Abstract

Besides the application of high pressure (HP) as a non-thermal preservation technology, HP could additionally have a deep impact on the material properties of the treated food. Especially the HP induced swelling and gelatinization of starch influences the processing properties of starch-based food systems and differs in comparison to thermal induced gelatinization. The aim of this study was to examine the impact of HP on starches under different conditions and to influence systematically the gelatinization and pasting properties of wheat starch by the addition of various types of sugar. Caused by limitation of conventional methods, this study also includes the development of an appreciate method based on the particle size measurements of the starch granules. Three methods of measuring particle sizes were examined for an application for pressure treated starches. Finally, an image analysis with microscope, camera and image processing software ImageJ was chosen to perform the analysis. Wheat, tapioca and potato starch with concentration of 5 (w/w) and 25 (w/w) were pressurized at 600 MPa for ten minutes at 20 °C as well as 60 °C, to reach treatment conditions which are suitable for HP food pasteurization. The results showed that ultra HP significantly increased the particle size of the starch granules, whereas the degree of swelling was starch type and temperature dependent. High starch concentrations resulted in a limited swelling caused of the limited water content. This effect is enhanced with increasing swelling properties. Besides this, sugar caused a significant decrease of the granules size. A dependence of this effect with the type of sugar was not examined. This work should be a contribution to expand the understanding of the swelling mechanisms of starch granules under HP and should facilitate a future process and product development of HP pasteurized starch based products.

Keywords: High pressure; Starch; Influence of sugars on the degree of gelatinization; Microscopic image analysis

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1. Introduction

During the last decades, the growing consumer demand for minimally processed, fresh-like, safe, and high quality food products triggered research efforts in the field of alternative food processing technologies. The application of high pressure (HP) has been evaluated as a promising food processing alternative to classical heat treatment technologies in several studies, which was first reported by Hite on 1899 [1], who achieved an extended shelf life of bovine milk after HP treatment.

However, besides the inactivation of microorganism, HP could additionally have a considerable impact on the material properties of the treated food and could, hence, well be used for food texture engineering, owing to its influence on the properties of food ingredients.

Generally, the primary structure of low molecular weight molecules such as peptides, lipids, vitamins, and saccharides is rarely affected by isostatic HP because of the very low compressibility of covalent bonds at pressures below 2 GPa. Conversely, HP can change the native structure of macromolecules such as proteins, enzymes and starch granules.

Especially the impact of HP on gelatinization of starch could influences the processing properties of starch-based food systems, whereas the pressure induced gelatinization differs in comparison to the thermal induced one [2]. The HP induced gelatinization effects limited swelling accompanied by retaining granular structure and loss of birefringence under polarized light [3]. The last attribute is used usually to determine the gelatinization degree [3]. The Extend of starch gelatinization is dependent on the applied pressure, the dwell time and the treatment temperature [4], whereas the type of starch has the highest impact on its gelatinization behavior under pressure. Starches with B-type crystalline structure, such as potato, which are generally found in roots are more resistant to pressure than A or C-type starches like e.g. wheat starch [2]. However, only a few studies have evaluated how other food components interfere with starch gelatinization under pressure, whereas this topic is of great importance for HP applications of starch containing food products [5].

In general one could point out that amount of free water plays an important role in the process of thermal starch gelatinization as the gelatinization temperature decreases with decreasing water content of the starch suspension. Additionally the presence of alcohol, alkali, lipids, salts, organic acids and sugars have an impact on the gelatinization temperature and, thus, affects the extended of gelatinization during thermal treatments [6]. Especially the effects of sucrose on the gelatinization behavior of several starches has been investigated in detail and it was found, that with an increasing sucrose concentration the gelatinization temperature increased as well [7-11]. Sucrose also caused a rise in the pasting temperature [12] and an increase in the starch melting enthalpy [9, 13]. Other low-molecular sugars such as fructose, glucose and maltose exhibited equal effects on the thermal starch gelatinization but influenced the gelatinization characteristics to different extents [7, 9, 14].

The influence of different low-molecular sugars on the pressure induced gelatinization of different types of starches showed, that sugars decreased in dependence to their number of equatorial OH-groups the loss of birefringence [4].

Therefore, this work should verify the idea to influence systematically the gelatinization and pasting properties of wheat, tapioca and potato starch by adding various sugars and vary treatment temperature during high pressure processing.

2. Materials & Methods

2.1. Sample preparation

Wheat, Tapioca and potato starch (5 w/w and 25 w/w) were suspended in deionized water. To prevent a sedimentation of the starch granules and resulting inhomogeneities, 0,1 % (w/w) Xanthan was added.

This concentration achieved sample stabilization for the treatment period. Furthermore, saccharose
(c = 0.438 mol g⁻¹) and maltose (c = 0.416 mol g⁻¹) were added all samples and additionally melizitose (c = 0.287 mol g⁻¹) to the wheat starch samples.

2.2. Pressure treatment

The HP treatments were performed at a pressure of 600 MPa for ten minutes at two different temperatures. Treatments at 20°C were performed in the U4000 High Pressure Single Vessel unit (Unipress, Warszaw, Poland) and treatments at 60°C were performed in the HP sterilization unit (0101-7000-S, Sitec Sieber Engineering AG, Zurich, Switzerland). After pressurization the samples were homogenized or resuspended if it was necessary, diluted to an applicable starch concentration and stored on ice.

2.3. Analytic Methods

An image analysis, realized with microscope (Nikon, Eclipse E400, Tokio, Japan) and camera (Pixellink fire wire camera PL-A662, Ottawa, Canada), was used to measure particle size distribution. Image processing was performed with ImageJ (V. 1.42q, US National Institute of Health, Bethesda, USA). Image processing included the steps of assembling, enhancing the contrast, threshold based segmentation, watershed operator in purpose of agglomerate separation and particle size measurement (Fig 1. B-D). To increase the contrast between sample and background, the starch granules were stained with potassium iodine iodide solution. At minimum 5000 particles were counted. This method was calibrated with 3 μm latex beads (Polystyren standard, Malvern Instruments GmbH, Herrenberg, Germany) and with an Abbe-Zeiss counting chamber. The described method was validated by a laser particle sizer (Laser Scattering Particle Size Distribution Analyzer LA-950, Horiba Ltd., Kyoto, Japan). The resulting particle size distributions were fitted with FFT-Filter.

Fig. 1. Image processing (100x magnification): native wheat starch (A), HP treated wheat starch at 600 MPa, 20°C, 10 min (B), threshold segmentation (C), binary picture (D).
3. Results & Discussion

The commonly used method to determine the degree of gelatinization of pressurized starch granules is to determine the loss birefringence under polarized light of each individual starch granule by counting them in a counting chamber. However, this method has some disadvantages like the low quantity of counted granules (usually less than 250), the fact that granules which are off focus in the light microscope sometimes appear not to be birefringent even if they are, which is accompanied by a subjective counting of each individual person. To avoid these problems, an image analysis particle size measurement system was developed. To calibrate and test this system transparent latex-beats with 3 μm diameter were used and analyses with 1000 x magnification. The determined mode diameter with the image analysis particle size measurement system was 2.75 μm and the average diameter was 2.78 μm. Hence, the deviation was less than 10 % and could be regarded as sufficient. Furthermore, the self developed system was validated by its comparison to a conventional particle size measurement system (Table 1).

Table 1. Specific values for native wheat and tapioca starch determined with the self developed microscopic image analyses system and a conventional laser particle sizer

<table>
<thead>
<tr>
<th></th>
<th>Image J particle size measurement system</th>
<th>Laser particle sizer</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Wheat starch</td>
<td>Tapioca starch</td>
</tr>
<tr>
<td>Average diameter [μm]</td>
<td>13.31</td>
<td>12.98</td>
</tr>
<tr>
<td>Minimal particle size [μm]</td>
<td>2.19</td>
<td>2.19</td>
</tr>
<tr>
<td>Maximal particle size [μm]</td>
<td>55.33</td>
<td>44.47</td>
</tr>
<tr>
<td>Range [μm]</td>
<td>53.14</td>
<td>42.27</td>
</tr>
<tr>
<td>Number of counted particles</td>
<td>17119</td>
<td>20187</td>
</tr>
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</table>

By the reason of the good correlation of all specific particle size values the developed particle size measurement system was regarded as suitable for the determination of particle size distributions for native and pressurized starches, in a measurement range from 2.19 μm up to 500 μm. The advantage of method is, besides the detection of quantitative values for changes in the particle size distribution, that also a visual analysis in regard to the formation of agglomerates and changes in the shape of individual granules is possible. Moreover, the previous staining with potassium iodine iodide solution, gives an indication for a possible leaching out of amylose from the starch granules after pressurization.

The results for the different kind of starches showed, that the granules increased their size significantly after pressure treatment (Fig. 1A, Fig. 2-4). Furthermore, the amount of available water in the wheat starch suspensions had an impact on particle size distribution. Limited availability of water in the high concentrated suspensions (25 %) caused presumably less swelling and consequently a decrease of particle size (Fig. 2 A).
This behavior was also found for potato and tapioca starch, where a decrease of the average diameter of 9.37 μm and 10.068 μm, respectively, was measured in the high concentrated (25 % w/w) starch suspensions.

However, in general the extend of particle size increase followed the thermal swelling power of the different kinds of starches. Therefore a stronger increase in the mean particle size for tapioca and potato starch was measured in comparison to wheat starch (Fig. 2-4).

Furthermore, especially for the high concentrated starch suspension of tapioca and potato starch an increased potassium iodine iodide staining of the background was observed, which could be an indicator for a fractional release of amylose during the pressure treatment. This behavior was also reported by Buckow and Heinz [3] for pressurized corn starch, whereas no background staining occurred for the wheat starch samples, which was also previously reported by Michel and Autio [15]. Hence, this is an indication for the different behavior of A-Type starches (mainly from roots) under pressure in comparison to the more pressure sensitive B- and C-type starches from cereals.

<table>
<thead>
<tr>
<th>Type of Sugar</th>
<th>Number of counted particles</th>
<th>Average diameter [μm]</th>
<th>Mode diameter [μm]</th>
<th>Minimal particle size [μm]</th>
<th>Maximal particle size [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>20187</td>
<td>12.276</td>
<td>10.75</td>
<td>2.192</td>
<td>44.265</td>
</tr>
<tr>
<td>native</td>
<td>1463</td>
<td>25.266</td>
<td>7.25</td>
<td>2.192</td>
<td>133.294</td>
</tr>
<tr>
<td>Sucrose</td>
<td>14201</td>
<td>19.663</td>
<td>10.75</td>
<td>2.192</td>
<td>86.141</td>
</tr>
<tr>
<td>Maltose</td>
<td>22689</td>
<td>21.558</td>
<td>10.75</td>
<td>2.192</td>
<td>119.889</td>
</tr>
</tbody>
</table>
The results for the addition of different types of sugars were contradictory. The added sugars decreased the granule swelling and all sugars cause approximately equal size distribution (Fig. 2-4). However, a loss of birefringence under polarized light of the individual starch granules was observed. Obviously, the number of equatorial OH-groups affected the gelatinization degree determined by a loss of birefringence, but did not affect the granule size. Presumably, these effects are based on different mechanism.

<table>
<thead>
<tr>
<th>Type of Sugar</th>
<th>Number of counted particles</th>
<th>Average diameter [μm]</th>
<th>Mode diameter [μm]</th>
<th>Minimal particle size [μm]</th>
<th>Maximal particle size [μm]</th>
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<td>28.311</td>
<td>14.75</td>
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<td>168.972</td>
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<td>native</td>
<td>3144</td>
<td>43.977</td>
<td>14.25</td>
<td>2.192</td>
<td>369.234</td>
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<tr>
<td>Sucrose</td>
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<td>28.975</td>
<td>14.75</td>
<td>2.192</td>
<td>134.946</td>
</tr>
<tr>
<td>Maltose</td>
<td>11633</td>
<td>26.038</td>
<td>14.75</td>
<td>2.192</td>
<td>185.151</td>
</tr>
</tbody>
</table>

Fig. 4. Particle size distribution of native (black, solid) and pressurized potato starch (5 % w/w) without the addition of sugar (blue, dashed) and with the addition of sucrose (red, dotted), maltose (green, dashed-dotted) at 20°C and its corresponding specific values from the optical particle size analysis.

However, an impact of the number of equatorial OH-groups of the different kind of sugars could not be verified based on the particle size distributions. This inhibitory effect of sugars on the increase of particle size can be attributed to the reduction of mobility of the solvent (water) and a decrease of the a_w-value, thereby impeding the penetration of water into the starch granules. Spies and Hoseney [8] suggested that sugar-starch interactions stabilize amorphous regions by sugar molecules forming bridges between starch chains and hence increase the energy requirement for starch gelatinization.

A direct comparison of the particle size distribution of pressurized starch suspensions measured with our self developed system and literature data is with best of the authors’ knowledge actually not possible, due to a lack published data. Only Stute et al. has published data for a particle size distribution of pressurized tapioca [2] and wheat starch [16] measured with a laser particle sizer. The range of the reported particle size distributions correlate quite well, but a direct comparison is not possible due to the lack of information about the concentration of the starch suspensions.

This work showed also, that the addition of sugars has not caused a phase transition in pressurized wheat starch. All wheat starch samples, which formed a starch gel, retained their pasty consistence even by adding sugars. Additional, rheological analyses underlined this fact and even showed an increase of viscosity after the addition of sugars. Nevertheless, the addition of sugars dramatically influenced the texture of the highly concentrated tapioca and potato starch suspensions. The pure starch suspensions formed a gel during the pressure treatment, whereas with the addition of sugars a gel formation was inhibited and only a paste like consistence was achieved.

4. Conclusions

The developed method to measure the particle sizes by image analysis proved to be an applicable method to examine influences on the particle size of pressurized starches. It was shown that sugars affected the particle size and consequently the swelling of the granules. Furthermore the water content is another important factor, in regard of the degree of swelling.
This study highlights the influence of various sugars on the properties of pressure induced starch gels and is relevant for HP pasteurization of starch-based food systems. Moreover, using controlled structure engineering in starch-based food systems possibly facilitates the food industry to use pressure-treated starches for different proposes, e.g. as substitute for fat in dietary foods.

References


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