All the baking boxes were covered with lids and transferred to the proofing chamber (MIWE Gäromat, MIWE Michael Wenz, Arnstein, Germany). The proofing condition was 38 °C with a humidity of 85% for all samples. Samples were checked after 45 min and if needed depending on loaf height, the proofing time was prolonged for an additional 10 min. The oven (MIWE Condo, MIWE Michael Wenz) used for baking was preheated for more than one hour before transferring the samples. It was programmed with the standard toast/sandwich program used by Lantmännens Schulstad.

2.3. Starch extraction procedure

The procedure to extract the starch was used as described previously (Goesaert, Leman, Bijttebier, & Delcour, 2009) with some minor changes. Frozen bread crumbs from each batch were decrusted before freeze-drying. Bread crumbs were freeze-dried
in a HETOSICC freeze-drier (HETO Birkerød, Denmark) for 3 days. Five grams of freeze-dried bread crumbs were crushed using mortar and pestle, mixed with 60 mL of Milli-Q water (Millipore, Billerica, MA, USA). The starch-water slurry was mixed for 2 min at high speed in a high shear mixer (Ystral, Ballreichs-Dottingen, Germany); then heated for 15 min in an autoclave at 120 °C. The slurry was allowed to cool down for 30 min in a water bath at room temperature after that, the sample was centrifuged (Beckman Coulter, Brea, CA, USA) at 5000g at 20 °C for 15 min. The aqueous phase was collected and the same procedure was repeated twice with the residue after re-dispersion in Milli-Q water. The total collected aqueous phase was mixed with methanol twice the volume of the aqueous phase in order to precipitate starch in solution. The mixture was left unperturbed at 5 °C overnight to allow the starch to sediment. The sample was carefully decanted and air-dried at 50 °C to further reduce the amount of liquid. The drying continued until approximately 50–70 mL of liquid remained before the sample was freeze-dried for 3 days.

2.4. Chemical analysis of extracted starch samples

Starch was extracted from bread samples baked with and without the addition of α-amylase (Novamyl 10000 BG) and the wheat flour used in the bread. An overview of all samples is shown in Table 2. Moisture content was determined using a moisture analyzer (MAC 110/WH, Radwag, Radom, Poland). Total starch content was determined enzymatically using a commercial total starch analysis kit (Megazyme amyloglucosidase/α-amylase, Megazyme International, Bray, Ireland) subtracting the free glucose content. Total protein content was determined by measuring nitrogen content using the elemental analyzer Flash EA 1112N (Thermo Fisher Scientific, Waltham, MA, USA). The sample (25–32 mg) was combusted in a sealed furnace and the nitrogen content was determined with thermal conductivity detection. Aspartic acid was used as standard. Nitrogen to protein factor of 6.25 was used in the calculations.

2.5. 1H NMR

The procedure was performed as described previously (Nilsson, Gorton, Bergquist, & Nilsson, 1996) with some minor adjustments. Samples were dissolved (5 mg/mL) in deuterated DMSO (100%, ≥99.96 atom % D, Sigma Aldrich, St Louis, MO, USA) in a screw-capped bottle at 100 °C for 15 min with continuous stirring at low speed, followed by freeze-drying. Exchange of hydroxyl protons was performed in order to reduce interference from the residual solvent resonance. Thus, 1.0 mL D₂O (isotropic purity >99.9 atom % D, Sigma Aldrich, St Louis, MO, USA) was added to 5 mg of the sample, heated at 100 °C for 15 min followed by a second freeze-drying. The dried, deuterated sample was dissolved in 1 mL of D₂O and heated for 30 min at 100 °C before analysis. The 1H NMR measurements were performed with a spectrometer (ARX 500, Bruker Fällanden, Switzerland) operating at 500 MHz. The average degree of branching (DB) was determined by comparing integrals of peaks for protons in H-1-{1→4} linkage positions and protons in H-1-{1→6} linkages, the former having a chemical shift of about 5.9 ppm and the latter about 5.5 ppm. The DB was determined according to Eq. (1).

\[
\text{degree of branching (DB\%)} = \frac{\text{integral } [H-1-{1→6}] \times 100}{\text{integral } [H-1-{1→4}] + \text{integral } [H-1-{1→6}] + \text{integral } [H-1-{1→6}]}
\]

where H-1-{1→6} refers to the anomeric proton in an α-{1→6} linkage, H-1-{1→4} refers to the anomeric proton in an α-{1→4} linkage and H-1(t) refers to the anomeric proton of a glucose residue at the terminal non-reducing end.

2.6. AF4-UV-MALS-dRI

Prior to AF4 analysis the samples were prepared as described elsewhere (Perez-Rea et al., 2015). The sample vial was heated at 100 °C for 1 h while still stirred. Finally the sample was diluted with Milli-Q water (Millipore Corp., Bedford, MA, USA) at 100 °C to a final concentration of 0.25 mg/mL. The dilution with hot water differs from the method described by (Perez-Rea et al., 2015) as dilution with room temperature water resulted in a very slightly turbid sample which was avoided with hot water. A sample aliquot was immediately injected on to the AF4 separation channel. The c performed using an Eclipse 3+ System (Wyatt Technology Europe, Dernbach, Germany) connected to a Dawn Heleos II multi-angle light scattering (MALS) detector (Wyatt Technology) operating at a wavelength of 658 nm, an Optilab T-rEX differential refractive index (dRI) detector (Wyatt Technology) operating at a wavelength of 658 nm and a UV-detector (Jasco Corporation, Tokyo, Japan) operating at a wavelength of 280 nm.

An Agilent 1100 series isocratic pump (Agilent Technologies, Waldbronn, Germany) with an in-line vacuum degasser and an Agilent 1100 series auto-sampler delivered the carrier flow and handled sample injection onto the AF4 separation channel. Between the pump and the channel a 100 nm pore size polyvinylidene fluoride membrane, (Millipore Corp., Bedford, MA, USA) was placed to ensure that particle free carrier entered the channel.

The AF4 channel was a short channel (Wyatt Technology) with trapezoidal geometry (tip-to-tip length of 17.4 cm and inlet and outlet width of 2.17 and 0.37 cm, respectively) and with a nominal thickness of 250 μm. The ultra-filtration membrane forming the accumulation wall was made of a hydrophilized polyethersulfone membrane (PES) with a cut-off of 10 kDa (Microdyn -Nadir GmbH, Wiesbaden, Germany). Validation of the performance of the AF4 system and experimentally determined channel thickness was performed with bovine serum albumin (BSA) (Sigma, A4378, St Louis, MO, USA) (1 mg/mL, w/v) according to the procedure described in literature using a MATLAB-based software (FFHY-dRad 2.2) (Håkansson, Magnusson, Bergstenstål, & Nilsson, 2012; Magnusson, Håkansson, Janiak, Bergstenstål, & Nilsson, 2012). The actual thickness was determined to be 199 μm for the channel used for sample S0, and 197 μm for the channel used for SBR, SBL, and SBH. The composition of the carrier liquid was 10 mM NaNO₃ (AppliChem, A3125, Darmstadt, Germany) and 0.02% NaN₃ (BDH, 10369, Poole, UK), dissolved in Milli-Q water.

The separation method used a constant detector flow at 1 mL/min. Injection onto the channel was performed with a flow rate of 0.2 mL/min for 4 min. The sample volume injected onto the channel was between 40 and 80 μl for an injected sample mass of approximately 10–20 μg. The injected amount was optimized in order to ensure no overloading occurred i.e. retention time independent of the injected amount. After injection, a 3 min focusing/relaxation step prior to elution with a focus flow identical to the initial cross flow was performed. The cross flow rate was programmed to decay exponentially using Eq. (2).

\[
\text{eq. (2)}
\]

Table 2

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample ID</th>
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</thead>
<tbody>
<tr>
<td>S0</td>
<td>S0</td>
</tr>
<tr>
<td>SBR</td>
<td>SBR</td>
</tr>
<tr>
<td>SBL</td>
<td>SBL</td>
</tr>
<tr>
<td>SBH</td>
<td>SBH</td>
</tr>
</tbody>
</table>

Overview of starch samples.
where $Q_c(t)$ is the crossflow rate as a function of time $t$ after elution starts, $Q_c(0)$ is the initial crossflow rate and $t_{1/2}$ is the half-life of the decay. For samples SBL and SBH, the elution started with an initial cross-flow of 0.30 mL/min and decreased exponentially with time to 0.07 mL/min with $t_{1/2} = 2$ min and then kept constant from 4 to 30 min. Finally, the channel was flushed without any cross-flow for 10 min before the next analysis. For samples S0 and SBR, elution started with an initial cross-flow of 0.75 mL/min and decreased exponentially with time to 0.07 mL/min with $t_{1/2} = 2$ min and then kept constant from 4 to 30 min. Finally, the channel was flushed without any cross-flow for 10 min before the next analysis. Data was recorded using Astra software version 5.3.4.20 (Wyatt Technology). Molar mass and $r_{\text{rms}}$ were obtained using the Berry method (Andersson, Wittgren, & Wahlund, 2003; Berry, 1966) by performing a 1st order fit to the data obtained at scattering detectors 7–14 (angles 69.3–121.2°) for samples SBL and SBH at scattering detectors 7–14 (angles 51.5–121.2°) for samples S0 and SBR. A specific refractive index increment ($dn/dc$) of 0.146 mL/g was used for amylpectin in water (Brandrup, Immergut, & Grulke, 1999). Apparent densities were calculated from molar mass and $r_{\text{rms}}$ data (Nilsson, 2013).

3. Results

3.1. Chemical analysis of extracted starch samples

The results from moisture content, total starch and total protein are shown in Table 3. The moisture content in the samples is rather similar and ranges between 11 and 12%. The total starch content ranges between 75 and 88%, with sample S0 having the highest starch content. It is possible that retrograded starch is lost already during the extraction procedure due to its poor aqueous solubility influencing the recovery. The protein content is higher in all samples extracted from bread (2.5–3.2%) compared to the sample extracted from the wheat flour (S0, 1.6%).

3.2. Degree of branching (DB)

Fig. 1 shows a representative $^1$H NMR spectrum for the samples (illustrated by sample SBL). The DB is 4.6%, the anomic protons of the terminal reducing ends, i.e. $\alpha$-H-1($\alpha$-$\beta$) linkage position (5.7–5.8 ppm) and $\beta$-H-1($\beta$-$\gamma$) linkage position (5.2 ppm), display integrated values 7.48 and 8.09 respectively. An overview of DB and values of anomic protons of the terminal reducing ends for all samples is given in Table 3. The results show a considerable difference in DB between S0 and all other samples, indicating that DB increases as a result of the baking process. Furthermore, addition of $\alpha$-amylase seems to increase the DB somewhat, i.e. SBL and SBH show higher DB than SBR (Table 3). It can also be noticed that the number of reducing ends H-1($\beta$-$\gamma$) and H-1($\alpha$-$\beta$), increases as a result of baking and increases even further as a result of $\alpha$-amylase addition.

3.3. Molar mass, size, and apparent density

AF4-UV-MALS-dRI was used to determine the molar mass (M), root- mean-square radius ($r_{\text{rms}}$) and apparent density ($p_{\text{app}}$), the analysis was performed in triplicate. The AF4-UV-MALS-dRI fractograms, with M overlayed are shown in Fig. 2. The results show that the samples have a wide M range i.e. from approximately $2 \cdot 10^4$ to $>10^8$ g/mol. At short elution times (less than approximately 10–11 min) it is not possible to determine the M in a reliable manner as the quality of the MALS-signal is insufficient. Furthermore, analytes eluting close to the void time ($t_0$) appear to contain both analytes eluting in the Brownian normal mode of AF4 as well as analytes co-eluting in the so called steric-hyperlayer mode (Caldwell, Nguyen, Myers, & Giddings, 1979) which makes the molar mass determination unreliable in this range. For all the samples, UV absorbance can be observed in the fractograms (Fig. 2) which could indicate the presence of UV-absorbing analytes. However, the UV-signal is non-straightforward to interpret for larger analytes as the absorbance is apparent due to light scattering contributions. Hence, the absorbance can only be interpreted for relatively small analytes (i.e. short elution times, approximately <12 min). In any case, it appears

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture a (%)</th>
<th>Total starch b (wt%)</th>
<th>Total protein b (wt%)</th>
<th>DB (%)</th>
<th>H-1(1→6)</th>
<th>H-1($\beta$-$\gamma$) c</th>
<th>H-1($\alpha$-$\beta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>11.4 ± 1.9</td>
<td>87.9 ± 1.5</td>
<td>1.6 ± 0.1</td>
<td>3.6</td>
<td>3.7</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>SBR</td>
<td>11.7 ± 1.1</td>
<td>79.7 ± 1.0</td>
<td>3.2 ± 0.1</td>
<td>4.2</td>
<td>4.4</td>
<td>4.9</td>
<td>5.6</td>
</tr>
<tr>
<td>SBL</td>
<td>11.9 ± 1.5</td>
<td>75.4 ± 1.0</td>
<td>2.6 ± 0.1</td>
<td>4.6</td>
<td>4.8</td>
<td>8.1</td>
<td>7.5</td>
</tr>
<tr>
<td>SBH</td>
<td>11.9 ± 1.5</td>
<td>77.8 ± 0.5</td>
<td>2.5 ± 0.05</td>
<td>4.6</td>
<td>4.8</td>
<td>9.1</td>
<td>9.4</td>
</tr>
</tbody>
</table>

a Moisture, total protein and total starch measurements include standard deviation (±) based on three (and for the starch analysis) six replicates.
b Values reported on dry basis.
c The H-1($\alpha$-$\beta$) and H-1($\beta$-$\gamma$) are from the $\alpha$ and $\beta$ anomic protons of the terminal reducing ends, respectively these were obtained from integration of the peak areas performed at fixed ppm values on the $^1$H NMR spectrum of each sample as explained in Section 2.5.
that there are (especially for the samples from bread) UV-absorbing species at short retention times (i.e. low MALS signal and considerable UV-signal). This may originate from the relatively low amount of protein present in the samples (Table 3) and could possibly, also be related to reaction products from the baking process.

Average data obtained are reported in Table 4. From the results it can be seen that all samples are high M species with an M weight-average of $M_w = 4.0 - 2.0 \times 10^7$ g/mol and $z$-average $r_{\text{rms}}$ between 59 and 115 nm. All reported $M_w$ were significantly different between all samples while the average $r_{\text{rms}}$ was significantly different between all samples, except for SBL and SBH, according to Tukey's test (Table 4). The average $q_{\text{app}}$ increases as a result of the baking process (SBR) and increases even further with addition of α-amylase. Table 4 also shows the mass recoveries from the separation channel. The mass recoveries are obtained from the integrated dRI-peak and based on the experimentally determined starch concentration (Table 3). The recoveries vary considerably between the samples, being low for S0 (51%) and high for SBH (126%). The reason for the low mass recovery of S0 is difficult to explain. A major challenge is related to the difficulty in drawing appropriate baselines for the dRI-signal as very low amounts are injected, in order to avoid overloading, making the mass recovery rather uncertain (Perez-Rea et al., 2015). Another reason for low mass recovery could be immobilization of the macromolecules at the accumulation wall which are then eluted as a “release peak” when the crossflow is turned off. However, no such release peaks were observed.

4. Discussion

Bread baking is a complex process, involving chemical as well as enzymatic reactions originating from both native and added enzymes which affect starch structure and size. These reactions can involve proteinaceous matter (Maillard type reactions) (Hill & Patton, 1947) which may not be detectable in the $^1$H NMR spectra as they could be present in the large unresolved peaks (approximately 5–4 ppm, Fig. 1). The presence of various reaction products is likely, as UV-absorption could, in some cases, be observed in the AF4 results (Fig. 2).

The DB of the starch extracted from wheat flour (S0) is based on the whole composition of the extracted starch sample i.e. including the largely unbranched amylose. Hence, in order to compare DB with values for amyllopectin from literature, it is necessary to correct DB for amylose content. Typical amylose content in wheat starch is approximately 20–30% (Cornell, 2004). Thus, by assuming an amylose content of 25% the DB (Table 3) for the amyllopectin in
the investigated sample can be estimated to 3.6/0.75 = 4.8%. This is comparable to DB reported in literature for waxy barley starch (DB 5.1%) (Fernandez, Rojas, & Nilsson, 2011) and maize amylopectin (DB 4.8%) (Nilsson et al., 1996).

The baking process results in an increase in DB (Table 3). The increase may appear puzzling as it would correspond to an increased number of branch points in the molecules. A likely explanation is that the increase in DB is due to the loss of (1→4) linkages during hydrolysis (i.e. the decrease in molar mass) which can result in products which are not extracted in the sample preparation procedure. Hence, this would give rise to a relative change in the ratio between (1→6) and (1→4) linkages.

From the AF4 results, there is a significant difference between the average molar mass of the samples (demonstrated by the statistical Tukay test). Also, this difference or variation is higher before the baking process and without the addition of the α-amylase (Table 4). Similarly, the same trend was observed for the average \( r_{\text{rms}} \), except between SBL and SBH samples, that there is not a significant difference. The reduction in M and size may be both due to the presence of intrinsic amylopectin enzymes.

Furthermore, the high temperature during the baking process may also give rise to some degradation of the starch molecules (Perez-Rea et al., 2015). The temperature is also supported by the higher number of reducing ends (H-1(α-r) and H-1(β-r)) (Table 3).

Furthermore, the apparent density of the molecules increases as a result of the baking process (Table 4). A possible interpretation is that the increased apparent density is caused by de-branched of the molecules, resulting in species with lower mass per volume ratio. However, there is no support for this hypothesis in the DB results (as it was mentioned). The increase in apparent density may indicate that the hydrolysis of the starch is non-random. After the baking process, the degradation is rather limited (i.e. the molar mass remains high) and, thus, a random degradation pattern would be expected to result in reaction products, smaller in size, but with similar density.

The increase in H-1(α-r) is logical considering the enzymatic action of α-amylase resulting in an α-configuration at the reducing end of the anomic carbon of the formed products (Robyt, 2009). Moreover, considering that Novamyl 10000 BG can act in an exo mechanism and bind glucose residues to the non-reducing ends of starch chains, frequently producing β-configuration at the anomic carbon (Christophersen et al., 1998; Robyt, 2009). Similarly, a decrease in M and \( r_{\text{rms}} \) is observed with the addition of α-amylase (possibly with a very weak dependence on the amount of added α-amylase) compared to bread baked without enzyme addition (Table 4).

5. Conclusion

This is the first time that techniques like AF4 and \(^1\)H NMR are combined in order to study the changes in molar mass, size and structure of starch during the bread baking process. The results show that we observe decrease in size and molar mass as an effect of baking while an increase in apparent density is observed. The effects are larger upon addition of α-amylase.

The reduction in molar mass is relatively modest and the starch remains as a very large macromolecule after the baking process. Furthermore, changes in the chemical structure of starch (as for instance an increase in DB) can be observed as a result of baking.

Conflict of interest

We declare that we have no conflicts of interest in the authorship or publication of this manuscript.

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References


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