Changes of Physical Properties of Wheat Gluten and Starch as a Function of Removing Some Attending Substances

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1 Introduction

The main components of wheat are protein (13 %) and starch (65 %). Wheat endosperm protein so called gluten is unique in possessing cohesive and viscoelastic properties. Physical properties of the gluten complex are related to all kinds of molecular interactions between very different protein components (gliadin- and glutenin subunits), carbohydrates and lipids [1–4].

Aggregates are stabilized by covalent disulfide and non-covalent bonds. Non covalent interactions such as hydrophobic, hydrophilic and ionic bonds are important too. The types of interactions are difficult to determine and good understanding of the mechanism of aggregation is missing [5–8].

Starch molecules can be characterized on the one hand by crystalline and amorphous structures of amylose and amylpectin molecules and on the other hand by a molecular degree of order which is fixed in starch granules. Shape and superstructure of wheat starch are enveloped with lipoproteins which are responsible for starch granules hydrophobic properties. These substances are important for interaction of gluten and starch in a dough. The aim of our experiments was to get some information about the influence of attending substances on physical properties and about mechanism of interaction behaviour between gluten and starch [9–17].

2 Materials and Methods

Commercial wheat gluten was extracted with ethanol by different procedures. Stirring in abs. ethanol and Soxhlet extraction with different numbers of cycles have been the main point of our interest regarding changes of gluten properties.

2.1 Preparation of single components by extraction

2.1.1 Effects of extraction on protein characteristics

The efficiency of the different extraction procedures can be correlated to the extractable amount of substances (Fig. 1).

Regarding the total amount of extractable material from native gluten it can be seen, that Soxhlet extraction with ethanol using 45 or 90 cycles is much more effective than magnetic stirring at room temperature even though when cysteine, which possesses a reductive force concerning gluten was added to the mixture. Although adding cysteine stirring doesn’t lead to an increased amount of extractable substances. The same trend can also be seen regarding the extractable amounts of nitrogen and lipids.

Properties of solubility reflecting hydrophobic or hydrophilic changes in gluten caused by extraction can be regarded as an indicator for different efficiencies of extraction methods.

The different procedures of extraction lead to a shift of pH values of maximum and minimum solubility compared to vital gluten (Table 1). The elution profile of phosphate buffer extracts from different gluten preparations can be regarded as an indicator for gluten alteration too (Fig. 2). For the effi-

<table>
<thead>
<tr>
<th>Solubility as function of pH highest sol.</th>
<th>lowest sol.</th>
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<tbody>
<tr>
<td>KA 2,4</td>
<td>7,8</td>
</tr>
<tr>
<td>K4 3,4</td>
<td>7,5</td>
</tr>
<tr>
<td>K5 2,1</td>
<td>7,2</td>
</tr>
<tr>
<td>K6 2,1</td>
<td>6,9</td>
</tr>
<tr>
<td>K7 2,2</td>
<td>5,7–9,2</td>
</tr>
</tbody>
</table>

Gluten and starch, the main components of a dough were extracted with different solvents and conditions, without heat or mechanical denaturation.

By removing substances especially lipids and lipoproteins physical properties of gluten and starch are altered. Solubility, water absorption capacity and rheological properties of ethanol extracted gluten were changed as a function of different conditions of extraction.

Extracting the lipids of starch alterations of the kernel starch surface properties can be observed by TEM. Most changes of physical properties become evident above all when starches are gelatinized by heating. But this behaviour is not important for binding starch and gluten during dough kneading.

Intensive extraction of lipid substances from gluten leads to an enhanced waterbinding capacity, but at the same time the ability to form a homogenous wet gluten structure is diminished. Rheological studies in ethanol-water slurries point to variations in viscosity behaviour.

For physical and microscopical studies doughs of gluten-starch mixtures were prepared from gluten, differently extracted starches and water soluble substances. Freshly prepared flour extract was added to dry mixtures (82 % starch, 18 % gluten) and the dough system was characterized in a mixograph and in a rotation viscosimeter. Doughs prepared with same amounts of extract showed differences in viscosity and viscoelastic properties. In general the extraction of lipids from gluten influenced dough properties in a higher degree than extraction of starch. More intensive extraction of gluten causes disturbance of dough structure. A certain restoration of viscoelastic properties of dough is attainable by adding more water soluble substances.
ciency of the extraction method profiles differ in their chromatographic behaviour especially the profile of cysteine treated gluten is characterized by a significant curve.

Surprisingly it became evident, that although different amounts of protein and lipid were removed, the rehydration capacity is not affected significantly (Fig. 3). Only gluten, treated with cysteine and subsequently Soxhlet extraction shows an increase in water binding capacity more than 100%.

Rheological behaviour measured in ethanolic suspension (70%) is shown in Fig. 4. Different procedures of treatment are reflected in altered flow curves compared to the original

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Fig. 1. Total extractable amount (%) from native gluten.

Fig. 2. Elution profiles of phosphate-buffer extracts from different gluten preparations.

Fig. 3. Rehydration behaviour of gluten preparations.
properties of vital gluten. In accordance to changed rehydration profiles cystein treated gluten possessed a significant decrease of flow curves compared to the vital gluten. This indicates the very important role of disulfide bond bridges responsible for the gluten structure. Extraction methods without cysteine influence viscosity behaviour in a very low degree. A systematical trend can not be recognized.

Thermoanalytical characterization of gluten preparations by DSC gives some differences in thermograms, which cannot be correlated to structural differences at the moment (Fig. 5).

2.1.2 Effects of extraction on starch properties

Extracting wheat starch, one main purpose, consists in preserving the granule structure of the molecules. Therefore, we have chosen conditions of extraction which does not change the starch granule structure. As native starch granules extracted with ethanol revealed strong adsorption of amido black on their surface, whereas starch granules extracted with SDS-solution didn’t absorb the dye. In all samples we observed double refraction microscopically, indicating that starch has not been gelatinized (Fig. 6). In this Figure the photographs marked with number 3 and 4 show native starch at different ranges of enlargement.

Figures 5 and 6 show starch granules extracted with 70% ethanol. Figures 7 and 8 show wheat starch granules after a treatment in the Twisselman apparatus. Photographs of starches treated with SDS respectively with SDS and mercaptoethanol are shown in Figures 9, 10, 11 and 12. Regarding these photographs in detail it can be seen that the starch granular structure was not disturbed. The extracts of starch contained lipids especially lysophosphatidylcholine and phosphatidylethanolamin and in smaller amounts free fatty acids and galactosyldiglycerides, known as characteristic lipids of thylacoid membranes.

Looking for the proteins of SDS- and ethanol extracts we found that molecular weights varied in the range between 35.000 and 45.000 Daltons; in acid buffer they had an electrophoretic pattern comparable to wheat gliadin, but the amino acid composition was quite different.

The ethanol extracts contained glucose, fructose, saccharose and maltose in small amounts, some of glucose was bound
to protein and could be separated by hydrolysis with sulfuric acid only indicating that there exists a real glycoprotein.

The rheological properties of extracted starches were significantly influenced by different extracting conditions (Table 2). Extraction with the polar ethanol/water solvent resulted in a decrease of the pasting temperature and in a considerable increase in peak viscosity and viscosity after cooling. It is apparent, that conditions of extraction affect the rate of decrease of pasting temperature and the rate of increase of peak viscosity after cooling. The extraction with 70 % ethanol at room temperature decreased the pasting temperature from 82.5 to 75 °C and by raising extraction temperature to 50 °C pasting temperature falls down to 69 °C. A drop to 56 °C was observed when extraction was performed with 1 % SDS + 1 % mercaptoethanol. In this case peak viscosity increases to more than 2,000 BU and the paste was transparent.

Quantitative rheological measurements on the Rheolab MC20 (Fig. 7) show the behaviour of shear tension as a function of temperature.

SDS-treated samples show a strong increase of shear tension within a small range of temperature to a maximum value and with increasing temperature a decrease, whereas the shear tension of the other samples increase slowly with different intensities within comparable broad temperature range.

DSC-measurements to determine transition temperature and enthalpy can only be correlated partly to different starch treatments (Table 3). Our DSC-thermograms of starch and extracted starch show only one endothermal desintegration peak. A second peak above 90 °C described in literature as

**Tab. 2.** Effect of extraction method on the rheological properties of wheat starch.

<table>
<thead>
<tr>
<th>Extraction Method</th>
<th>Pasting Temp. (°C)</th>
<th>Peak viscosity (BU (M1))</th>
<th>Viscosity after cooling (BU (M2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Starch</td>
<td>82,5</td>
<td>380</td>
<td>119</td>
</tr>
<tr>
<td>Starch, 70 % ETOH/RT</td>
<td>75,0</td>
<td>460</td>
<td>1570</td>
</tr>
<tr>
<td>Starch, 50 °C/70 % ETOH</td>
<td>69,0</td>
<td>585</td>
<td>2130</td>
</tr>
<tr>
<td>Starch, 160 h/80 % ETOH</td>
<td>67,5</td>
<td>655</td>
<td>2280</td>
</tr>
<tr>
<td>Starch, 1 % SDS + 1 % ME</td>
<td>56,0</td>
<td>2000</td>
<td>1680</td>
</tr>
</tbody>
</table>

**Tab. 3.** Effect of extraction method on the thermodynamical properties of wheat starch.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Transition temp. (°C)</th>
<th>Enthalpy (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Starch</td>
<td>57,85</td>
<td>7,27</td>
</tr>
<tr>
<td>Starch, 1 % SDS</td>
<td>58,95</td>
<td>9,01</td>
</tr>
<tr>
<td>Starch, 50 °C/70 % ETOH</td>
<td>59,95</td>
<td>7,25</td>
</tr>
<tr>
<td>Starch, 160 h/80 % ETOH</td>
<td>57,45</td>
<td>7,00</td>
</tr>
<tr>
<td>Starch, 1 % SDS + 1 % ME</td>
<td>58,25</td>
<td>7,70</td>
</tr>
</tbody>
</table>

**Fig. 6.** Microscopic pictures of different treated starches.

**Fig. 7.** Rheological behaviour as a function of extraction procedure.
reversible desintegration peak of the amyllose-lipid complex [18], could not be measured (Fig. 8).

From our results of physical and rheological measurements it can be assumed, that extraction might also affect the structural organisation of granules.

In order to proof our assumption we studied thin sections of starch granules after different extraction procedures by TEM. After fixing and contrasting with potassium permanganat starch granules exhibited a fibrous basic structure in different extent. The ethanol-treated starch, especially, revealed a markedly fibrous matrix (Fig. 9).

Treated starch granules showed a shell-like structure additionally, indicated by electron dense rings, alternating with more electron-transparent ones. The rings and lamellae of ethanol extracted starches seemed to be more contrasted than in the SDS-starch (Fig. 10). Comparing thin sections of SDS- and ethanol-treated starch granules it becomes evident, that treatment with detergent like SDS brings more profound alterations in structural organization of the granules. It can be suggested, that SDS-treatment is much efficient.

2.2 Gluten starch interaction as a model system

The recombination of modified single components to a gluten starch system similar to a wheat flour dough leads to a significant change of energy in comparison to a gluten starch mixture with unmodified components.

It is noticeable that exhausted extraction of the lipid material from gluten leads to a decrease of energy units (Fig. 11).

Measurements in dough-like systems with a mixograph demonstrate the influence of modified gluten as well as the role of modified starch in systems of recombination. At the moment the influence of SDS-treated starch in gluten-starch model systems is mysterious, but it is well known that SDS influences wheat starch characteristics in different manner. We cannot explain that on one hand native gluten and SDS-starch show under corresponding conditions a higher viscosity than the standard system. It may be, that the SDS-extraction increases the hydrophilic character of the starch granule and therefore the attraction forces between gluten and starch are reinforced. In contrast extracted gluten and SDS-starch have a small or no measurable viscosity. Similar results with same correlations are demonstrable in systems measured with a rotation viscosimeter. It is obvious, that forces which are responsible for sticking starch and gluten are influenced by lipids and or lipoproteins from gluten and starch. The TEM photographs of doughs show this in an impressive manner (Fig. 12).
In the left picture, a normal gluten/starch mixture is shown whereas the right one shows a system consisting of modified components. Both systems are prepared under corresponding conditions. It can be seen that the interactions within the systems are quite different.

3 Conclusion

The remove of extractable material from gluten under non denaturating conditions with ethanol, with and without reduction by cysteic acid, changes physical properties of gluten, especially functional interactions which lead to aggregates of macromolecules are influenced in different degree. Exhaustive extraction of gluten with ethanol results in a nearly lipid free product. Depending on the lipid content solubility characteristics and water binding capacity of gluten are influenced. By reduction and extraction gluten becomes water resistant and high voluminous when water is added. Reduced gluten loses its viscous and elastic characteristics. This demonstrates, that attending substances like lipids are very important for gluten properties but it is confirmed that disulfide bridges are mainly responsible for building up the structure of gluten.

Lipids and proteins, present as a film on the surface of wheat starch kernels influence the properties of the wheat carbohydrates. The question, if the increase of viscosity by removing off the “granule film” is caused by variation of molecular arrangement of starch, by an increased penetration of solvent into the starch kernel or by some unknown factors cannot be answered. We cannot explain, that different physical measurements like viscosimetric data and DSC-thermograms of extracted starches do not agree completely.

According to our results with DSC, we only found one endothermal desintegration peak independent from the extraction method. Regarding this experimental result and keeping in mind, that starch lipids have been extracted without gelatinization of wheat starch we believe, that a part of these lipids is associated with starch components inside the granule without forming helical inclusion complexes of amylose.

The interaction between starch and gluten is dependent from the presence of the protein film and phospholipids on the starch kernel, which give them hydrophilic character and on the other hand from the status of the gluten complex. The viscoelastic behaviour of a dough made from native gluten with SDS-extracted wheat starch was superior to the standard but TEM photographs of dough with SDS starch show a very limited protein network embedding starch kernels. It seems there is a different mechanism which promotes the viscosity and the starch protein interaction.

Bibliography

The influence of high pressure on gel forming maltodextrins. Part II: Investigation of the primary structure, discussion and conclusions. The influence of static high pressure (600 MPa) on physical and chemical properties of maltodextrin powders, which have had low degrees of hydrolysis (DE 2-8%) and water contents of 5-24% w/w, and maltodextrin gels (15% w/w) have been investigated. No cleavage of covalent bonds has been proved. High pressure treatment results in structural changes depending on water content, ageing and specification of the material. Structural changes concern the physical micro- and macro-structure. In comparison with the untreated material, a more compact maltodextrin powder with an increased solid-density has been produced. The physico-chemical properties have changed after high pressure treatment. We found a series of weak glass transitions with pressure-treated as well as non-treated maltodextrin powders, which appear as series of local extreme values in the first derivative of DSC thermograms. Maltodextrin powders, which have aged after high pressure treatment, show increased peak temperatures as well as hydrated glass transition temperatures in DSC above a water content of 12% w/w. Glass transition temperatures between 22°C and 155°C have been determined within the investigated moisture range of 24-5% w/w. Maltodextrin gels are more homogeneous after high pressure treatment and keep a stable consistency, also after ageing (48h), whereas the untreated sample separates out. It has been shown, that pressure-treated maltodextrin gels have a higher mechanical and thermal stability. Presumably, the use of pressure results in a deformation of the network of the concentrated solution and primary gel respectively, but chances for restructuring arise from this deformation.

1 Einleitung