Effect of Acetylated Retrograded Starch (Resistant Starch RS4) On the Nutritional Value and Microstructure of the Crumb (SEM) of Wheat Bread

Agata Wojciechowicz-Budzisz* , Zygmunst Gil, Radoslaw Spychaj, Anna Czaja, Ewa Pejcz, Anna Czubaszek and Mirosław Zmiżewski

Department of Fruit, Vegetables and Grain Technology, Faculty of Grain Technology, Wrocław University of Environmental and Life Sciences, Chełmiskiego, Wrocław, Poland

Abstract

The objective of this study was to evaluate nutritional value and the microstructure of crumb of bread baked with three types of wheat flour with acetylated retrograded resistant starch preparation (RS4) obtained in the laboratory.

The research material was three wheat flour type 550, 750 and 2000 incorporated with acetylated retrograded starch obtained in the laboratory with a degree of substitution DS = 0,16. Content of RS4 preparations in the achieved flour samples was 10, 20, 30 and 40%. The control sample was wheat flour without additives (0%). The study assessed the nutritional value of bread on the basis of energy value, the total amount of digested starch (TDS) and total dietary fiber (TDF). Also carried pictures of bread crumb investigated using scanning electron microscopy (SEM).

Breads baked with whole meal flour type 2000 were characterized by higher energy than breads made from flour lower type. Increasing the type of wheat flour resulted in a gradual decrease in the total amount of digested starch (TDS) in the breads. The consequence of increasing the share of resistant starch in the samples was a gradual increase in total dietary fiber (TDF). With the increase in the share of resistant starch in the samples were reduced energy value, and the total amount of digested starch (TDS). In the photograph of the control bread crumb made using a scanning electron microscope is shown forming the gluten network of pores of medium and large.

Keywords: Wheat bread resistant; Starch RS4; Dietary fiber energy value; SEM

Introduction

Over the past five decades is a noticeable trend to seek new sources of dietary fiber which have been used to enrich bakery products. Starch plays an important role in textures of many kinds of food products and serves as a major source of energy for humans. Starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) according to the rate of glucose release and its absorption in the gastrointestinal tract Chung et al. [1]. RDS is the starch fraction that causes a rapid increase in blood glucose level after ingestion. SDS is the starch fraction that is digested slowly but completely in the human small intestine. RS is the starch portion that cannot be digested in the small intestine, but may be fermented in the large intestine by Hoover et al. [2].

In the recent years physiologists, nutritionists and technologists have been increasingly interested in starch resistant to the hydrolytic influence of the human body's digestive enzymes. Resistant starch (RS) is thus the sum of starch and its breakdown products which are not absorbed in the small intestine of a healthy human being Champ [3]. Different forms of resistant starch can be distinguished. Resistant starch type 1 (RS 1) is the kind of starch found in plant cells with intact cell walls, for example in whole wheat grains. Resistant starch type 2 (RS 2) is the starch found in raw (unelatinized) granules of some plant species, like potato or banana, and type 3 (RS 3) is retrograded starch. Resistant starch type 4 (RS 4) is chemically or physically modified starch Englyst and Cummings [4], Haralampu [5].

Resistant starch has been attributed numerous health benefits. Therefore, it is regarded as a component of dietary fiber Fuentes – Zaragoza et al. [6], Ohr [7]. The beneficial influence of resistant starch on human digesting process is revealed in better glucose tolerance (glycemic index reduction), lowering lipids level in blood and the increase of chyme mass. Indigested resistant starch is fermented in the large intestine, as a result of which short chain fatty acids are created (acetate, propionate and butyrate). This in turn results in significantly reducing pH in the bowel, and consequently a selection of microorganisms takes place in the large intestine (thus providing its antineoplastic protection, prevention of colon cancer) [8-10]. The health benefits of RS have been also reported as substrate for the growth of the probiotics, reduction of gall stone formation, hypocholesterolemic effects, inhibition of fat accumulation, and increasing absorption of minerals [11].

Breads and cereal products are a large part of daily human nutrition, thus they may play an important role in the diet of an ill person, aiding their treatment. The importance is no lesser in the case of a healthy human being, where their role might be preventive [12]. Both the nutritional value and technological properties of resistant starch are important in the potential development of a wide range of fiber-enriched food.

The aim of this study was to investigate the effect of different RS4 share on the nutritional value of wheat bread (energy value, the total amount of digested starch (TDS) and total dietary fiber (TDF)) and microstructure of the crumb (SEM).

*Corresponding author: Agata Wojciechowicz-Budzisz, Department of Fruit, Vegetables and Grain Technology, Faculty of Grain Technology, Wrocław University of Environmental and Life Sciences, Chełmiskiego, Wrocław, Poland, Tel: +48 71 3207713; E-mail: agata.wojciechowicz@up.wroc.pl

Received March 11, 2015; Accepted April 08, 2015; Published April 15, 2015


Copyright: © 2015 Wojciechowicz-Budzisz A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Experimental

Materials

The research material was wheat flour type 550 (Polish Mills Brzeg, Poland) (information about chemical composition of flours in Table 1, 750 (Diamant Stradunia Mill Ltd., Poland) and 2000 (Eco Company, Piotr Hillar, Tuczki 35, Rybno, Poland) incorporated with acetylated retrograded starch (resistant starch RS4) obtained in the laboratory with a degree of substitution DS=0,16, moisture =10,9%, total protein=0,2%, total amount of digested starch (TDS)=30,0%. The starch had been obtained according to the methodology guidelines developed by the Department of Food Storage and Technology at the Wrocław University of Environmental And Life Sciences. The starch preparations had been obtained according to the P-382126 patent application [13]. Potato starch Superior Standard had been retrograded and then acetylated. The acetylation process had been carried out analogically to the way it is done in Polish starch production plants [14]. The obtained resistant starch preparations had been crumbled and sifted with a sieve with meshes of 265 μm. The content of resistant starch in the achieved flour samples (separately for each flour type) was 10, 20, 30 and 40%. The control sample was wheat flour without the addition of resistant starch (0%). Other ingredients, such as salt were purchased from a local store. Compressed yeast were supplied by Lesaffre Bio-Corporation Inc. (Łódź, Poland)

Preparation of bread

Preparation of resistant starch and wheat flour blends used in laboratory bread baking in the proportions of 0/100, 10/90, 20/80, 30/70, 40/60. The bread was baked using the single-phase method according to the method developed by the Cereal Technology Institute at the Wroclaw University Of Environmental And Life Sciences. The bread was baked using the single-phase method according to the method developed by the Cereal Technology Institute in the proportions of 0/100, 10/90, 20/80, 30/70, 40/60. The bread was baked using the single-phase method according to the method developed by the Cereal Technology Institute according to the method developed by the Cereal Technology Institute (Łódź, Poland) incorporated with acetylated retrograded starch (resistant starch RS4) obtained in the laboratory with a degree of substitution DS=0,16, moisture =10,9%, total protein=0,2%, total amount of digested starch (TDS)=30,0%. The starch had been obtained according to the methodology guidelines developed by the Department of Food Storage and Technology at the Wrocław University of Environmental And Life Sciences. The starch preparations had been obtained according to the P-382126 patent application [13]. Potato starch Superior Standard had been retrograded and then acetylated. The acetylation process had been carried out analogically to the way it is done in Polish starch production plants [14]. The obtained resistant starch preparations had been crumbled and sifted with a sieve with meshes of 265 μm. The content of resistant starch in the achieved flour samples (separately for each flour type) was 10, 20, 30 and 40%. The control sample was wheat flour without the addition of resistant starch (0%). Other ingredients, such as salt were purchased from a local store. Compressed yeast were supplied by Lesaffre Bio-Corporation Inc. (Łódź, Poland)

Preparation of bread

Preparation of resistant starch and wheat flour blends used in laboratory bread baking in the proportions of 0/100, 10/90, 20/80, 30/70, 40/60. The bread was baked using the single-phase method according to the method developed by the Cereal Technology Institute at the Wroclaw University Of Environmental And Life Sciences, described in the P-384873 patent application [15] and based on the following recipe: wheat flour or mixture of wheat flour and resistant starch – 250 g, yeasts – 3 g/100 g of flour, salt – 1,5 g/100g of flour, water in amounts allowing to obtain 300 FU consistency (Table 2). That is, the dry ingredients (wheat flour and RS4) were mixed, first. Yeast and salt were dissolved in water at 30°C. All the ingredients were mixed by yeast and acetic acid. After that, the dough (about 400 grams) was placed in a baking tin and fermented in a temperature of 30-35°C for 60 minutes. After this one-hour fermentation period the dough was kneaded and placed back in the fermentation chamber. After about 30 minutes the dough was kneaded once more and left to final fermentation (35-45 minutes) under the same incubation conditions. The prepared dough samples were baked in the laboratory stove (Brabender, Duisburg, Germany) for 30 minutes in a temperature of about 240°C. Right after putting dough in forms into the oven the steam was injected.

Chemical analysis

Energy value of bread was determined by the Rozental’s method [16]. Total dietary fiber (TDF) was determined in bread baked from flour type 750 by the enzymatic-gravimetric AOAC method [17]. Total amount of digested starch (TDS) was determined in breads baked from flour type 550, 750 and 2000 with amount of resistant starch 0, 10 and 20% by its hydrolysis with the mixture of pancreatic alpha-amylase and glucoamylase at the temperature of 37°C for 16 hours, followed by the measurement of the released glucose using glucose oxidase. Porcine pancreatic alpha-amylase types VI-B (Sigma) as well as glucoamylase AMG 300L (Novozymes) were used for the analyses. The amount of released glucose was determined colorimetrically at the λ=500 nm using LiquickCor-Glucose diagnostic kit (Cormay, Poland). Four replicates were made for each probe and standard deviations were calculated [18].

Scanning electron microscopy (SEM)

For preparation of bread samples baked from flour type 750, small pieces of crumb (10x10x5mm) were cut from the centre of the slice with a sharp knife and frozen in liquid nitrogen. Then, the test material was placed in a chamber dissecting and freeze-dried (t=10 min, temperature -90°C). All prepared specimens were sputter coated with mixture of gold and platinum (Quorum Technologies business model PP2000 Cryo - SEM Preparation Systems). The preparations were then viewed and photographed using a ZISEE scanning electron microscope apparatus model EVO LS15 at -130°C. Photographic documentation was performed at 1500X (1.50 KX) and 3000X (3.00 KX).

Results and Discussion

Breads baked with resistant starch characterized by a lighter crust and crumb color compared with the control bread (Figures 1-6). With increasing RS4 share in samples crumb color was becoming clearer, and the skin pale and less ruddy. The increase in resistant starch content in the samples caused clear differences in elasticity of the crumb. Bread baked with blends containing 10 and 20% of the RS4 was comparable in terms of flexibility of wheat bread. Greater participation of starch resulted in the creation of dense, wet and sticky crumb of fine porosity.

According to Eerlingen RC [19], adding components containing an artificially increased content of resistant starch in the process of bread baking does not lower the quality of the resulting products, and what is more, it results in reducing the caloric value of the makings. Enriching wheat flour with resistant starch, as it has been done in this experiment, has caused change in nutritional properties of wheat breads (Figures 7-9).

Energy value of bread

Kawka [20] and Piecut [21] reported that the energy value of 100 g of bread ranges from 213 kcal (wholemeal rye bread) to 410 calories (half-french bread with jam). In our study, the energy value of received loaves does not exceed 185 kcal/100 g bread (Figure 7). Breads baked with wholemeal flour type 2000 were characterized by higher energy than bread made from flour lower type. This is due to high fat content in wholemeal flour. With the increase of the quantity of resistant starch, a

### Table 1: Chemical composition of flours

<table>
<thead>
<tr>
<th></th>
<th>flour type 550</th>
<th>flour type 750</th>
<th>flour type 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>ash [%]</td>
<td>0,54 ± 0,009</td>
<td>0,77 ± 0,008</td>
<td>1,93 ± 0,007</td>
</tr>
<tr>
<td>total protein [%]</td>
<td>11,3 ± 0,05</td>
<td>12,2 ± 0,09</td>
<td>12 ± 0,06</td>
</tr>
<tr>
<td>wet gluten [%]</td>
<td>30,8 ± 0,75</td>
<td>31,1 ± 0,94</td>
<td>19,4 ± 0,48</td>
</tr>
<tr>
<td>Zeleny's sedimentation test [ml]</td>
<td>35 ± 0,71</td>
<td>38 ± 0,71</td>
<td>30 ± 0,35</td>
</tr>
<tr>
<td>falling number [s]</td>
<td>360 ± 1,41</td>
<td>381 ± 2,82</td>
<td>344 ± 2,12</td>
</tr>
</tbody>
</table>

### Table 2: Amount of added water to flour to obtain 300 FU consistency of dough [%]

<table>
<thead>
<tr>
<th></th>
<th>flour type 550</th>
<th>flour type 750</th>
<th>flour type 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>62,4 ± 0,42</td>
<td>69,2 ± 0,48</td>
<td>61,6 ± 0,36</td>
</tr>
<tr>
<td>10% RS4</td>
<td>67,2 ± 0,53</td>
<td>80 ± 0,65</td>
<td>66,8 ± 0,48</td>
</tr>
<tr>
<td>20% RS4</td>
<td>73,2 ± 0,57</td>
<td>88,8 ± 0,72</td>
<td>72,4 ± 0,56</td>
</tr>
<tr>
<td>30% RS4</td>
<td>82 ± 0,68</td>
<td>98 ± 0,98</td>
<td>77,6 ± 0,61</td>
</tr>
<tr>
<td>40% RS4</td>
<td>90,4 ± 0,89</td>
<td>102,8 ± 1,21</td>
<td>84 ± 0,70</td>
</tr>
</tbody>
</table>

significant decrease of energy value of bread has been observed (Figure 7). This decrease is directly related to larger water absorption of the mixtures containing RS4, overbake of bread and total dietary fiber content in bread with the increase of acetylated retrograded starch content in the samples. This relationship is also apparent from the growing resistance to enzymatic degradation of chemically modified starches [22]. A similar dependence has also been observed by [23,24].
Total dietary fiber (TDF)

With the increased content of resistant starch in the samples, the total dietary fiber content in bread has increased (Figure 8). Because of fact that resistant starch is regarded as a component of dietary fiber, it's increasing participation in breads causes increase of total dietary fiber content. The increase in total dietary fiber (TDF) in processed cereals under the influence of different types of additive preparations has been reported by many authors [25-32].

Fiber content in the control sample (5.1%) was higher than in the studies performed by Kawka [20] for wheat bread (2.7%). Increasing the fiber content up to about 11.5% corresponds to or exceeds the content of this physiologically valuable ingredient in traditional whholemeal (9.4g/100g bread) or rye bread (9.1g/100g bread) which shows the growth of its nutritional value.

Total amount of digested starch (TDS)

Increasing the type of wheat flour resulted in a gradual decrease in the total amount of digested starch (TDS) in the breads (Figure 9). The consequence of increasing participation of resistant starch in tests was a decrease in the total amount of digested starch (TDS), which has been caused by increase resistance to enzymatic degradation of chemically modified starches. This relationship is apparent, due, as in the case of energy value, an increase in water absorption of mixtures, overtake of bread and total dietary fiber content in bread. Zabidi and Aziz [32] also noted a reduction of the amount of starch digested in bread with increasing content of Cempedak seed flour (CSF).

Scanning electron microscopy (SEM)

In the case of the photograph (Figure 9) made using a scanning electron microscope of the control bread crumb visible is open and developed gluten network, where it can be seen a few partially gelatinized, thus distorted, starch grains (Photo 1A,A). Similar SEM micrographs of fresh and semi-baked different bread types obtained [33]. As a result of an addition of resistant starch to the dough structure of starch granules disappeared, creating a uniform matrix. It can be noticed a gel-like structure formed by completely gelatinized, unstructured starch grains (Photo 1B, B, D, D, E, E). Some starch knobs retained their individuality, even though they appear to be heavily distorted due to partial gelatinization (Photo 1C, D). The degree of starch gelatinization can be attributed to differences in the amount of water in the dough [34]. Increasing content of modified starch in bread resulted in a significant increase in water absorption of the flour (Table 2), and thus increases the degree of starch gelatinization during baking. In doughs with the participation of material with the high content of dietary fiber the protein system is weakened, and has less ability to retain gas. This adverse effects associated with an increased amount of soluble proteins and non-protein nitrogen fraction, and reducing the amount of prolamin type proteins, the presence of non-starch polysaccharides, dispersion of gluten proteins and the interaction between the fiber and gluten [35]. The addition of resistant starch in an amount up to 20% effect on the establishment of a rough, porous matrix of irregular appearance (Photo 1B, B, C, C). Similar images from electron scanning microscope of cooked rice obtained [36]. At 30 and 40% share of the preparation appears to be continuous, coherent matrix protein – starch, like at the picture of frozen bread crumb with 3% inulin addition. Starch granules in the crumb samples were fully gelatinized and hardly distinguishable in the matrix (Photo 1D, D, E, E). However, the starch granules were enveloped in a coating of muciilage and were indistinct from each other. This suggest that at some stage during the bread preparing process, during dough mixing and moulding, some of the resistant starch inclusions had become dispersed and mixed intimately with the starch granules and protein matrix. Thus, the overall appearance was one in which the starch granules appeared to be ‘glued’ together by the resistant starch [37].

Conclusions

The consequence of increasing the share of resistant starch in the samples was a gradual increase in total dietary fiber (TDF). With the increase in the share of resistant starch in the samples were reduced energy value, and the total amount of digested starch (TDS). In the photograph of the control bread crumb made using a scanning electron microscope the structure appears to be open and developed, in which it can be seen a few partially gelatinized, thus distorted, starch grains. The addition of resistant starch in an amount up to 20% effect on the establishment of a rough porous matrix of irregular appearance. At 30 and 40% share of the preparation the surface does not appear bumpy and has only small gas cells.

Acknowledgement

The Project is supported by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018.

References

17. AOAC Official Method 985.29 (1997) Total Dietary Fiber in Foods-Enzymatic-


