GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research / Araştırma
GIDA (2024) 49 (5) 946-959
doi: 10.15237/gida.GD24056

INVESTIGATION OF THE EFFECT OF ENZYME APPLICATION ON THE STRUCTURE OF WAFERS IN THE FOOD INDUSTRY

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Received/Geliş: 31.05.2024; *Accepted*/Kabul: 25.09.2024; *Published online*/Online baskı: 28.09.2024

Poyraz Ekinci, F., Kökyar, N., Sener, D. (2024) Investigation of the effect of enzyme application on the structure of wafers in the food industry. GIDA (2024) 49 (5) 946-959 doi: 10.15237/ gida.GD24056

Poyraz Ekinci, F., Kökyar, N., Sener, D. (2024) Enzim uygulamasının gıda endüstrisinde gofretlerin yapısı üzerindeki etkisinin incelenmesi. GIDA (2024) 49 (5) 946-959 doi: 10.15237/ gida.GD24056

ABSTRACT
The study aimed to assess the impact of 13 different enzymes, including protease, amylase, hemicellulase, and xylanase, on wafer dough and sheets, aiming to identify the most effective enzyme combination. Commercial protease, amylase, hemicellulase, and xylanase enzymes were applied to wafer dough following manufacturer instructions, and their flow behaviors were analyzed. Subsequently, the doughs were baked into wafer sheets, and various parameters such as water activity (aw), moisture content (%), weight loss (%), color parameters (L*, a*, b*), hardness (g), and sensory attributes were evaluated. Protease 4, Hemicellulase 2, and Xylanase 2 enzymes were selected for combination testing based on the analysis of flow behavior and characterization parameters of the wafer doughs. Among the tested combinations, the wafer dough containing 80 ppm Xylanase 2 + 300 ppm Protease 4 enzyme group exhibited promising potential for industrial applications due to its favorable flow behavior, while the resulting wafer sheets demonstrated desirable crispness and a brown-caramel color, suggesting suitability for commercial use.
Keywords: Wafer sheet, enzyme, protease, amylase, hemicellulase, xylanase

ENZİM UYGULAMASININ GIDA ENDÜSTRİSİNDE GOFRETLERİN YAPISI ÜZERİNDEKİ ETKİSİNİN İNCELENMESİ

ÖZ
Çalışmanın amacı proteaz, amilaz, hemiselülaz ve ksilanazdan oluşan 13 farklı enzimin gofret hamur ve plakasına olan etkisinin incelenmesi ve etkili olan enzim kombinasyonunun belirlenmesidir. Bu amaçla üreticinin talimatlarına göre belirlenen dozlarda denenen ticari proteaz, amilaz, hemiselülaz ve ksilanaz enzimleri gofret hamuruna uygulanmış ve akış davranışları incelenmiştir. Daha sonrasında pişirilerek gofret plakası haline getirilen hamurların su aktivitesi, nem (%), ağırlık kaybı (%), renk parametreleri (L*, a*, b*), sertlik (g) ve duyusal analizleri gerçekleştirilmiştir. Gofret hamurlarının akış davranışları ve karakterizasyon parametreleri değerlendirildiğinde Proteaz 4, Hemiselülaz 2, Ksilanaz 2 enzimleri kombinasyon olarak denenmek üzere seçilmiştir. Denenen kombinasyonlar arasından 80 ppm Ksilanaz 2 + 300 ppm Proteaz 4 enzim grubu içeren gofret hamuru akış davranışı, gofret plakası ise gevrekliği ve kahverengi- karamel rengi dolayısıyla endüstride kullanılmak üzere potansiyel göstermektedir.
Anahtar kelimeler: Gofret yaprağı, enzim, proteaz, amilaz, hemiselülaz, ksilanaz

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INTRODUCTION

Chemical additives and/or physical methods are generally used to produce improved bakery products. Physical methods are easy and cost-effective, including heat and humidity treatments, freezing and ultra-pressure treatments, while chemical methods involve the incorporation of functional groups into starch molecules through processes such as etherification, esterification, crosslinking and acid treatment. In recent years, microbial-based enzymes have started to replace chemical and physical methods as they are recognized as natural and safe by both the environment and food manufacturers. This is due to the fact that enzyme-catalyzed reactions are substrate-specific and occur easily under suitable conditions (Zhou et al., 2014; Park et al., 2018).

Different enzymes such as amylase, protease, hemicellulase and xylanase are used in the food industry to improve dough properties and product quality of bakery products (Zhou et al., 2014; Park et al., 2018; Stemler et al., 2023; Barrera et al., 2016). Enzymes used in the food industry represent the largest share of the enzyme market (Park et al., 2018).

Amylase, one of the most valuable enzymes in the industry, has the ability to hydrolyze starch molecules into polymers containing glucose units (Barrera et al., 2016; Ahmad et al., 2019). In bakery products, α-amylase is generally used to improve textural properties and reduce elasticity. Mechanical damage during wheat milling affects starch granule integrity. Damaged starch granules have a higher water absorption capacity compared to natural starch granules. This negatively affects the rheological behavior of the dough and the quality of the products. Damaged starch granules are more hydrolyzed than natural starch granules due to their sensitivity to the enzyme α- amylase. A-amylase enzyme helps to reduce the negative effect of damaged starch granules (Barrera et al., 2016).

Proteases, one of the most frequently used enzymes in the bakery industry, refer to a group of enzymes whose catalytic function is to hydrolyze the peptide bonds of proteins (Jabalia et al., 2014). Proteases are used to ensure contact with flour proteins with shortened chain length during the dough fermentation phase (Philipps-Wiemann, 2018). Protease treatment of the dough makes it easier to process the dough and results in a wider range of products. In addition, mixing time is shortened and energy input to the process is reduced. Additional benefits of proteases are improved pan flow, grain and crumb texture, water absorption, color and taste in products such as wafers (Stauffer CE, 1987; Lindahl L, 1992).

Hemicellulases, another enzyme used in industry, consist of a group of enzymes that are effective in the hydrolysis of hemicelluloses, a complex polysaccharide group. Since xylan is the largest component in the hemicellulose structure, endoxylanases are one of the most important hemicellulosic enzymes. Xylanases are used to improve the gluten structure of bread, dough volume, viscosity, softness and elasticity, and to improve texture, taste and flavor in biscuit production. Hemicellulases are mostly used in the baking industry and in the production of prebiotic oligosaccharides (de Souza and Kawaguti, 2021; Pandit B, 2023).

Wafer, one of the most consumed bakery products, requires a low-protein, soft wheat flour with a weak gluten structure that allows it to be molded correctly. The protein levels of flours and the amount of damaged starch significantly affect the water absorption of flours. Hard wheats contain more protein and undergo more starch damage when milled. Soft wheats, on the other hand, contain less protein and suffer less starch damage during milling. Thus, the water holding capacity of flour made from soft wheat is lower. If strong flour is used in wafer production in the industry, the dough lumps during the process due to the gluten network formation of the flour, clogging the pipes and depositor, making mixing difficult. In addition, it was observed that wafers prepared with strong flour formed irregular shapes due to their hard texture. The water holding capacity and gluten network of wheat also affect the viscosity of the wafer batter. It is ensured that the wafer prepared with weak flour with low water holding capacity spreads

sufficiently on the cooking plate and the product is crispy as desired by the consumer. For this purpose, both environmentally friendly and flexible product enzymes are used in wafer production to provide the desired texture and viscosity (D. Manley, 2011; Jabalia et al., 2014; Barrera et al., 2016; Ahmad et al., 2019; S. Ahmad et al., 2022; Naderi, B. et al., 2023).

Wafer is a light, thin and crispy product obtained by pouring the liquid dough between the heating plates and baking it quickly. Although the term wafer is used for the product group consisting of plain wafers, hollow wafers, cones, wafer bars, plain wafer sheets will be evaluated in this study. In this study, Elvan Gıda (Istanbul, Turkey) aimed to determine the enzyme or enzyme combinations that facilitate the workability of wafer dough during the production process and provide crunchy properties.

MATERIAL METHOD

Material

The wafer wheat flour used in the study was purchased from Ulusoy Un Ticaret A.Ş., palm oil from Cargill, Incorporated, soy lecithin from Archer Daniels Midland Company, sodium bicarbonate from Sisecam Soda Lukavac, natural cocoa powder from Altınmarka Gıda San. ve Tic. A.Ş., edible salt from Med-Mar Tuz San. Tic. A.Ş. The enzymes used in the study, their sources, strengths and amounts used in the studies are given in Table 1 according to their groups.

Table 1. Enzymes used in the study, their sources, potencies and amounts used in the studies

Usage amount	Enzyme	Enzyme source and power
300 ppm	Protease 1	Bacterial protease, 9-11 u/g
	Protease 2	Bacterial protease, 120 u/g
	Protease 3	Fungal protease, 227 UHb/g
	Protease 4	Bacterial protease, 1126 UHb/g
15 ppm	Amylase 1	Fungal amylase, 378000 -462000 u/g
	Amylase 2	Fungal gluco-amylase, 5400-6600 u/g
	Amylase 3	Fungal alpha-amylase, 1728 AZ/g
125 ppm	Hemicellulase 1	Fungal hemicellulase, bacterial hemicellulase, 1650 u/g
	Hemicellulase 2	Fungal hemicellulase, 3420-4180 u/g
	Hemicellulase 3	Fungal hemicellulase, 1650 u/g
80 ppm	Xylanase 1	Microbial xylanase, 170000-230000 u/g
	Xylanase 2	Bacterial xylanase, 568 XylH/g
	Xylanase 3	Fungal xylanase, 1700 XylH/g

Method

Characterization of wafer flour

In order to analyze the effect of the wafer flour used in the study, moisture content (%), protein content (%), gluten index (%) and ash content (%) were determined.

Determination of moisture content

Moisture content determination of the wafer flour used in the study was carried out with Precisa XM 60 moisture analyzer. The moisture content of wafer flour was determined according to the method 930.15 (AOAC, 2000).

Protein content determination of wafer flour

Protein content determination of wafer flour was carried out by Kjeldahl method with Buchi KjelFlex K-360. Protein content was calculated according to the following formula (AOAC International, 2000).

$[\{(V_{H2HSO4} \times 14 \times 2 \times 0.1)/m\}1000x100 =$ % Nitrogen content

% Nitrogen content x 6,25= Protein content (%)

Total wet gluten, dry gluten and gluten index

The total wet gluten, dry gluten and gluten index of wafer flour was determined according to the

method 38-12.02 (AOAC, 2000). After centrifugation, the amounts of weak gluten (a) in the small chamber and strong gluten (b) in the large chamber were weighed separately and noted. Total wet gluten was dried for 4 min and weighed (d). They was calculated according to the following formula.
(a + b) x 10 = Total Wet Gluten (c)
(d) x 10 = Dry gluten
(b / c) x 100 = GI (Gluten Index)

Ash determination
The gluten index of wafer flour was determined according to the method 942.05 (AOAC, 2000). Considering the moisture content of the wafer flour, the % ash content was determined according to the formula given below. The studies were carried out in two parallels.
%Ash (m/m) = [((M2-M0)/M1)x100]x[100/(100-N)]

Preparation of wafer batter
The formulation of the wafer dough used in the study is given in Table 2. According to the formulation given in Table 2, salt and sodium bicarbonate were homogenized in the specified amount of water, then wafer wheat flour, palm oil, soy lecithin and natural cocoa powder were added and homogenized with a hand blender (Tefal Eco Respect Powelix 600W, France) for 60 seconds. Then, the determined amount of enzymes were added to the wafer dough prepared separately for each enzyme and mixed with a hand blender for 30 seconds.

Table 2. Formulation of the wafer dough used in the study

Ingredient	Concentration (%)
Water (g)	55.272
Wafer wheat flour (g)	42.517
Palm oil (g)	1.275
Soy lecithin (g)	0.425
Natural cocoa powder (g)	0.170
Sodium Bicarbonate (g)	0.170
Salt (g)	0.170
Total (g)	100

In order to examine the effect of the enzymes supplied from the market on the wafer sheets, the manufacturer's recommended doses were taken into consideration for each enzyme group used in the study.

From the wafer dough prepared with different enzymes, 10 ml was drawn using an Ayset brand injector and weighed. Afterwards, 10 ml of wafer batters taken with the injector were cooked at 180°C for 150 seconds in a wafer plate cooker (Çiftçioğlu Makina, Türkiye) heated at 180°C for 30 minutes. After the baking was completed, the wafers were cooled at room temperature for 15 minutes and weighed with a balance and the weight loss due to baking was calculated.

Flow behavior of wafer batter (s)
Although viscosity can vary over time, it is the resistance of a substance to flow that indicates the quality of a fluid (Abbas et al., 2010). In this study, flow behavior measurements were carried out in order to correlate the dough viscosity changed by the effect of enzyme with the friability of wafer sheets. The flow behavior of the wafer batters was evaluated with the discharge times of the flow container viscometer as suggested in Altinok, E., et al (2022). After the wafer batters prepared with different enzymes were homogeneously prepared, the flow time at 0 minute was measured and recorded in seconds with a UVE DIN 8 mm handle cup at 25 °C. At 30 minutes after the dough was prepared, the flow times were measured and recorded again with a UVE DIN 8 mm handled cup. The measurements were carried out in three repetitions.

Characterization of the wafer sheets
In order to perform comparative characterization of the wafer sheets prepared with different enzymes, water activity, moisture content (%), weight loss (%), color parameters, hardness and sensory analysis were tested.

Water activity (aw)
In order to measure the water activity of wafer sheets produced with different enzymes, the samples were crushed into powder and filled up to the specified place of the plastic sample container of the water activity device. The sample container was placed in the relevant compartment in the device and the measurement was taken. The

experiment was carried out in 2 parallels. If the difference between the results of two parallel samples was more than 0.05, the measurement was repeated (AOAC International, 2000).

Determination of moisture content

In the study, the classical method was used to determine the moisture content of wafers produced with different enzymes. The study was carried out in 2 parallels. The result was calculated according to the following formula (AOAC International, 2000).

%dry matter (m/m)= (M2-M0)/M1x100

M0: Weight of the glass petri dish

M1: Initial weight of the sample

M2: Weight of sample and glass petri dish after incineration

Moisture content (%)=100- % dry matter weight

Weight loss (%)

In order to measure the weight loss of the wafer sheets, the injector was used as a dough depositor. For this purpose, the injector was tared, 10 ml of wafer dough was drawn into the injector, weighed again with a precision balance and the value was recorded. The dough in the injector was then baked in the wafer machine and allowed to cool for 15 minutes. The cooked wafer sheets were weighed on a precision balance and the value was recorded. The studies were carried out in 3 parallels for each sample. Weight loss was determined by using the equation (1); (Berk et al., 2017).

Weight loss (%) = 100 x (W0- W1)/W0 (1)

W0: Weight of the wafer batter before baking

W1: Weight of the wafer sheet after baking

Color parameters

Color measurements of wafer sheets produced with different enzyme combinations and without enzyme were carried out with Konika Minolta brand color measuring device. The measurements were performed by touching the device directly to the product surface in 3 parallels for each sample. CIE L* (lightness), a* (redness) and b* (yellowness) evaluation system was used to determine the color parameters (Sahin and Sumnu, 2006).

Hardness

The hardness of the wafer sheets was analyzed with CT3 Texture Analyzer 30 minutes after baking. For this purpose, the samples were placed centered on the apparatus of the texturing device. The TA40 probe (12 mm radius) was brought to the closest point to the sample so as not to touch the sample and the program was run. The results were recorded as hardness values in grams and the analyses were carried out in 3 parallels (AOAC International, 2000).

Sensory analysis

Sensory analysis test was performed on wafer sheets produced with and without different enzyme combinations. The wafer sheet samples numbered with three-digit random numbers were evaluated by 10 trained panelists aged between 25 and 42 years who were Elvan Gıda personnel. Panelists evaluated the samples for general liking using a 9-point hedonic test. Very much like=9, Not at all like=1 (Resurreccion, 2008).

Statistical analysis

The experimental data obtained throughout the study were evaluated by analysis of variance (ANOVA) to detect the significant differences in flour types and concentrations ($p \leq 0.05$). If significant difference was found, Tukey's Test with 95% confidence level was performed for comparison. SPSS (Version 20, IBM, U.S.A.) was used in order to determine the Pearson correlation coefficients with 5% confidence level.

RESULT AND DISCUSSION

Characterization of wafer flour

In the food industry, technological recommendations for the quality of wafer production start with the quality of flour (Meleshkina E. P., 2016). In order to facilitate the processability of wafers in the process and to ensure homogeneous baking, wafers should be made from low protein soft wheat flour with a weak gluten structure (Naderi, B. et al., 2023). For this reason, in order to evaluate the effect of the wafer wheat flour used in the study on the results of the study, moisture content (%), protein content (%), gluten index (%) and ash content (%) analyzes were performed. According to the

analysis, the moisture content of wafer wheat flour was determined as 11.6%, protein content as 8.79%, gluten index as 92.6% and ash content as 0.51%.

E. P. Meleshkina et al. (2021) mentioned the values of the quality criteria of flour in the study of the application of the alveograph device in order to improve the requirements for the quality of flour for the production of wafer sheets. In the study, it is stated that the moisture content should not exceed 14.5%, while the wafer flour we used in our study is suitable for wafer use with a moisture content of 11.6%.

Although the nutritional quality of wheat protein is lower than that of other cereals, wheat flour is used as the primary source to form the desired structure in wafer. Soft wheat flour, which is also used in wafer flours, has more spreading properties than hard wheat flour. This is due to the low protein level, low water absorption and fineness of soft wheat flour. In studies in the literature, the protein content of soft wheat flour was reported to be between 8-10%, which is consistent with our study (Delcour et al., 2012). In addition, while the wet gluten content of soft wheat flour is reported to be between 23-24% in the literature, the wet gluten content of the flour we used in this study was 23.3%, which is in line with this range (Meleshkina, E. P. et al., 2021).

When starch and water-soluble proteins are washed out of the dough, most of the remaining viscoelastic mass contains water-insoluble protein fractions called gluten. These fractions are essential for industrial quality. Gluten plays an important role in determining the baking quality of wheat by imparting water holding capacity, stickiness, viscosity and elasticity (Oikonomou, N. A. et al., 2015). Gluten index is used to define whether gluten quality is poor (<30%), normal (=30-80%) or strong (>80%) (Cubadda, R. et al., 1992). The wafer flour used in the study had 23.5% wet gluten, 8.6% dry gluten and 92.6% gluten index, indicating that the flour had strong gluten quality.

Ash is one of the main indicators of the quality and utilization of wheat flour (Cardoso et al., 2019; Carson et al., 2009). Flour with high ash levels is generally characterized as less purified. Therefore, it is a commonly used parameter for determining flour purity and extraction rate (Piironen, V. and Salmenkallio-Marttila, 2009). According to Turkish Food Codex Wheat Flour Communiqué, ash value is not required for special purpose wheat flour. According to Elvan Food quality criteria, the ash value in wafer wheat flour should be maximum 0.6%. Therefore, the wafer wheat flour used in the study complies with Elvan Gıda quality criteria.

Flow behavior of wafer batter

The viscosity of wafer dough varies depending on the components of the product, particle size of flour, water content and temperature (Xue, J. and Ngadi, M., 2006). According to the literature, by relating rheological results with hardness, it can be said that the increase in the viscosity of the wafer dough results in wafers with low hardness values (Altinok, E. et al., 2022). Based on the flow time of wafer batters, we can comment on the viscosity of the batters. The length of the flow time indicates the high water holding capacity and viscosity. In the wafer samples in our study, it was observed that high viscosity appeared with a decrease in hardness values in line with the literature.

The data on the flow times of the wafer doughs with and without enzymes from the viscometer container at 0 and 30 minutes after preparation are given in Table 3. The viscosities of commercial enzymes belonging to each enzyme group were statistically significant (P<0.05). It was observed that Protease 4 enzyme provided significantly the lowest flow time at 0 and 30 minutes in wafer dough. This data is due to the fact that proteases break down peptide bonds as well as proline residues, which often occur in gluten proteins, increasing the solubility of proteins and decreasing viscosity. Compared to the original protein, the use of protease enzyme resulted in smaller peptides and fewer secondary structures, so the control group had higher viscosity values at 0 and 30 minutes compared to

the wafer doughs containing protease enzyme (Naderi, B. et al., 2023).

It was determined that none of the wafer doughs containing amylase enzyme could provide a decrease in flow time at the end of 30 minutes. In the literature, it is seen that the addition of alpha-amylase does not change the viscosity of the dough or increases the final viscosity (Hung, P. V. et al., 2007; Doğan, İ. S., 2002).

It was observed that the wafer dough containing hemicellulase 2 enzyme provided significantly lower flow time at 30 minutes than the wafer dough containing other hemicellulase enzymes. Among the wafer doughs containing xylanase enzyme, it was observed that the wafer dough containing Xylanase 2 enzyme provided a significant decrease in the flow time from minute 0 to minute 30. As a control, it was observed that the wafer dough without enzyme had a high flow time compared to the wafer doughs containing enzyme at 0 and 30 minutes and there was no decrease in flow time after 30 minutes of waiting time.

Table 3. Flow behavior times of wafer doughs at 0 and 30

Enzyme	0. min flow time (s)	30. min flow time (s)
Protease 1	68 ± 2^{b}	61 ± 1^{b}
Protease 2	78 ± 3^{a}	58 ± 3^{b}
Protease 3	81 ± 6^{a}	80 ± 1^{a}
Protease 4	28 ± 1^{c}	25 ± 2^{c}
Amylase 1	95 ± 4^{a}	101 ± 4^{a}
Amylase 2	70 ± 5^{b}	70 ± 3^{b}
Amylase 3	61 ± 8^{b}	61 ± 3^{c}
Hemicellulase 1	139 ± 3^{b}	138 ± 6^{b}
Hemicellulase 2	52 ± 6^{c}	49 ± 3^{c}
Hemicellulase 3	227 ± 3^{a}	225 ± 6^{a}
Xylanase 1	100 ± 5^{c}	90 ± 1^{b}
Xylanase 2	140 ± 9^{b}	43 ± 6^{c}
Xylanase 3	158 ± 4^{a}	220 ± 5^{a}

Significantly different at $P<0.05$ in the column for each enzyme group are lettered

Characterization of the wafer sheets

In order to examine the effect of enzymes on wafer sheets, the data of the characterization studies were examined. The data on the characteristics of the wafer sheets consisting of 13 different enzymes and a control group are given in Table 4. shows the aw, moisture content (%), loss (%), weight loss (%), hardness (g) and color parameter values of the wafer sheets containing the enzymes. All enzymes except hemicellulase 3 and xylanase 3 increased the hardness value of wafer sheets. Enzyme use increased weight loss. According to color measurement results, Protease 4 and xylanase 2 darkened the product color significantly.

Water activity (aw) and moisture content (%)

Protease enzymes break down proteins in the wafer layer, releasing amino acids and peptides. Amylase enzymes break down starch into simpler sugars. Hemicellulases and xylanases break down hemicellulose and xylan in plant material, releasing small molecules. Protein, starch and hemicelluloses, which are large molecules, retain more moisture. Therefore, the breakdown of these molecules can potentially release some bound water, which can increase the overall moisture content and water activity of the wafer layer (Troller, 2012). Moisture content, one of the most important factors of wafers, directly affects the basic textural properties of the product (Dogan, 2006).

Table 4. The effect of enzymes on the characteristics of wafer sheets (Significantly different at $P<0.05$ in the column are lettered)

Enzyme	aw	Moisture content (%)	Weight loss (%)	Color parameters	Hardness (g)
Protease 1	0.178±0.001^{i}	2.51±0.001^{e}	60.55±0.040^{de}	68.08±0.030^{i} 3.49±0.020^{d} 25.67±0.030^{d}	681.7±0.500^{e}
Protease 2	0.164±0.002^{j}	2.66±0.010^{d}	60.51±0.220^{def}	70.91±0.010^{d} 1.23±0.030^{fg} 18.37±0.020^{m}	725.2±0.105^{c}
Protease 3	0.324±0.004^{d}	4.28±0.020^{b}	57.52±0.040^{i}	71.10±0.100^{cd} 0.90±0.100^{g} 20.21±0.020^{i}	674.8±0.200^{f}
Protease 4	0.217±0.004^{h}	2.26±0.060^{f}	61.31±0.035^{c}	54.19±0.040^{l} 9.70±0.200^{a} 27.86±0.020^{c}	926.7±0.491^{a}
Amylase 1	0.453±0.002^{a}	3.83±0.040^{c}	59.55±0.070^{h}	70.23±0.030^{f} 1.64±0.040^{e} 20.00±0.030^{j}	574.7±0.800^{h}
Amylase 2	0.449±0.001^{a}	4.41±0.010^{b}	60.17±0.065^{g}	67.00±0.040^{j} 4.87±0.020^{c} 28.47±0.060^{b}	524.66±0.509^{j}
Amylase 3	0.432±0.001^{b}	2.05±0.020^{g}	59.53±0.110^{h}	68.39±0.050^{h} 2.89±0.030^{d} 24.77±0.050^{e}	529.5±0.400^{i}
Hemicellulase 1	0.432±0.003^{b}	5.16±0.050^{a}	60.27±0.025^{fg}	72±0.020^{a} 1.38±0.050^{ef} 18.62±0.090^{l}	492±0.750^{k}
Hemicellulase 2	0.184±0.004^{i}	2.26±0.050^{f}	64.09±0.060^{a}	70.91±0.060^{d} 2.30±100^{d} 23.00±0.020^{f}	703±0.420^{d}
Hemicellulase 3	0.297±0.005^{e}	1.51±0.040^{h}	59.53±0.045^{h}	70.63±0.040^{e} 1.24±0.020^{f} 21.67±0.030^{h}	383.66±0.480^{l}
Xylanase 1	0.187±0.007^{i}	1.28±0.050^{i}	62.18±0.060^{b}	69.11±0.040^{g} 2.50±0.200^{d} 22.44±0.040^{g}	624±0.730^{g}
Xylanase 2	0.232±0.002^{g}	0.63±0.030^{j}	60.69±0.125^{d}	66.53±0.030^{k} 6.34±0.050^{b} 29.67±0.030^{a}	772.7±0.200^{b}
Xylanase 3	0.397±0.005^{c}	5.176±0.006^{a}	60.36±0.085^{efg}	71.05±0.020^{c} 2.21±0.010^{d} 22.39±0.040^{g}	353.33±0.060^{m}
Control	0.25±0.003^{f}	3.87±0.050^{c}	52.80±0.080^{j}	71.79±0.010^{b} 1.11±0.020^{fg} 19.48±0.040^{k}	327.5±0.0390^{n}

Table 4 shows the water activity and moisture content (%) values of enzyme added wafers. Water activity and moisture content of wafer sheets containing Protease 3, Amylase 2, Hemicellulase 1, Xylanase 3 enzymes were significantly higher than the control. Protease 1, Protease 2, Protease 4, Hemicellulase 2, Xylanase 2 enzymes have significantly lower water activity and moisture content (%) values compared to the control.

The critical water activity value for the glass transition of the product at 20°C is 0.591. (Martínez-Navarrete et al., 2004). It is seen that the critical water activity value for the glass transition at which softening starts in all enzyme groups and control wafer sheets is lower than the critical water activity value. If the water activity exceeds the critical value for the glass transition, friability is lost (Tufan, 2018). One of the methods used to reduce the water holding capacity of flour is the addition of enzymes (Dogan, 2006).

Weight loss (%)

Data on the weight loss of the wafer doughs used in the study are given in Table 4. When the results were evaluated statistically, it was seen that the control dough without enzyme had significantly less weight loss with 52.80% compared to the wafer doughs containing enzyme. At 0 and 30 minutes, the control dough, which had one of the highest flow behavior time values, retained more moisture than the enzyme-containing doughs, resulting in less weight loss during baking. Wafer sheets containing Hemicellulase 2, Xylanase 1, Protease 4, Xylanase 2 enzymes had the highest weight loss.

According to studies in the literature, the reason for the higher weight loss in the enzyme-free wafer sheets compared to the enzyme-containing wafer sheets is thought to be due to the higher gluten and protein content and lower viscosity of the dough due to the absence of enzymes. While the high water holding capacity of protein and gluten prevents the removal of water from the final product, the high viscosity prevents the water from rising to the surface and mixing with the atmosphere with pressure (Tufan et al., 2020; Naderi, B. et al., 2023).

Color parameters

Maillard reactions due to amino acids and reducing sugars affect the brownness of wafer sheets prepared and cooked under the same conditions. The color data of the wafer sheets are given in Table 4. According to the table, the lightness index (L*) of the doughs prepared by adding enzyme was significantly lower than the control, while the redness index (a*) was significantly higher in the enzyme added groups compared to the control. This is due to the fact that protease enzyme increases amino acids, while hemicellulase, xylanase and amylase enzymes increase reducing sugars (Tufan et al., 2020; Naderi, B. et al., 2023).

The redness value of the wafer sheet with the highest lightness value, Hemicellulase 1 enzyme added, was determined to be lower than the other groups. The lightness value of the wafer sheets with the highest redness value, the wafer sheet with the addition of Protease 4 enzyme, was determined to be lower than the other groups. The lightness value of the control wafer sheet is the highest value after the wafer sheet added with Hemicellulase 1.

Hardness

Textural characteristics observed during biting or chewing, such as crispness, are one of the critical characteristics indicating the freshness of the wafer and are affected by wafer formulation and processing conditions (Altinok, E. et al., 2022). The crispness felt during the consumption of the wafer is characterized by the consumer as fresh and pleasant due to the sound it makes (Çarşanba, et al., 2018).

When we look at Tables 3 and 4, it is seen that the low flow behavior value is compatible with the high hardness value. It is seen that the wafer dough containing Protease 4 enzyme, which has significantly the lowest flow behavior values, gives the highest hardness value ($P > 0.05$). Wafer doughs containing Xylanase 2, Protease 2, Hemicellulase 2 enzymes, which had the highest hardness value, showed similarly low flow behavior ($P > 0.05$). It is seen that all enzymes used in the study have significantly higher hardness value and therefore friability compared to the control wafer sheet because the enzymes reduce the water holding capacity in the wafer dough. Similarly, Altinok, E. et al. (2022) observed that the decrease in flow behavior time was observed with an increase in hardness value.

Sensory analysis

In this study, panelists sensory evaluated wafer sheets containing different enzymes. The data of the panelists' evaluation in terms of general taste are given in Figure 1.

According to the results of the sensory analysis test performed by the panel, it was determined that the samples containing Protease 4, Hemicellulase 2 and Xylanase 2 enzymes were the most liked wafer sheets on average. Panelists stated that they considered crispness and brown caramel color formation in their evaluations. Similar to Aslam et al. (2014), it was observed that higher hardness value caused higher crispness and therefore more sensory appreciation. In addition, according to the sensory analysis results, it was determined that the most liked wafer sheets also had a significantly high redness index.

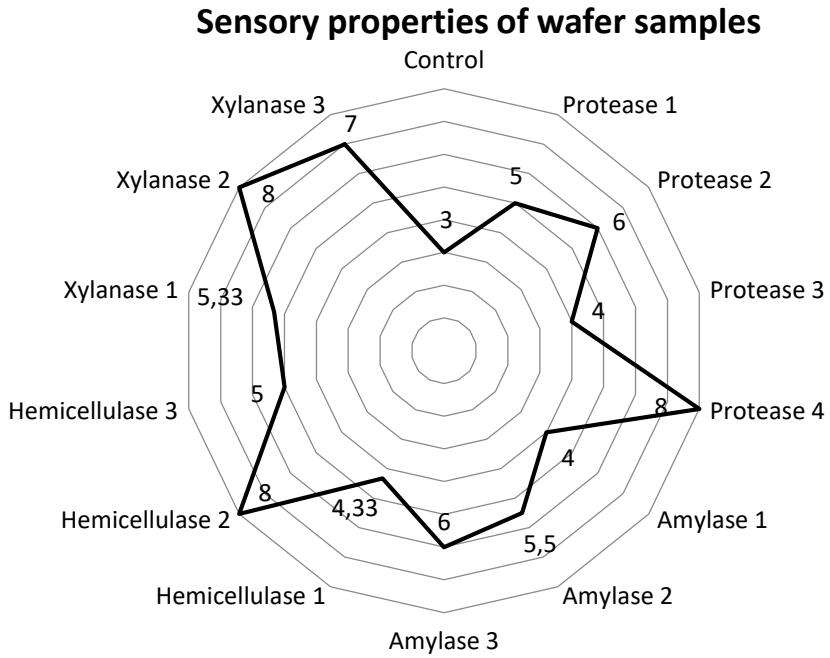

Figure 1. Effect of commercial enzymes on sensory appreciation of wafer sheets

Characterization of Wafer Sheets Containing Enzyme Combinations

Protease 4, Hemicellulase 2 and Xylanase 2 enzymes were selected to be used in combinations in the next wafer sheet studies because they significantly reduced the dough flow behavior time at 30 minutes compared to 0 minutes, had significantly lower aw and moisture content (%) compared to the control, had significantly higher weight loss (%) compared to other enzymes, and produced the most sensory pleasing wafer sheets due to crispness and brown caramel color.

Data on the flow behavior times of wafer doughs containing combined enzyme groups are given in Table 5. When the dough flow behaviors of the groups were examined, it was seen that the group containing only Xylanase 2+ Protease 4 enzyme combination had a significantly lower flow time of 47±1.5 seconds at 0 minute. At 30 minutes, the doughs containing Hemicellulase 2+ Xylanase 2+ Protease 4 and Xylanase 2+ Protease 4 groups had significantly similar lowest flow behavior time with 11±1 and 13±2 seconds. Hemicellulase 2+ Xylanase 2+ Protease 4 and Xylanase 2+ Protease 4 groups were determined as the most advantageous ones at the dough stage in the study we conducted in order to find the most advantageous enzyme group in terms of process ease and speed in industrial wafer production.

The data of the characterization studies of the combinations are given in table 6. When the hardness (g) values, which is an indicator of friability, of the wafer sheets containing the combinations with significantly similar weight loss were examined, it was observed that the Hemicellulase 2+ Xylanase 2 group was the highest with 735.5±0.370 g, Hemicellulase 2+ Xylanase 2+ Protease 4 group had the second highest value with 722.83±0.260 g, while Hemicellulase 2+ Protease 4 and Xylanase 2+ Protease 4 groups had similarly the lowest values with 715.66±0.290 and 714.5±0.250.

Table 5. Flow behavior times of wafer doughs containing combined enzyme groups at 0 and 30 minutes (Significantly different at $P<0.05$ in the columns are indicated by letters)

Enzyme combinations	0. min flow time (s)	30.min flow time (s)
Hemicellulase 2+ Xylanase 2	55±1^{a}	23±1.5^{b}
Hemicellulase 2+ Xylanase 2+ Protease 4	53±1.5^{a}	11±1^{c}
Hemicellulase 2+ Protease 4	53±2.5^{a}	30±1.5^{a}
Xylanase 2+ Protease 4	47±1.5^{b}	13±2^{c}

Table 6. The effect of the selected enzyme combinations on the characterization of wafer sheets (Significantly different at $P<0.05$ in the columns)

Enzyme combinations	aw	Moisture content (%)	Weight loss (%)	Color parameters	Hardness
Hemicellulase 2+ Xylanase 2	0.349±0.004^{a}	5.01±0.230^{a}	60.66±0.140^{a}	68.00±0.500^{a} 1.79±0.040^{d} 13.50±0.520^{d}	735.5±0.370^{a}
Hemicellulase 2+ Xylanase 2+ Protease 4	0.258±0.007^{b}	2.58±0.190^{c}	60.86±0.050^{a}	67.40±0.400^{a} 3.11±0.030^{c} 19.78±0.170^{c}	722.83±0.260^{b}
Hemicellulase 2+ Protease 4	0.203±0.003^{c}	3.66±0.120^{b}	60.68±0.410^{a}	63.41±0.030^{b} 5.09±0.050^{b} 24.20±0.350^{b}	715.66±0.290^{c}
Xylanase 2+ Protease 4	0.155±0.075^{d}	3.67±0.050^{b}	60.8±0.800^{a}	58.54±0.090^{c} 7.31±0.200^{a} 26.81±0.350^{a}	714.5±0.250^{c}

According to the analysis of color parameters, which is one of the factors affecting sensory evaluation, it was observed that the wafer sheets containing Hemicellulase 2+ Xylanase 2 and Hemicellulase 2+ Xylanase 2+ Protease 4 groups had the highest lightness index (L), while the wafer sheets containing Xylanase 2+ Protease 4 group had the highest redness index (a). Images of the wafer sheets containing the combined enzyme groups and the control wafer sheets without enzymes are given in Figure 2.

As determined in the color parameters analysis, the wafer sheet containing Xylanase 2+ Protease 4 group, which has the highest redness index as determined in the color parameters analysis, is seen in an intense brown-caramel color in accordance with the image. In the sensory analysis test of the wafer sheets containing the combined enzyme groups by a panel of 10 people, it was observed that the wafer sheets containing the Xylanase 2+ Protease 4 enzyme group were significantly the most admired in the evaluation of the panelists considering the crispness and brown caramelized color.

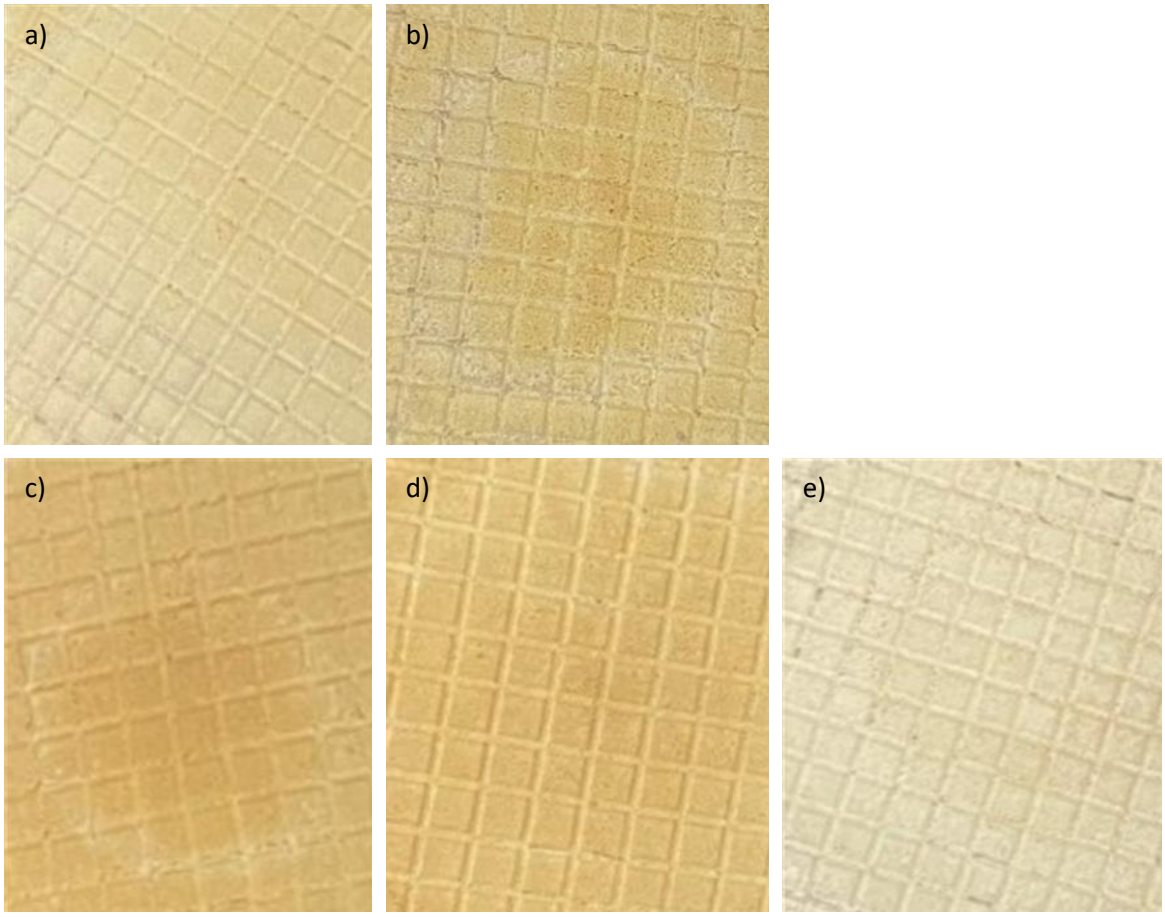

Figure 2. Images of wafer sheets containing combined enzymes and control group (a: Hemicellulase 2+ Xylanase 2, b: Hemicellulase 2+ Xylanase 2+ Protease 4, c: Hemicellulase

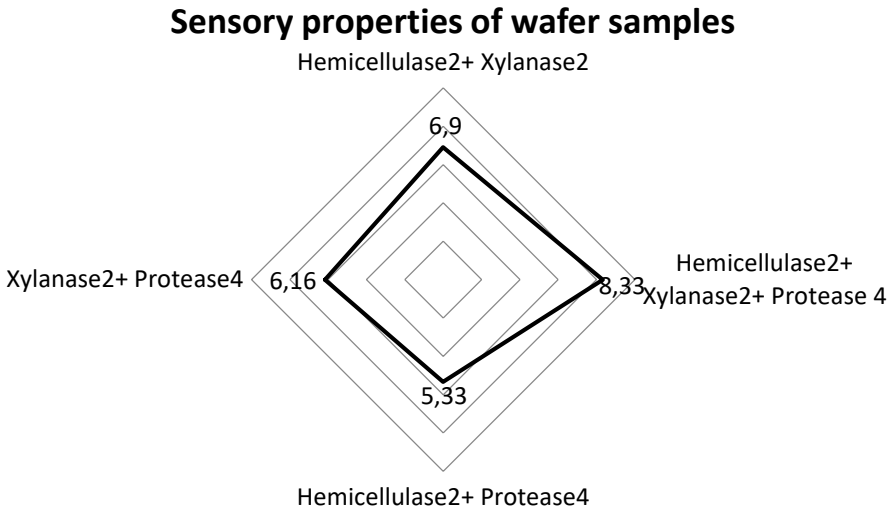

Figure 3. Effect of enzyme combinations on sensory appreciation of wafer sheets

CONCLUSION

In the wafer industry, enzymes are used in order to make the depositor process easily and to save time and cost. For this reason, commercial protease, amylase, hemicellulase and xylanase enzymes, which have the potential to be used in the industry, were applied to wafer dough alone and in combination in our study. Protease 4, Hemicellulase 2, Xylanase 2 enzyme combinations were selected to be tested as a result of the characterization studies carried out in order to determine the effect of the applied enzymes on the processability, color, crispness and sensory appeal of the wafer dough. Among the tried enzyme combinations, 80 ppm Xylanase 2 + 300 ppm Protease 4 group was selected with the sensory desired crispness and brown-caramel color of the wafer sheet as well as the low flow time of the dough containing the group. Xylanase 2+ Protease 4 enzyme group, which reduces water holding capacity and viscosity by breaking down xylan and protein in wheat, is promising for use in industry.

CONFLICT OF INTEREST

The authors declared that they have no actual, potential or perceived conflict of interest for this article.

AUTHORS CONTRIBUTIONS

Fatmanur Poyraz Ekinci: Methodology, Formal analysis, Investigation, Writing – Original Draft Nagihan Kökyar: Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing; Dilek Şener: Conceptualization, Project administration, Supervision, Editing. All authors approved the final manuscript and accepted to be held responsible for the content.

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