საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 18, №2, 2024
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 18, no. 2, 2024

Biotechnology

Enzymatic Degradation of Gluten Wheat Dough and Sensory Analysis of Gluten-Free Bread

Tripon Parjanadze[*], Tamriko Khobelia[**], Kristine Museliani[**], Malkhaz Dolidze[§], Tamar Sachaneli[§], Edisher Kvesitadze[§]

[*] *Scientific Research Institute of Food Industry, Georgian Technical University, Tbilisi, Georgia*
[**] *Research Center "Biomed", Georgian Technical University, Tbilisi, Georgia*
[§] *Faculty of Agricultural Sciences and Biosystems Engineering, Georgian Technical University, Tbilisi, Georgia*

(Presented by Academy Member Giorgi Kvesitadze)

The work aimed to dissolve gluten in the dough made from the wheat flour to prepare gluten-free wheat bread from it. For this, a dough was prepared from wheat flour and washed in running water to remove the existing starch, soluble proteins, etc. from the flour and get purified gluten. A broad-spectrum protease was selected to degrade the wheat dough gluten. The gluten was used as a substrate to select a specific protease that disintegrates gluten more efficiently. Hydrolysis of the separated gluten was carried out. The dough was prepared at room temperature and left for 1.5 h. At this stage, glucose, total sugars, and protein were determined. The doses of the selected enzyme were defined from purified gluten obtained from 100 g of flour. In this experiment were two unknown parameters: the amount of enzyme and time. Therefore, based on the activity indicator, it was taken 0.75 U/ml of activity and time was a research parameter, and from the visual indicator of baked bread, we tried to select the least leavened bread. The sample with 0.75 U/ml of protease activity and an incubation time of 2 hours had the least increase in volume. During this time, the dough slightly increased in volume. In addition, gluten-free bread is made from Buckwheat flour. A comparison of wheat gluten-free bread with buckwheat bread according to the hedonic scale was done. The results prove that gluten-free wheat bread has better taste and rheological properties than buckwheat bread and is organoleptically close to traditional bread. *© 2024 Bull. Georg. Natl. Acad. Sci.*

buckwheat bread, gluten-free wheat bread, enzyme, hydrolysis of the gluten, protease, sensory analysis of bread

For an effective bread-making process gluten plays an important role, ensuring proper structure and a desirable texture in the final product. Nowadays, celiac disease is known as an autoimmune disorder, triggered by the ingestion of gluten, and the only cure from it is consuming gluten-free foods. The main treatment option is a gluten-free diet [1,2].

The worldwide increasing occurrence of gluten-related disorders promotes interest in the development of gluten-free bread and other baked goods

without compromising individuals with gluten-related disorders [3].

Gluten, a protein fraction derived from wheat, rye, barley, oats, and their hybrids, and derivatives, is very important in baking technology. Its unique properties contribute to the structure, texture, and elasticity of various baked goods. Glutens are potential proteins with multifunctional therapeutic effects [4,5].

A gluten-free diet is unbalanced and usually has a higher percentage of calories from fat, less carbohydrates, as well a low intake of non-starch polysaccharides. Protease is an effective food additive for improving the quality of gluten-free rice bread [6,7].

Proteolytic events during sourdough fermentation liberate amino acids, which improve bread quality flavor, however, proteolytic degradation of gluten proteins may adversely affect the rheological characteristics of wheat doughs and bread texture [8].

Different attempts have been made to reduce of the immunogenic gluten sequences of wheat while it was keeping its baking technological properties. In the last decade, several studies have shown the capacity of proteolytic enzymes, mainly peptidases, to degrade gluten during food processing. It was shown that selected *Lactobacillus* in combination with fungal and/or malt proteases could decrease the residual concentration of gluten immunogenic sequences during extended fermentation time [9].

Materials and Methods

To assess the glucose concentration in the research sample, the GOD-POD Kit was employed following the manufacturer's instructions (DiaSys GmbH). Optical density was measured at 546 nm. The concentration of the glucose was calculated using a formula provided by the manufacturer.

Total sugars were determined using the DNS method [10]. The samples were measured on a spectrophotometer at 546 nm, against a control test tube. Total carbohydrate concentration was determined on a calibration curve constructed with glucose standard solutions.

The amount of total protein in the liquid solution was determined using the Biuret method [11]. The samples were measured using a spectrometer (Perkin Elmer, USA) at 546 nm against the reagent blank. The protein concentration was determined on a calibration curve constructed with standard solutions of bovine serum albumin.

Gluten hydrolysis: 25 grams of dough was taken and in the first case, 5 ml of distilled water was added, and in the second case, 0.8 grams of commercial enzyme (Chymoral Forte) dissolved in 5 ml of water was added. 10 mg of solid mass was taken from the obtained mixture and 10 ml of pH 7 buffer was added. The mixture was centrifuged. A standard solution was prepared with a concentration of 5 mg/ml. The optical density of the standard solution at 546 nm was determined to be 0.198. The optical density of the test solution was determined at 546 nm.

The sensory analysis included the evaluation of bread obtained from dough treated with protease enzyme. Each person participating in the evaluation (10 users were selected) met all the qualification requirements. They evaluated the resulting product according to color, taste, texture, smell, and aroma. A five-point hedonic scale ranging from very good to very bad (1 – very good, 2 – good, 3 – neither good nor bad, 4 – bad, 5 – very bad) was used to indicate the level of approval of each characteristic [12].

To prepare gluten-free wheat bread, proteases from various biological sources (fungal, plant, and commercial origin) were utilized. For complete digestion of gluten, enzyme activity was determined by the colorimetric method [13].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to study proteins. Protein molecular masses were visualized in SDS-PAGE under denaturing conditions [14].

Results and Discussions

The goal of the work was to dissolve gluten in the dough traditionally made from wheat flour, to obtain gluten-free dough, and to prepare gluten-free wheat bread from it.

The dough was prepared from wheat flour and washed in running water to remove the existing starch, soluble proteins, etc. from the flour and get purified gluten. A broad-spectrum protease was selected to degrade the wheat dough gluten. The gluten was used as a substrate to select a specific protease that disintegrates gluten more efficiently. Hydrolysis of the separated gluten was carried out, where after centrifugation, water-soluble phenolic amino acids were measured by the Biuret method, which indicated the transfer of hydrolysis products from insoluble gluten to soluble state as a result of hydrolysis (see Table 1).

Table 1. Yeast, pharmaceutical and plant protease activity

Protease/ Parameter	Protein mg/ml	Enzyme activity U/ml	Specific activity U/mg
Yeast protease	1.75	0.4	0.228
Protease pharmaceutical reparation	2.8	0.75	0.267
Plant protease	1.1	0.25	0.227

As shown in Table 1, the pharmaceutical preparation after dissolving contained more protein – 2.8 mg/ml, which is higher than Yeast and Plant Proteases. Accordingly, the U/ml of this preparation was higher. The higher amount of enzyme gave us a higher activity per U/ml. We chose the protease with the highest specific activity index. In the case of the protease pharmaceutical preparation, this indicator was the highest – 0.267 U/mg, which exceeded the specific activity of the other two proteases. That is why the pharmaceutical preparation was chosen as the optimal option. For comparison, as described, 1 tablet of the pharmaceutical preparation contains 100000 armour/unit of proteases (trypsin, chymotrypsin). We used a solution corresponding to 2 tablets.

Determination by electrophoresis was performed to visualize gluten degradation:

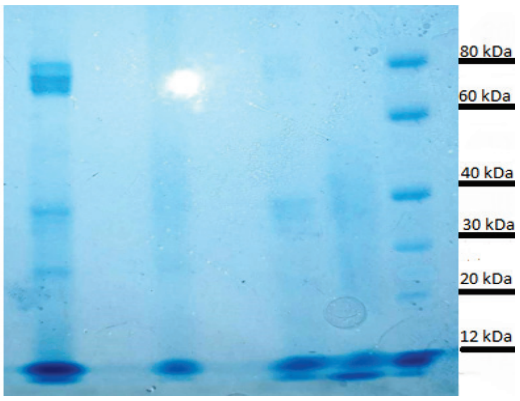

Fig. 1. Denaturing polyacrylamide gel electrophoresis (SDS-PAGE). Lane 1 – electrophoresis of gluten under denatured conditions, Lane 2 – the result of 30-minute incubation of gluten, Lane 3 – a result of 10-minute incubation of gluten, Lane 4 – a result of 20-minute incubation of gluten, Lane 5 – molecular mass markers.

As can be seen from the electrophorogram, the first lane shows the electrophoresis of gluten under denatured conditions, the second lane shows the result after 30 minutes of gluten incubation, the third and fourth lanes show the results after 10 minutes and 20 minutes of incubation with protease. The fifth lane contains molecular mass markers. After 30 minutes of incubation in the second path, no fraction of gluten remained, all gluten subunits were broken down and converted into peptides and amino acids. Since the molecular mass of the lowest gluten subunit is more than 31,000 kDa, the electrophorogram clearly shows that there are no protein fragments from 12 kDa to 30 kDa and above in the hydrolyzate, which unequivocally indicates the complete breakdown of gluten, both LMW and HMW – gluten subunits in molecular fractions. Although after 10 minutes and 20 minutes of incubation, it should be said that gluten fragments are still visible as traces. The dough was prepared at room temperature and left

for 1.5 h. At this stage, we determined glucose, total sugars, and protein, the results are given in Table 2.

Table 2. Composition of gluten-free and traditional wheat dough

Dough/Parameter	Glucose mg/ml	Total sugars mg/ml	Protein mg/ml
Wheat dough	0.78	1.416	19.419
Gluten-free wheat dough	0.95	1.605	31.0606

As can be seen from Table 2, glucose and total sugars are slightly increased in gluten-free wheat dough. This may be due to trial deviation.

As for protein, the content is particularly high in gluten-free wheat bread, which indicates that due to the specificity of the method, the protein in the dough extract could not be transferred into the solution, because gluten is insoluble in water. The protease-treated polypeptides were easily extracted into the solution, resulting in a significant increase in the amount of protein in the mixture compared to the wheat dough extract.

Accordingly, we determined the doses of the selected enzyme, for which were used purified gluten as a substrate from the amount of dough obtained from 100 g of flour. In this experiment, we had two unknown parameters: amount of enzyme and time. Accordingly, based on the activity indicator, we took 0.75 units of activity and left time as a research parameter, and from the visual indicator of baked bread, we tried to select the least leavened bread, since gluten directly participates in the increase in dough volume. The sample with 0.75 of protease activity and an incubation time of 2 hours had the least increase of dough volume.

The production of gluten-free products in the world is carried out mainly from gluten-free raw materials such as buckwheat flour [15]. We compared the wheat gluten-free bread with the buckwheat bread according to the hedonic scale. Ten experts were interviewed. According to the them, based on the taste properties, wheat gluten-free bread turned out to be slightly sweet due to the high content of peptides and aminoacids.

The obtained product was characterized according to color, taste, aroma, smell, and texture, finally, gluten-free wheat bread was evaluated: taste, smell, aroma, texture, color – characteristic of wheat bread, color – brownish for buckwheat bread.

Conclusions

According to the screening, a broad-spectrum protease was selected and its dosages were determined to break down gluten. The work shows that broad-specificity proteases, especially those used in terms of digesting food in the stomach, effectively cleave gluten. Based on the experiments, a specific enzyme – protease was selected, through which it was possible to completely break down the isolated gluten.

Based on the research, it was possible to break down gluten and probably other proteins during the fermentation process simultaneously, resulting in gluten-free bread. Gluten-free bread was compared to bread made from gluten-free material (buckwheat) and showed better taste and rheological properties.

ბიოტექნოლოგია

ხორბლის ცომში გლუტენის ფერმენტული დაშლა და მიღებული უგლუტენო პურის სენსორული ანალიზი

ტ. ფარჯანაძე*, თ. ხობელია**, ქ. მუსელიანი**, მ. დოლიძე§, თ. საჩანელი§, ე. კვესიტაძე§

** საქართველოს ტექნიკური უნივერსიტეტი, კვების მრეწველობის სამეცნიერო-კვლევითი ინსტიტუტი, თბილისი, საქართველო*
*** საქართველოს ტექნიკური უნივერსიტეტი, კვლევითი ცენტრი „ბიომედი“, თბილისი, საქართველო*
§ საქართველოს ტექნიკური უნივერსიტეტი, აგრარული მეცნიერებებისა და ბიოსისტემების ინჟინერინგის ფაკულტეტი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის გ. კვესიტაძის მიერ)

სამუშაო მიზნად ისახავდა ხორბლის ფქვილისგან დამზადებულ ცომში გლუტენის დაშლას და მისგან უგლუტენო ხორბლის პურის მიღებას. ექსპერიმენტული პურის მისაღებად ხორბლის ფქვილისგან მომზადდა ცომი და გაირეცხა გამდინარე წყალში, რათა ფქვილიდან გამორეცხილიყო მასში არსებული სახამებელი, წყალში ხსნადი ცილები და ა.შ. რის შედეგადაც მიღებულ იქნა გასუფთავებული გლუტენი. ფართო მოქმედების მქონე პროტეაზა შეირჩა ხორბლის ცომში გლუტენის დასაშლელად. გლუტენი გამოიყენებოდა როგორც სუბსტრატი სპეციფიკური პროტეაზას შერჩევის დროს. ჩატარდა გამოყოფილი გლუტენის ჰიდროლიზი. ცომი მომზადდა ოთახის ტემპერატურაზე და მოხდა მისი დაყოვნება 1,5 საათი. ამ ეტაპზე განისაზღვრა გლუკოზა, საერთო შაქრები და ცილა. შერჩეული ფერმენტის დოზები განისაზღვრა 100 გ ფქვილისგან მიღებულ გასუფთავებულ გლუტენზე გადაანგარიშებით. ამ ექსპერიმენტში იყო ორი უცნობი პარამეტრი: ფერმენტის რაოდენობა და დრო. აქედან გამომდინარე, ხვედრითი აქტივობის მონაცემზე დაყრდნობით, აღებულ იქნა 0,75 ერთეული/მლ აქტივობა და საკვლევი პარამეტრი იყო დრო. ცომის ვიზუალური მაჩვენებლიდან გამომდინარე, შევეცადეთ შეგვერჩია ყველაზე ნაკლებად მოცულობაში მომატებული ცომი. ნიმუშს, სადაც დამატებულ იქნა 0,75 ერთეული/მლ აქტივობით პროტეაზა და ინკუბაციის დრო იყო 2 საათი, ახასიათებდა ცომის მოცულობის ყველაზე ნაკლები ზრდა. გარდა ამისა, უგლუტენო პური მზადდება წიწიბურას ფქვილისგან. განხორციელდა ხორბლის უგლუტენო პურის შედარება წიწიბურას პურთან ჰედონური სკალის მიხედვით. შედეგები ადასტურებს, რომ უგლუტენო ხორბლის პურს აქვს უკეთესი საგემოვნო თვისებები, ვიდრე წიწიბურას პურს და ორგანოლეპტიკურად ახლოსაა ტრადიციული მეთოდით მიღებულ პურთან.

REFERENCES

1. Moradi M., Bolandi M., Arabameri M., Karimi M., Baghaei H., Nahidi F., Eslami Kanafi M. (2021) Semi-volume gluten-free bread: effect of guar gum, sodium caseinate and transglutaminase enzyme on the quality parameters. *Journal of Food Measurement and Characterization,* 15: 2344-2351.
2. Kõiv V, Tenson T. (2021) Gluten-degrading bacteria: availability and applications. *Applied Microbiology and Biotechnology,* 105: 3045-3059.
3. Muzaffar K., Sofi S. A., Mir M. B., Bhat N. A., Jan R., Singh A., Majid D. (2021) Technological aspects of gluten free bread. Gluten-free bread technology. springer, Cham. 29-48. https://doi.org/10.1007/978-3-030-73898-3_3
4. Šmídová Z., Rysová J. (2022) Gluten-free bread and bakery products technology. *Foods,* **11**(3): 480.
5. Saadi S., Saari N., Ghazali H. M., Abdulkarim S. M., Hamid A. A., Anwar F. (2022) Gluten proteins: Enzymatic modification, functional and therapeutic properties. *Journal of Proteomics,* 251, 104395.
6. Bradauskiene V., Vaiciulyte-Funk L., Mazoniene E., Cernauskas D. (2019) Fermentation with Lactobacillus strains for elimination of gluten in wheat (*Triticum aestivum*) by-products. In Baltic Conference on Food Science and Technology: *conference proceedings.* LLU, 109-114.
7. Honda Y., Inoue N., Kurita M., Okunishi T. (2021) Alpha-glutelin degradation and its hydrolysate by protease enhance the specific volume of gluten-free rice starch bread. *Journal of Cereal Science,* 102, 103338.
8. Thiele C., Grassl S., Gänzle M. (2004) Gluten hydrolysis and depolymerization during sourdough fermentation. *Journal of Agricultural and Food Chemistry,* **52**(5): 1307-1314.
9. Heredia-Sandoval N. G., Valencia-Tapia M. Y., Calderón A. M., Islas-Rubio A. R. (2016) Microbial proteases in baked goods: modification of gluten and effects on immunogenicity and product quality. *Foods,* **5**(3): 59. DOI: 10.3390/foods5030059
10. Miller G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry,* **31**(3): 426-428.
11. Chang Sam KC., Zhang Y. (2017) Protein analysis. Food analysis, 315-331.
12. Muchiri M. N., McCartney A. L., Methven L. (2020) Sensory profile and consumer preference of novel probiotic yoghurt enriched with orange sweet potato (Ipomoea batatas). *African Journal of Food, Agriculture, Nutrition and Development,* **20**(5): 16471-16489.
13. Khobelia T., Museliani K., Ninua T., Kvesitadze E. (2022) Colorimetric assay to determine total proteolytic activity. *Bull. Georg. Natl. Acad. Sci.,* **16**(2): 106-114.
14. Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227(5259): 680-685.
15. Zhang J., Yao Y., Li J., Ju X., Wang, L. (2023) Impact of exopolysaccharides-producing lactic acid bacteria on the chemical, rheological properties of buckwheat sourdough and the quality of buckwheat bread. *Food Chemistry,* 425, 136369.

Received April, 2024