Chapter 5

To develop a fiber-rich functional food and its quality evaluation

5.1 Introduction:

By-products are typically viewed as agro-industrial waste, they harbor significant potential as sources of bioactive compounds suitable for use as value-added compounds (Helkar et al., 2016). Various food manufacturing sectors, including cereal, dairy, brewing, marine, meat, fruit, and vegetable processing, produce substantial quantities of by-products. However, the utility of these by-products hinges on their specific composition, which can render them valuable reservoirs of compounds beneficial for consumption by humans (Subiria-Cueto et al., 2021). For instance, certain wastes boast high concentrations of organic acids (such as ascorbic, malic, and phosphoric acid) and phenolic compounds, making them ideal as antibrowning agents. Additionally, their essential oil and phenolic compound content endows them with antimicrobial properties. Furthermore, by-products serve as reservoirs of specific bioactive compounds like dietary fiber, phenolic compounds, and essential minerals (Subiria-Cueto et al., 2021; Olivas-Aguirre et al., 2017).

Pineapple, scientifically known as *Ananas comosus*, is a tropical fruit made up of fused berries, belonging to Bromeliaceae family, and is considered the most cost-effective plant in this family (Dhar et al., 2023). Pineapples are well-known for their delicious taste and balanced sugar-acid ratio, making them highly valued fruits. Pineapples, with their sweetness, offer low calories yet abound in vitamins, antioxidants, and enzymes. They support bone health, bolster the immune system, and facilitate digestion (Jose et al., 2022). Pineapple by-products contain a significant amount of dietary fiber, constituting 76% of the total content, with 99.2% being insoluble fiber and 0.8% soluble fiber, offering considerable nutritional benefits (Meena et al., 2021). In 2019, global pineapple production soared to 28.17 million tonnes, with India ranking as the 6th largest supplier of 1.71 million tonnes approximately (Dhar et al., 2023). The industrial processing of pineapples yields significant biowaste, primarily composed of peel (30%), pomace (50%), core (7%), and crown (13%), collectively representing approximately 25–35% of the total weight of pineapple (Banerjee et al., 2018). These by-products, rich in secondary metabolites, predominantly originate from the cannery and other pineapple processing industries. They hold immense potential for recycling into raw materials or for conversion into higher-value products. Through innovative and technological approaches, pineapple by-products can be transformed into value-added items, offering greater social and economic benefits compared to the main fruit itself (Roda et al., 2019). This highlights the opportunity to harness waste streams from pineapple processing to generate valuable resources and promote sustainability in the pineapple industry.

Dietary fiber is increasingly acknowledged as a vital constituent in food products due to the growing emphasis on creating healthier food options (Fuller et al., 2016). Notably, common food staples like bread are now being utilized to incorporate dietary fiber. This fiber comprises a mixture of plant carbohydrate polymers such as polysaccharides, oligosaccharides, hemicelluloses, cellulose, resistant starch, pectin substances, inulin, and gums. Beyond its resistance to digestion, absorption, and hydrolysis, dietary fiber plays a role in augmenting fecal bulk, encouraging colonic fermentation, and reducing both Pre-prandial cholesterol and post-prandial blood glucose levels within the body (Singh et al., 2018). The utilization of dietary fiber in bread production stems from its advantageous technological properties. For instance, its capacity to retain water aids in preventing bread from undergoing staleness, thereby prolonging its shelf life (Sharma et al., 2016). This analysis explores the importance of dietary fiber in human nutrition, its primary sources in bread production, its influence on dough rheology, and its impact on bread shelf life, among other significant facets.

Enzymes are widely employed for dietary fiber extraction and modification due to their notable efficiency, specificity, and gentle processing conditions (Li et al., 2017). Insoluble dietary fiber (IDF) primarily comprises cellulose and hemicellulose, forming a dense polymeric structure necessitating appropriate treatments for modification. These treatments aim to convert the polymer matrix into simpler carbohydrates while also loosening the compact structure, enhancing porosity, and facilitating partial degradation to enhance the physicochemical and biochemical attributes of insoluble dietary fiber (IDF) (Guo et al., 2018). Various methods have been utilized to modify IDF owing to its coarse texture and limited utilization prospects (Zhao et al., 2018). Among these methods, enzymatic treatment stands out as a straightforward, cost-effective, and environmentally friendly approach. Studies have shown that complex enzymatic hydrolysis significantly enhances physicochemical properties (Zhang et al., 2019).

Bakery products are staples in numerous cultures globally, frequently consumed (Arranz-Otaegui et al., 2018). Yet, their considerable carbohydrate (starch and sugars) and fat content can contribute to weight issues such as overweight or obesity in consumers (Serra-Majem & Bautista-Castaño, 2015). Consequently, there has been a growing interest among researchers and the food industry in incorporating functional components sourced from agro-industrial waste into bakery formulations.

Several bakery products have undergone enrichment through the incorporation of by-products. For example, bread has been supplemented with dietary fiber and phenolic compounds through the incorporation of pomegranate seed powder (Gül and Sen, 2017) and white cabbage residue (Pop et al., 2021). Gluten-free foods have been prepared using unripe plantain peel flour to increase the dietary fiber and antioxidant compounds (Agama-Acevedo et al., 2016). Moreover, white bread has been prepared with raw mango peel powder to increase the bioactive compounds (Pathak et al., 2017), while bread has been fortified with dietary fiber using jackfruit rind powder (Feili, 2014). Additionally, bakery products such as muffins, bread, and brownies have been enhanced with dietary fiber and enriched with phenolic compounds by incorporating red and white wine grape pomace (Walker et al., 2014), among other instances. These interventions underscore a trend in utilizing by-products to augment the nutritional profile of bakery items, emphasizing the potential for waste reduction and value addition in the food industry.

The primary motive behind incorporating waste-derived dietary fiber into bread formulations is to enhance their bioactive content. However, limited research has explored the interplay of dietary fiber in bakery goods incorporated with by-products. Thus, it is imperative to assess how various bread-making factors such as fermentation, baking, and storage directly impact bioactive contents and interactions. Furthermore, comparing the bioactive compound levels of enriched products with those of unenriched counterparts is crucial (Santos et al., 2022; Aiello et al., 2020; Vasileva et al., 2018; Spiker et al., 2017;). This approach ensures a comprehensive understanding of the effects of waste-derived dietary fiber enrichment on the nutritional composition of bakery goods.

In summary, while traditionally viewed as waste, agro-industrial by-products represent a rich source of bioactive compounds with diverse functionalities. Their potential applications span from preventing browning in food products to combating microbial contamination and enriching food formulations with essential nutrients and bioactive compounds. Thus, harnessing these by-products can not only mitigate waste but also contribute to the development of healthier and more sustainable food products.

5.2.Methodology:

5.2.1 Materials

α-amylase (porcine pancreatin), amyloglucosidase, protease, and glucose assay kit (GAGO-20) were used for the present experiment. All the chemicals used were of high-purity analytical grade (SIGMA ALDRICH, USA).

5.2.2 Preparation of functional bread

The preparation of functional bread was described by Begum et al., 2020 with slight modifications. The control bread was made by using 100g of refined flour, 60 ml water, 2g instant dry yeast, 2g salt, 5 g white refined sugar, and 4g fat. The enzyme-modified dietary fiber (EMDF) was added at different levels of 1, 2, 5, and 10% to substitute the flour in the formation of functional bread (Table 1). The enzyme-modified dietary fiber (EMDF) levels were selected based on our preliminary trials. Preparation of dough was done after mixing all the above-mentioned components and proofed for 90-95 min at ambient temperature. Further, the dough was placed in a pan using butter paper after molding. Finally, the dough was beaked for 15min at 210-220ºC after the final proofing time of 40-45 min. Functional Bread loaves were then removed from the pan and let the loave to cool at room temperature. The functional bread was then stored in polyethylene pouches for further investigation.

Table 5.1: Functional bread formulation with enzyme modified dietary fiber:

Sample code	EMDF (%)
Product A	1
Product B	2
Product C	5
Product D	10

5.2.3 Chemical constituents of functional bread

Functional bread was further analyzed for its chemical constituents through proximate analysis (crude fat, crude protein, crude fiber, moisture content, ash) according to the AOAC method. The carbohydrate content was calculated by the difference of all the other chemical compositions. The soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) content were determined using the enzymatic-gravimetric method.

5.2.3.1 Water retention capacity of functional bread

The water retention capacity (WRC) of functional bread was calculated using the below equation:

$$WRC\ (\%) = \frac{Wa}{wb} \times 100 \qquad (1)$$

W_a represents the total weight of the sample after baking

W_b represents the total weight of the sample before baking

5.2.3.2 Specific volume of functional bread

The following formula was applied in order to calculate the specific volume (Sv) of a sample using the rapeseed displacement method:

$$Sv\ (cm3\ g-1)\ =\ \frac{Loaf\ volume}{Loaf\ weight} \qquad (2)$$

5.2.4 Color Characteristics of Functional Bread

Color characteristics of developed functional bread (crumb and crust) were determined by using a Minolta CR-410 portable colorimeter (Konica Minolta, Japan). The experimental statistics were presented as the following: L* (lightness/brightness), a* (positive and negative a represents redness and greenness respectively), and b* (positive and negative b represents yellowness and blueness respectively).

5.2.5 Texture profile analysis of functional bread

Using a Probe Texture Analyzer (TAHD plus, stable microsystem, UK) fitted with a 50 N cell weight, the hardness and extensibility of the functional bread were evaluated by texture profile analysis (Arora et al., 2023). The centre of the functioning bread was where the experimental sample was taken out. At a test speed of 1 mm/s, the sample was compressed to 50% of its initial elevation using a cylindrical probe with a 25 mm diameter. Hardness (N) was the highest pressure that was applied to the functioning bread during compression. Springiness was calculated as the ratio of the height of the sample that rebounded to its original position after the first compression or to the highest distortion. Gumminess, chewiness, cohesiveness, and springiness were analyzed accordingly.

$$Gumminess\ =\frac{G2}{G1}\times\ Hardness \qquad (3)$$

$$Springiness\ =\frac{T2}{T1} \qquad (4)$$

$$Chewiness\ =\ Gumminess\ \times\ Springiness \qquad (5)$$

$$Cohesiveness\ =\ \frac{G2}{G1} \qquad (6)$$

Where G1 and G2 represent the area of the 1st compression and 2nd compression cycle respectively.

T1 and T2 represent the time variation between the 1st and 2nd compression cycles respectively.

5.2.6 Sensory evaluation

The sensory assessment of functional bread took place instantly after the loaves had cooled to ambient temperature. Slices of the functional bread were prepared for sensory evaluation using a hedonic scale. Fifteen panelists were involved in the evaluation process. The sensory attributes considered for the functional bread included crumb color, appearance, taste, tenderness, and overall quality, rated on a 9-point scale. Ratings ranged from 1, indicating extreme dislike, to 9, indicating extreme liking. Panelists were instructed to evaluate each sample independently without comparing them to one another.

5.2.7 In vitro analysis of functional bread

5.2.7.1 *In vitro* starch digestibility of functional bread

In vitro, starch digestibility of the prepared functional bread was analyzed according to the procedure of Devi et al., 2023 with slight modification. The functional bread sample was dried till the equilibrium moisture content and prepared as a powder for further analysis. The powdered sample was mixed with sodium acetate buffer (0.5 M, pH 5.2) in a ratio of 1:10 (w/v). To formulate working solution A, α-amylase (porcine pancreatin) was solubilized in distilled water at a ratio of 1:7; w/v. Subsequently, solution B was created by solubilizing deionized water with amyloglucosidase in a ratio (1:1; v/v). Then, 50 mL of solution A was combined with 10 mL of solution B to produce the final solution. Following a 10-minute incubation at 37.7°C, 5 mL of the resultant solution was introduced into the prepared sample suspensions. These suspensions underwent incubation in a water bath with continuous agitation at 37°C and 200 rpm. Sampling occurred at specific time intervals (0, 20, 60, 120, 180, and 240 minutes), with 1 mL of the starch hydrolyzed solution withdrawn and combined with 20 mL of 70% ethyl alcohol to stop the enzyme activity. Total starch (TS) hydrolysis was assessed by suspending the residue in 6 mL of 2M KOH, afterward incubation in a water bath for 30 minutes at 200 rpm. Subsequently, centrifugation at 5000 rpm for 15 minutes was performed, and the supernatant was utilized for glucose analysis using a GOPOD kit (GAGO-20) at 500 nm. The determination of total starch (TS), rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) was accomplished utilizing the subsequent formulas:

$$TS = Tg \times 0.9 \quad (7)$$

$$RDS = \frac{(G20 - G0) \times 0.9}{TS} \times 100 \quad (8)$$

$$SDS = \frac{(G120 - G20) \times 0.9}{TS} \times 100 \quad (9)$$

$$RS = \frac{TS - (G120 \times 0.9)}{TS} \times 100 \quad (10)$$

Where, G_0, G_{20}, and G_{120} represent the release of glucose at 0, 20, and 120 min of hydrolysis respectively.

5.2.7.2 Approach to Kinetic modeling:

The percentage of total starch hydrolyzed at different time intervals (0, 20, 60, 120, 180, and 240 minutes) was used to calculate the starch digestion rate. We evaluated starch hydrolysis kinetics using the first-order kinetic equation that Goni et al. (1997) proposed:

$$C = C\infty (1 - e - kt) \quad (11)$$

Where t is the selected time, k is the kinetic constant, C is the starch hydrolysis (%) at time t, and $C\infty$ is the equilibrium concentration.

The following equation was used to analyze the starch hydrolysis curve by calculating the area under the curve based on starch hydrolysis over time:

$$AUC = C\infty (tf - t0) - (C\infty / k) [1 - exp \{-k (tf - t0)\}] \quad (12)$$

$C\infty$ denotes the percentage of starch hydrolysis at 240 minutes, t_f stands for the total time (240 minutes), t_0 for the start time, and k for the kinetic constant.

The AUC was used to determine the hydrolysis index (HI) for each sample using the following equation:

$$HI = \frac{AUC\ of\ each\ sample}{AUC\ of\ control\ bread} \times 100 \quad (13)$$

The predicted glycaemic index (pGI) was determined using the following equation:

$$pGI = 39.71 + 0.549\, HI \quad (14)$$

5.2.8 Statistical analysis:

The triplicate of each trial was conducted. Using SPSS 16, the data's mean values were examined using Duncan's multiple range test to look for any significant differences at a significance level of 0.05 ($p < 0.05$).

5.3 Result and Discussion

5.3.1 Chemical composition of Functional bread

The chemical composition (moisture content, fat, protein, crude fiber, ash, and total carbohydrate) of Queen pineapple waste extracted enzyme-modified dietary fiber incorporated functional bread is shown in Table 5.2.

Table 5.2: Chemical composition of functional bread

Sample code	Moisture content (%)	Fat (%)	Protein (%)	Crude fiber (%)	Ash (%)	Total carbohydrate
Control	36.17 ± 0.05^{a}	7.01± 0.02^{a}	11.21 ± 0.06^{a}	3.84 ± 0.18^{a}	1.82 ± 0.14^{a}	39.95 ±0.07^{a}
Product-A	34.78± 0.11^{ab}	7.67±0.12^{b}	10.21 ± 0.12^{ab}	4.21 ± 0.21^{ab}	1.93 ± 0.21^{b}	41.20 ± 0.18^{b}
Product-B	33.35 ± 0.12^{abc}	7.88 ± 0.08^{bc}	9.32±0.20^{c}	5.89 ± 0.25^{abc}	2.01 ± 0.42^{c}	41.55 ± 0.21^{bc}
Product-C	31.83 ± 0.25^{d}	7.52 ± 0.21^{d}	8.68 ± 0.28^{cd}	6.41 ± 0.32^{d}	1.98 ±0.35^{d}	43.58 ± 0.33^{d}
Product-D	30.11 ± 0.35^{e}	7.21±0.35^{e}	8.12 ± 0.37^{e}	8.98 ± 0.43^{e}	2.09 ± 0.48^{e}	43.49 ± 0.51^{e}

(Mean values with differing superscript letters in the same column represent a statistically significant difference at a significance level of $p < 0.05$. The values are presented as mean ± standard deviation; n = 3).

Enzyme-modified dietary fiber (EMDF) incorporation into the bread modifies the chemical constituents of the functional bread as compared to the control bread. The moisture content varies from 36.17 to 30.11 %, Product D having the lowest moisture content. The fat content

(7.01% for control and 7.21 % for product D) did not vary greatly among all the samples and was in a range from 7.01 to 7.21%. The protein content was highest in the control sample 11.21%, lowest in the sample coded as product D 8.12%, and 10.21%, 9.32%, and 8.68% for product A, product B, and product C respectively. The addition of dietary fiber from 1 to 10% influences gluten formation and also results in a weak protein network during dough formation. The ash content, denoting the mineral residue after complete combustion, exhibited minimal variance across the bread samples, falling within the range of 1.82% to 2.09%. The carbohydrate composition exhibited variability among all samples as compared to the control, ranging from 39.95% to 43.49%. Crude fiber increased with the addition of dietary fiber from 1 to 10%. The amount of crude fiber in the control sample was 3.84% and for 1,2,5, and 10% of addition of dietary fiber resulted in the increase of crude fiber from 4.21% 5.89%, 6.41%, and 8.98% for Product A, Product B, Product C, and Product D respectively. The total dietary fiber content along with IDF and SDF of the functional bread is shown in Table 5.3.

Table 5.3: Dietary fiber composition of functional bread

Sample code	Insoluble dietary fiber (%)	Soluble dietary fiber (%)	Total dietary fiber (%)
Control	7.19 ± 0.12^{a}	0.47 ± 0.08^{a}	7.66 ± 0.05^{a}
Product A	12.52 ± 0.15^{ab}	0.87 ± 0.21^{b}	14.39 ± 0.12^{b}
Product B	14.12 ± 0.32^{c}	0.96 ± 0.25^{bc}	15.08 ± 0.21^{c}
Product C	17.85 ± 0.38^{cd}	1.12 ± 0.35^{d}	18.97 ± 0.28^{d}
Product D	18.87 ± 0.21^{e}	1.68 ± 0.41^{e}	20.55 ± 0.36^{e}

(Mean values with differing superscript letters in the same column represent a statistically significant difference at a significance level of $p < 0.05$. The values are presented as mean ± standard deviation; n = 3).

5.3.2 Physical properties of Functional bread:

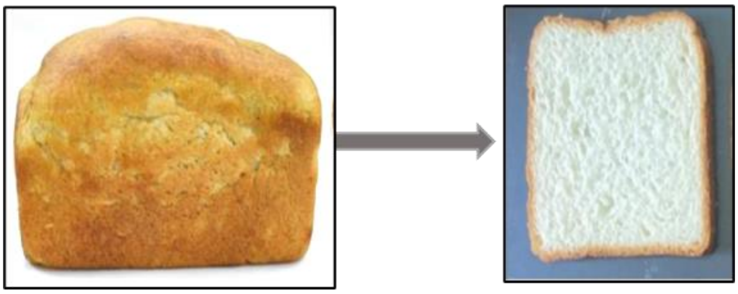

Fig 5.1: Control bread sample (Without fortification)

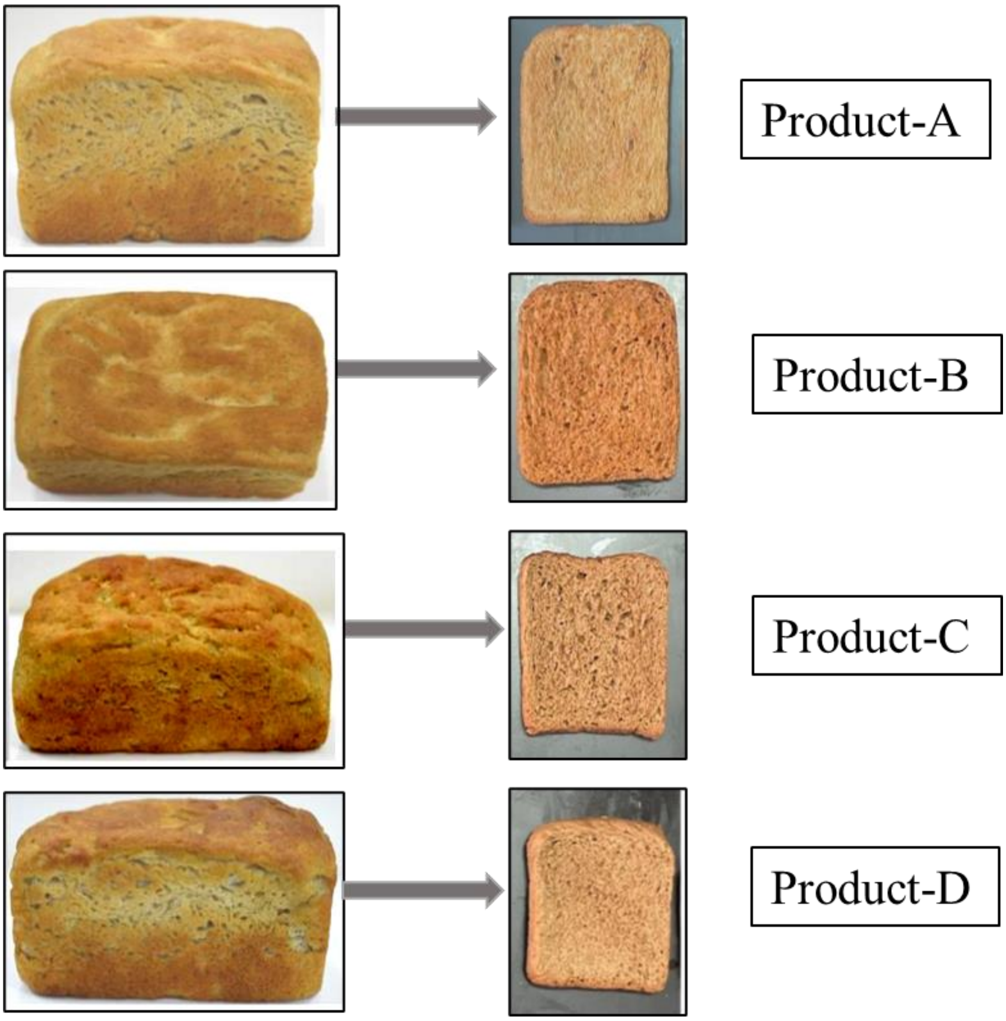

Fig 5.2: Bread fortified with Pineapple waste-extracted enzyme-modified dietary fiber

5.3.2.1 Water retention capacity and specific volume of functional bread

The water retention capacity (WRC) of functional bread ranged from 88.11% to 83.21 % for control and product-D respectively shown in Table 5.4. The WRC illustrated an inconsistent water absorption among all the samples. WRC of all the samples decreased as the increase in the incorporation of enzyme-modified dietary fiber (EMDF). High EMDF addition participates in the gluten matrix of bread during the dough formation for the absorption of free water molecules, resulting in less water content for extending the gluten matrix in the dough of the functional bread.

Table 5.4: Physical properties of functional bread

Sample code	**Specific volume (cm^3/g)**	**Water retention capacity (%)**
Control	5.88 ± 0.05^a	88.11 ± 0.04^a
Product-A	5.74 ± 0.06^{ab}	86.12 ± 0.15^b
Product-B	5.58 ± 0.08^c	85.27 ± 0.21^c
Product-C	5.41 ± 0.21^d	84.65 ± 0.29^d
Product-D	5.28 ± 0.35^e	83.21 ± 0.35^e

(Mean values with differing superscript letters in the same column represent a statistically significant difference at a significance level of $p < 0.05$. The values are presented as mean ± standard deviation; n = 3).

The specific volume represents a crucial quality parameter of bread, representing the capacity of dough for expansion. The specific volume of the EMDF-incorporated functional bread ranged from 5.88 to 5.28 (cm^3/g), as shown in Table 5.4. The specific volume of the control sample was highest as compared to the other EMDF-incorporated bread samples. With the increase in EMDF content from 1 to 10% in the bread flour level the specific volume was consecutively decreased from 5.74 to 5.28 cm^3/g. During dough mixing, yeast-produced CO_2 is entrapped within the dough matrix, primarily held by inter- and intra-disulfide bonds within the gluten network. This entrapment facilitates dough expansion during fermentation, contributing to the final bread volume. The incorporation of dietary fiber (DF) in bread can dilute gluten, weakening its network and reducing its ability to trap air during fermentation. This can lead to lower bread loaf volume due to compromised dough expansion. The reduction in bread specific volume observed upon fortification with wheat fiber, psyllium husk fiber, and partially hydrolyzed guar gum may stem from their interference with gluten development and gas retention, resulting in a denser crumb structure (Mudgil et al., 2016).

5.3.2.2 Color characteristics of functional bread:

The color characteristics of functional bread can be altered after incorporating EMDF due to enzymatic activity breaking down complex molecules, potentially leading to changes in browning reactions. Additionally, the interaction between the modified fiber and other bread components may influence the overall color profile, potentially resulting in variations compared to unmodified fiber or control formulations. The color characteristics control and EMDF incorporated functional bread sample are shown in Table 5.5.

Table 5.5: Color characteristics of functional bread:

Sample code	Crust L*	Crust a*	Crust b*	Crumb L*	Crumb a*	Crumb b*
Control	48.85 ±0.73[a]	6.10 ±0.98[a]	12. 12 ±1.23[a]	72.35 ±0.93[a]	-0.40 ±0.03[a]	8.76 ±0.27[a]
Product-A	46.12 ±0.25[a]	7.01 ±0.73[ab]	14.02 ±0.98[a]	68.12 ±0.83[b]	1.10 ±0.09[b]	18.71 ±0.97[d]
Product-B	45.67 ±0.45[a]	7.56 ±1.13[ab]	11.87 ±1.45[a]	64.23 ±0.53[bc]	1.61 ±0.10[bc]	16.02 ±0.73[c]
Product-C	43.93 ±0.15[a]	10.78 ±1.83[c]	13.92 ±1.03[a]	59.05 ±0.38[bc]	2.01 ±0.13[bc]	15.32 ±0.75[c]
Product-D	41.87 ±0.31[a]	12.98 ±1.73[c]	12.89 ±0.79[a]	52.11 ±1.18[d]	3.39 ±0.17[d]	10.52 ±0.57[d]

(Mean values with differing superscript letters in the same column represent a statistically significant difference at a significance level of $p < 0.05$. The values are presented as mean ± standard deviation; n = 3).

As the level of enzyme-modified dietary fiber (EMDF) increases in the functional bread, there is a visible increase in the redness (a* value) of the crumb, accompanied by a reduction in lightness (L* value). The LAB values of the crumb of EMDF-incorporated functional bread are significantly different from those of the control. This suggests a substantial alteration in the bread's color profile attributed to the presence of EMDF, likely due to its impact on Maillard reactions and pigment formation during baking. The outcomes reported by Huang et al., 2020 with our current findings, indicate a direct correlation between changes in the a* and b* values and the inherent color properties of the raw materials utilized in bread production. This suggests that variations in these color parameters can be attributed to differences in the composition and characteristics of the ingredients incorporated into the bread formulation, thereby influencing the final color of the bread product (Xu et al., 2021). The incorporation of EMDF did not result in a significant difference in the color of the crust. This observation is consistent with previous studies on bread fortified with rice bran dietary fiber, as reported by Sheikholeslami et al. (2021) and Wen et al. (2017). These findings suggest that certain types of dietary fiber may have limited influence on the color characteristics of the crust in bread formulations.

5.3.2.3 Textural properties of functional bread:

The textural characteristics of functional bread were greatly impacted by the amount of EMDF added to functional bread flour. Table 5.6 displays the textural characteristics of the functional bread.

Table 5.6: Textural properties of functional bread:

Sample code	Hardness (N)	Springiness	Chewiness (N)
Control	2.45 ±0.01^{a}	0.86 ±0.08^{a}	2.12 ±0.04^{a}
Product-A	3.21 ±0.05^{ab}	0.82 ±0.10^{ab}	1.04 ±0.05^{ab}
Product-B	3.67 ±0.12^{c}	0.77 ±0.18^{c}	0.94±0.15^{c}
Product-C	3.88 ±0.22^{d}	0.71 ±0.24^{d}	0.85 ±0.22^{d}
Product-D	4.01 ±0.38^{e}	0.62 ±0.38^{e}	0.78 ±0.31^{e}

(Mean values with differing superscript letters in the same column represent a statistically significant difference at a significance level of $p < 0.05$. The values are presented as mean ± standard deviation; $n = 3$).

Hardness, a characteristic influenced by the force required to compress bread, correlates with bite force and serves as a key metric for assessing bread quality. This parameter offers insight into the structural integrity and texture of the bread, guiding evaluations of its overall acceptability and consumer preference (Ma et al., 2020). The hardness of the functional bread sample ranged from 1.45 to 4.01 N which varied significantly among all the EMDF-incorporated samples as compared to the control. With the increase of EMDF incorporation the hardness of functional bread sample was observed. Product-D, containing 10% EMDF, exhibited the highest hardness, attributed to the gluten dilution effect, which compromises the gluten network and ability to retain air during fermentation. Consequently, this leads to a denser bread crumb structure characterized by reduced porosity and increased firmness.

The springiness values of functional bread varied from 0.96 to 0.62 across different levels of enzyme-modified dietary fiber (EMDF) incorporation. Although the control sample exhibited the highest springiness (0.96), differences were statistically non-significant up to 1% EMDF incorporation. However, the lowest springiness value (0.62) was observed with 10% EMDF incorporation, attributed to a weakened gluten matrix resulting from reduced bread loaf volume (Kiumarsi et al., 2019).

In textural properties, the chewiness is directly proportional to the hardness. The chewiness of functional bread displayed a range from 1.12 to 0.78 N. Notably, Product-D containing 10% EMDF showed the lowest chewiness at 0.78 N, suggesting a weakening of the internal bonds among bread components (Begum et al., 2020). This decline in chewiness may be associated with a reduction in specific volume, indicating a potential interrelationship between textural properties and change in specific volume. Some studies have reported that the inclusion of dietary fiber can lead to increased hardness and chewiness of bread (Bouaziz et al., 2020), a finding consistent with our observations.

5.3.3 Sensory evaluation of functional bread:

The assessment of functional bread quality through sensory evaluation relies on subjective qualitative judgments, reflecting consumer preferences rather than absolute measures. Fig. 5.2 illustrates the impact of enzyme-modified dietary fiber (EMDF) on the sensory characteristics (appearance, color, taste, tenderness, and overall acceptability) of control bread and functional bread with varying levels of EMDF incorporation. Sensory evaluation results indicated that both control bread and EMDF-incorporated (1%, 2%, 5%, and 10%) functional bread had a significant effect on quality and overall acceptability.

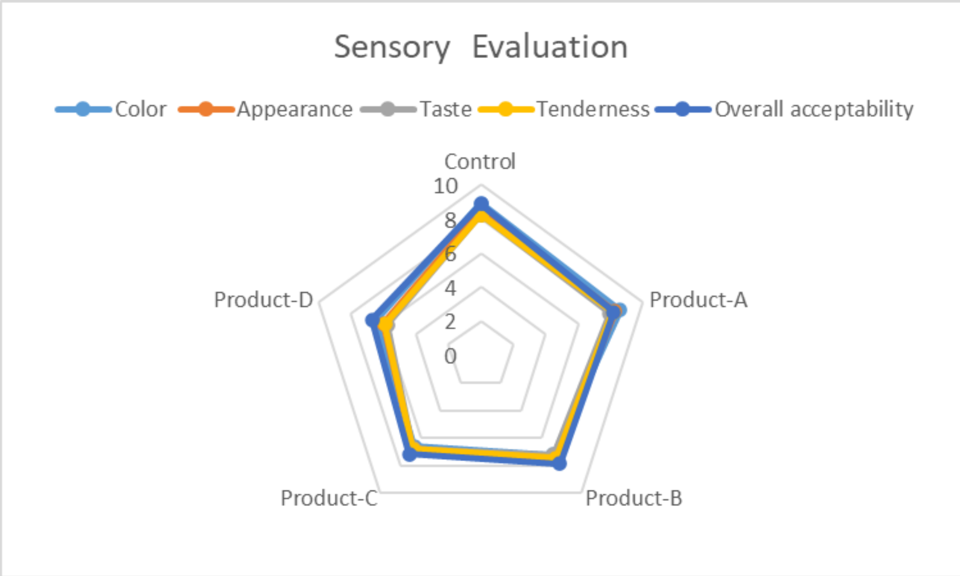

Fig 5.3: Sensory evaluation of functional bread

The control bread formulation received the highest scores across all sensory characteristics assessed (Table 5.7), consistent with findings by Jeddou et al. (2017). Their study demonstrated that replacing wheat flour with EMDF from queen pineapple waste kept the functional and sensory qualities of the bread. Similarly, it was reported that up to 10% apple pomace flour could be incorporated into cake formulations (Valkova et al., 2022), further supporting the potential for utilizing dietary fibers in bakery products without compromising sensory appeal.

Table 5.7: Sensory evaluation of functional bread:

Sample code	Color	Appearance	Taste	Tenderness	Overall acceptability
Control	8.92 ± 0.36^{a}	8.46 ± 0.18^{a}	8.23 ± 0.20^{a}	8.23 ± 0.16^{a}	8.87 ± 0.28^{c}
Product-A	8.58 ± 0.52^{b}	8.27 ± 0.23^{ab}	7.92 ± 0.23^{ab}	8.15 ± 0.22^{b}	8.18 ± 0.41^{e}
Product-B	7.23 ± 0.63^{c}	7.53 ± 0.24^{abc}	7.23 ± 0.20^{ab}	7.46 ± 0.21^{bc}	7.88 ± 0.11^{a}
Product-C	6.61 ± 0.68^{d}	6.76 ± 0.25^{bcd}	6.75 ± 0.33^{c}	6.78 ± 0.25^{bcd}	7.18 ± 0.21^{b}
Product-D	6.46 ± 0.80^{e}	6.00 ± 0.33^{e}	5.76 ± 0.42^{d}	5.92 ± 0.39^{e}	6.7 ± 0.35^{d}

(Mean values with differing superscript letters in the same column represent a statistically significant difference at a significance level of $p < 0.05$. The values are presented as mean ± standard deviation; n = 3).

5.3.4 *In vitro* analysis of functional bread:

5.3.4.1 *In vitro* starch digestibility:

The percentages of total starch (TS), rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) in the prepared functional bread are illustrated in Table 8. The developed EMDF-incorporated functional bread has lower TS (44.07% for product-D) as compared to the control bread (66.12%).

Table 5.8: Starch digestible fraction of functional bread

Sample	TS (%)	RDS (%)	SDS (%)	RS (%)
Control	66.12 ± 0.12^{a}	50.71 ± 0.28^{b}	50.08 ± 0.11^{a}	4.11 ± 0.38^{c}
Product-A	56.41 ± 0.08^{ab}	46.32 ± 0.33^{c}	54.26 ± 0.21^{ab}	6.5 ± 1.02^{d}
Product-B	50.28 ± 0.31^{c}	45.89 ± 0.04^{a}	55.72 ± 0.48^{c}	8.82 ± 0.08^{a}
Product-C	47.76 ± 0.47^{d}	44.56 ± 0.51^{d}	57.04 ± 1.65^{d}	11.23 ± 0.21^{b}
Product-D	44.07 ± 0.55^{e}	42.38 ± 0.64^{e}	58.32 ± 2.23^{e}	14.08 ± 1.43^{e}

(Mean values with differing superscript letters in the same column represent a statistically significant difference at a significance level of $p < 0.05$. The values are presented as mean ± standard deviation; $n = 3$).

The starch digestion curve of functional bread samples is shown in Fig 4. Total starch hydrolysis kinetics adhere to first-order kinetics, exhibiting varying rates of starch digestion in the functional bread samples. The inclusion of EMDF resulted in a reduction in starch hydrolysis, with the decrease being directly proportional to the quantity of EMDF added. The rapidly digestible starch (RDS) content in the functional bread samples decreased from 50.71% in the control bread to 42.38% in Product-D. On the other hand, the SDS increased from 50.08% (control) to 58.32% (product-D). SDS, or slowly digestible starch, is considered more advantageous than RDS, or rapidly digestible starch, as it leads to a gradual elevation in postprandial glucose levels, thus aiding in the regulation of blood glucose levels (Noro et al., 2016). This trend is represented in the behavior of resistant starch (RS). In the control sample, RS was measured at 4.11%, while in product-D, with increasing amounts of enzyme-modified dietary fiber (EMDF), RS levels rose to 14.08%. These outcomes are reported by Sciarini et al. (2017).

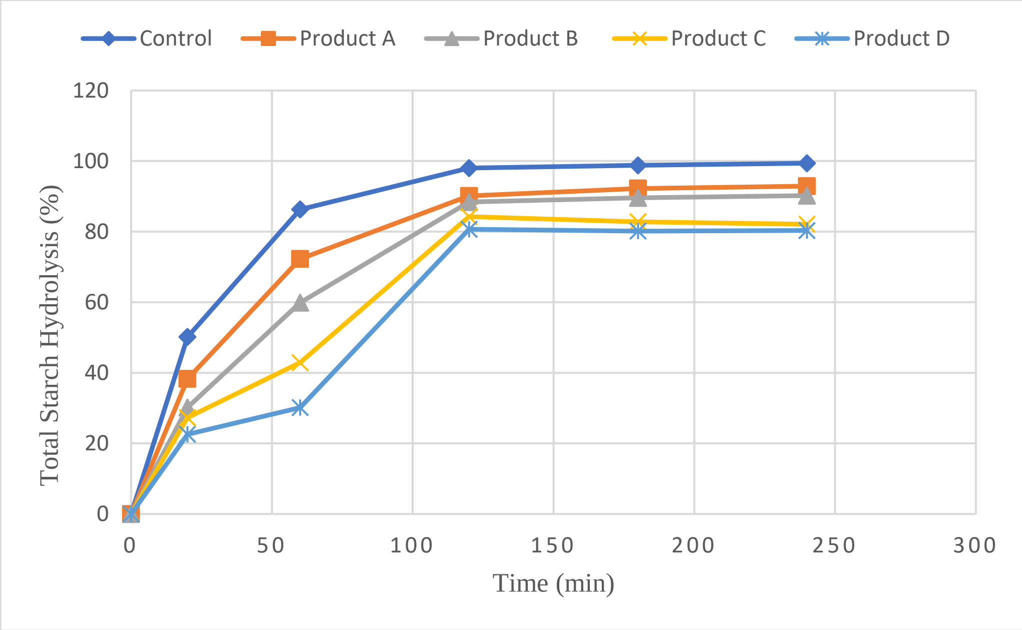

Fig 5.4: Rate of total starch hydrolysis of functional bread

The initial 20 minutes and the subsequent 20 to 120 minutes were designated for assessing rapidly digestible starch (RDS) and slowly digestible starch (SDS), respectively. RDS and SDS serve as indicators of the absorption rate within the small intestine. Incorporating insoluble dietary fiber (DF) in fiber-enriched cakes tended to diminish RDS while augmenting SDS compared to the control, as observed by Bae and Lee (2014).

5.3.4.2 Predicted glycemic index:

The predicted glycemic index (pGI) of each functional bread sample was calculated using reaction rate constant data according to the equation (), as depicted in Table 9. Significantly reduced pGI values were observed for EMDF-incorporated functional bread compared to the control bread. Among these samples, product-D exhibited the lowest pGI value of 67.03. The increased level of enzyme-modified dietary fiber (EMDF) notably delays starch digestion, primarily attributable to its substantial water-holding capacity, consequently leading to a reduction in the predicted glycemic index (pGI) value (Begum et al., 2020). Similarly, pasta and extruded products enriched with insulin as a soluble dietary fiber source demonstrate reduced carbohydrate digestibility, resulting in lower glycemic index values (Goh et al., 2015). Diets characterized by low glycemic index and elevated resistant starch content have been associated with the prevention and management of conditions linked to glucose metabolism (Goh et al., 2015). Accordingly, health-conscious consumers tend to prefer foods with lower glycemic index values (Chi et al., 2019). Additionally, insoluble dietary fiber (IDF) has been reported to be more effective than soluble dietary fiber in reducing pGI values (Bae and Lee et al., 2014).

Table 5.9: Determination of pGI of functional bread

Sample	AUC	HI (%)	pGI
Control	15176.19 ± 132.12^{a}	101.76 ±1.89^{a}	95.58 ^{cd}
Product-A	13218±120.76^{b}	85.23±1.43^{b}	86.50^{c}
Product-B	11321.89 ± 95.32^{c}	77.54 ± 1.11^{c}	82.28^{b}
Product-C	9034.76± 73.34^{d}	61.78±1.06^{d}	73.63^{cde}
Product-D	6345.65±61.45^{e}	49.76 ± 0.97^{e}	67.03^{a}

(Mean values denoted by distinct superscript letters within the same column indicate a significant distinction at $p<0.05$.

5.4 Conclusion:

Incorporating various levels of enzyme-modified dietary fiber (EMDF) - 1%, 2%, 5%, and 10% of the flour mixture exerts notable impacts on both the chemical and physical characteristics of bread. It significantly influences attributes such as starch digestibility and glycemic index (pGI). Increased EMDF incorporation correlates with heightened bread hardness, with the highest total dietary fiber content observed at the 10% EMDF level. Furthermore, escalating EMDF levels result in decreased specific volume (5.28 cm^3/g) and yield firmer bread characterized by elevated chewiness and reduced springiness, as evidenced by texture analysis data. Choosing an EMDF incorporation level of 5% of the flour mixture yields nutritionally balanced bread comparable to the control variant in terms of specific volume, hardness, and springiness. Moreover, functional bread maintains acceptable sensory characteristics concerning color, taste, tenderness, appearance, and overall acceptability. EMDF supplementation alters starch digestibility, notably reducing rapidly digestible starch (RDS) and increasing slowly digestible starch (SDS) content compared to the control. Additionally, functional bread fortified with EMDF exhibits higher resistant starch (RS) content compared to the control, indicative of its potential health benefits. The glycemic index (pGI) diminishes with increasing EMDF incorporation, with the lowest value observed in bread fortified with 10% EMDF, underscoring its potential as a low-glycemic option.

Bibliography:

1. Agama-Acevedo, E., Sañudo-Barajas, J. A., Vélez De La Rocha, R., González-Aguilar, G. A., and Bello-Perez, L. A. (2016). The potential of plantain peels flour (Musa paradisiaca L.) as a source of dietary fiber and antioxidant compound. *CyTA-Journal of Food*, 14(1), 117-123.
2. Aiello, F., Restuccia, D., Spizzirri, U. G., Carullo, G., Leporini, M., and Loizzo, M. R. (2020). Improving kefir bioactive properties by functional enrichment with plant and agro-food waste extracts. *Fermentation*, 6(3), 83.
3. Arora, K., Tlais, A. Z. A., Augustin, G., Grano, D., Filannino, P., Gobbetti, M., & Di Cagno, R. (2023). Physicochemical, nutritional, and functional characterization of gluten-free ingredients and their impact on the bread texture. *LWT*, *177*, 114566.
4. Bae, I. Y., and Lee, H. G. (2014). In vitro starch digestion and cake quality: Impact of the ratio of soluble and insoluble dietary fiber. *International Journal of Biological Macromolecules*, 63, 98-103.
5. Begum, Y. A., Chakraborty, S., and Deka, S. C. (2020). Bread fortified with dietary fibre extracted from culinary banana bract: Its quality attributes and in vitro starch digestibility. *International Journal of Food Science & Technology*, 55(6), 2359-2369.
6. Bouaziz, F., Ben Abdeddayem, A., Koubaa, M., Ellouz Ghorbel, R., and Ellouz Chaabouni, S. (2020). Date seeds as a natural source of dietary fibers to improve the texture and sensory properties of wheat bread. *Foods*, 9(6), 737.
7. Chi, C., Li, X., Zhang, Y., Chen, L., Xie, F., Li, L., and Bai, G. (2019). Modulating the in vitro digestibility and predicted glycemic index of rice starch gels by complexation with gallic acid. *Food Hydrocolloids*, 89: 821-828.
8. Feili, R. (2014). Utilization of jackfruit (Artocarpus Heterophyllus Lam.) rind powder as value added ingredient in bread (Doctoral dissertation, Universiti Sains Malaysia).
9. Fuller, S., Beck, E., Salman, H., and Tapsell, L. (2016). New horizons for the study of dietary fiber and health: a review. *Plant Foods for Human Nutrition*, 71, 1-12.
10. Goh, R., Gao, J., Ananingsih, V. K., Ranawana, V., Henry, C. J., and Zhou, W. (2015). Green tea catechins reduced the glycemic potential of bread: An in vitro digestibility study. *Food Chemistry*, 180: 203-210.
11. Goñi, I., Garcia-Alonso, A., and Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, 17(3), 427-437.

12. Gül, H.; Sen, H. (2017) Efectos de la harina de semilla de granada en la reología de la masa y en la calidad del pan. *CYTA Journal of Food*, 15, 622–628.
13. Huang, Z., Wang, J. J., Chen, Y., Wei, N., Hou, Y., Bai, W., and Hu, S. Q. (2020). Effect of water-soluble dietary fiber resistant dextrin on flour and bread qualities. *Food Chemistry*, 317, 126452.
14. Jeddou, K. B., Bouaziz, F., Zouari-Ellouzi, S., Chaari, F., Ellouz-Chaabouni, S., Ellouz-Ghorbel, R., and Nouri-Ellouz, O. (2017). Improvement of texture and sensory properties of cakes by addition of potato peel powder with high level of dietary fiber and protein. *Food Chemistry*, 217, 668-677.
15. Jose, M., Himashree, P., Sengar, A. S., and Sunil, C. K. (2022). Valorization of food industry by-product (Pineapple Pomace): A study to evaluate its effect on physicochemical and textural properties of developed cookies. *Measurement: Food*, 6, 100031.
16. Kiumarsi, M., Shahbazi, M., Yeganehzad, S., Majchrzak, D., Lieleg, O., and Winkeljann, B. (2019). Relation between structural, mechanical and sensory properties of gluten-free bread as affected by modified dietary fibers. *Food Chemistry*, 277, 664-673.
17. Meena, L., Sengar, A. S., Neog, R., and Sunil, C. K. (2022). Pineapple processing waste (PPW): bioactive compounds, their extraction, and utilisation: a review. *Journal of Food Science and Technology*, 1-13.
18. Ma, Q., Cai, S., Jia, Y., Sun, X., Yi, J., and Du, J. (2020). Effects of hot water extract from vine tea (Ampelopsis grossedentata) on acrylamide formation, quality, and consumer acceptability of bread. *Foods*, 9(3): 373.
19. Maibam, B. D., Chakraborty, S., Nickhil, C., and Deka, S. C. (2023). Effect of Euryale ferox seed shell extract addition on the in vitro starch digestibility and predicted glycemic index of wheat-based bread. *International Journal of Biological Macromolecules*, 226, 1066-1078.
20. Mudgil, D., Barak, S., and Khatkar, B. S. (2016). Optimization of bread firmness, specific loaf volume and sensory acceptability of bread with soluble fiber and different water levels. *Journal of Cereal Science*, 70: 186-191.
21. Noro, W., Akaishi, R., Nakamura, S., Ohtsubo, K. I., Maeda, H., and Yoshii, Y. (2016). Prevention of abrupt increases in postprandial blood glucose levels by rice bread made with the novel rice cultivar “Konayukinomai”. *Food Science and Technology Research*, 22(6), 793-799.

22. Pathak, D., Majumdar, J., Raychaudhuri, U., and Chakraborty, R. (2017). Study on enrichment of whole wheat bread quality with the incorporation of tropical fruit by-product. *International Food Research Journal*, 24(1), 238.
23. Pop, C., Suharoschi, R., and Pop, O. L. (2021). Dietary fiber and prebiotic compounds in fruits and vegetables food waste. *Sustainability*, 13(13), 7219.
24. Roda, A., and Lambri, M. (2019). Food uses of pineapple waste and by-products: a review. *International Journal of Food Science & Technology*, 54(4), 1009-1017.
25. Banerjee, S., Ranganathan, V., Patti, A., and Arora, A. (2018). Valorisation of pineapple wastes for food and therapeutic applications. *Trends in Food Science & Technology*, 82, 60-70.
26. Santos, D., Pintado, M., and da Silva, J. A. L. (2022). Potential nutritional and functional improvement of extruded breakfast cereals based on incorporation of fruit and vegetable by-products-A review. *Trends in Food Science & Technology*, 125, 136-153.
27. Sciarini, L. S., Bustos, M. C., Vignola, M. B., Paesani, C., Salinas, C. N., and Perez, G. T. (2017). A study on fibre addition to gluten-free bread: Its effects on bread quality and in vitro digestibility. *Journal of Food Science and Technology*, 54, 244-252.
28. Sharma, S. K., Bansal, S., Mangal, M., Dixit, A. K., Gupta, R. K., and Mangal, A. K. (2016). Utilization of food processing by-products as dietary, functional, and novel fiber: A review. *Critical reviews in food science and nutrition*, 56(10), 1647-1661.
29. Sheikholeslami, Z., Mahfouzi, M., Karimi, M., and Ghiafehdavoodi, M. (2021). Modification of dough characteristics and baking quality based on whole wheat flour by enzymes and emulsifiers supplementation. *LWT-Food Science and Technology*, 139, 110794.
30. Singh, A., Kaur, V., and Kaler, R. S. S. (2018). A review on dietary fiber in cereals and its characterization. *Journal of Applied and Natural Science*, 10(4), 1216-1225.
31. Spiker, M. L., Hiza, H. A., Siddiqi, S. M., and Neff, R. A. (2017). Wasted food, wasted nutrients: nutrient loss from wasted food in the United States and comparison to gaps in dietary intake. *Journal of the Academy of Nutrition and Dietetics*, 117(7), 1031-1040.
32. Subiria-Cueto, R., Coria-Oliveros, A. J., Wall-Medrano, A., Rodrigo-Garcia, J., González-Aguilar, G. A., Martinez-ruiz, N. D. R., and Alvarez-Parrilla, E. (2021). Antioxidant dietary fiber-based bakery products: a new alternative for using plant-by-products. *Food Science and Technology*, 42, ctaAR57520.
33. Valková, V., Ďúranová, H., Havrlentová, M., Ivanišová, E., Mezey, J., Tóthová, Z., ... and Kačániová, M. (2022). Selected physico-chemical, nutritional, antioxidant and sensory

properties of wheat bread supplemented with apple pomace powder as a by-product from juice production. *Plants*, 11(9), 1256.

34. Walker, R., Tseng, A., Cavender, G., Ross, A., and Zhao, Y. (2014). Physicochemical, nutritional, and sensory qualities of wine grape pomace fortified baked goods. *Journal of Food Science*, 79(9), S1811-S1822.
35. Wen, Y., Niu, M., Zhang, B., Zhao, S., and Xiong, S. (2017). Structural characteristics and functional properties of rice bran dietary fiber modified by enzymatic and enzyme-micronization treatments. *LWT-Food Science and Technology*, 75, 344-351.
36. Xu, J., Li, Y., Zhao, Y., Wang, D., and Wang, W. (2021). Influence of antioxidant dietary fiber on dough properties and bread qualities: A review. *Journal of Functional Foods*, 80, 104434.