Chapter 6 To incorporate starch-polyphenol complex from *Euryale ferox* seed in gluten free bread formulation

6.1 Introduction

Celiac disease is one the most prevalent food induced intestinal obstruction provoked by the consumption of gluten in genetically prone individuals [10]. Celiac disease has been recognized as a crucial general health problem, as the occurrence is escalating worldwide. Despite the significant advances that have been achieved in comprehending the etiology, treatment, and dietary management of celiac disease (CD), the only successful and safe management now accessible for celiac disease patients is a careful, gluten-free (GF) diet [5]. As a result, a substantial growth of gluten free market was recorded in recent years and is still expected to rise [8]. However, gluten free cereal-based foods generally had relatively lower dietary fiber, resistant starch (RS), vitamins, minerals, bioactive compounds, and higher glycemic index (GI) than their counterparts [8,10]. Consumption of these energy dense, low nutritious foods aggravated the incidence of diabetes among celiac patients [23]. Therefore, one of the most effective strategies for improving the nutritional value is the partial replacement of conventional gluten free flours with health beneficial flour [6].

Among all the gluten free products, gluten free bread is one of the most popular staple products [2]. In the recent years, there has been a remarkable research attempt to upgrade the physical, structural, as well as nutritional attributes of gluten-free breads by incorporating a wide range of ingredients, such as pregelatinized starch [12, 15, 16, 17, 18], dietary fiber [13], resistant starch [19], and modified starch [7, 25]. The utilization of pre-gelatinized starch in gluten free products is acceptable because it is a physically modified starch, thus it is safe. Further, the application of resistant starch in gluten-free bread is gaining interest [13, 19]. Therefore, the purpose of the current study was to utilize pre-gelatinized *Euryale ferox* kernel starch-*Euryale ferox* seed shell extract complex, a novel antioxidant rich resistant starch so as to improve the texture, organoleptic, and nutritional properties of gluten-free bread.

6.2 Materials and Methods

6.2.1 Preparation of gluten-free bread

The gluten-free bread used as a control was prepared with 200 g of gluten-free flour mix (rice flour/corn starch, 50:50), 6 g of fresh baker's yeast, 3 g of salt, 12 g of sugar, 4 g of baking powder, 10 mL of oil, and 360 mL of water. Gluten free bread flour was gradually replaced by *Euryale ferox* starch-*Euryale ferox* seed shell extract complex at a rate of 10,

20, 30, 40 and 50% and samples were coded as EFCB-10, EFCB-20, EFCB-30, EFCB-40 and EFCB-50 respectively. The quantity of water needed to make bread was established based on preliminary trials and the characteristics of the dough. The ingredients for the bread were blended to make dough. After that, fermentation occurred for 30 min at 30 °C and 85% relative humidity. The fermented dough was baked for 25 min at 175°C. After baking, the breads were and left to cool for an hour, and sealed in plastic bags for future use [24].

6.2.2 Moisture retention, and specific volume of gluten-free bread loaves

After baking, bread loaves were brought to room temperature and recorded its weight. The moisture retention (%) was calculated as the ration of weight of dough and weight of baked loaves.

Moisture retention (%) =
$$\frac{W_a}{W_b} \times 100$$
 (1)

Where, W_a and W_b are weight of bread loaf before and after baking respectively.

The AACC [1] rapeseed dispersion technique was used to calculate the volume of the bread loaves.

Specific volume
$$(cm^{3}/g) = \frac{loaf \ volume \ (cm_{3})}{loaf \ weight \ (g)}$$
 (2)

6.2.3 Color attributes of gluten-free bread

Color of the crumb and crust of the bread was analyzed by Hunter colorimeter (Ultrascan VIS, Hunterlab, USA).

6.2.4 Texture profile analysis of gluten-free bread

The textural characteristic of the bread was assessed using a Texture Analyzer (TA-HD plus; Stable Micro Systems, UK) with a 50 N load cell [3]. [Explained in Section 5.2.3]

6.2.5 *In vitro* starch digestibility, and predicted glycemic index (pGI) of gluten-free bread

The starch TS contained in the bread samples were estimated following the method of Goni et al. [11]. According to the equation of Goni et al. [11], the kinetics of starch hydrolysis were calculated. [Explained in section 5.2.5]

6.2.6 Extraction of phytochemicals

Each sample of dried bread powder (5 g) was infused into a different solvent of 250 mL. The phytochemicals were extracted using four different solvents: 100% ethanol, water, 50% aqueous ethanol, and 25% aqueous ethanol. The extracts were centrifuged for 10 min at 7,500 rpm at 4°C. The supernatant thus obtained was collected, dried, and kept at -20°C for further analyses.

6.2.7 Total phenolic content

The TPC in the sample was evaluated by the Folin-Ciocalteu method of Singleton as described by Singelton et al. [21]. The results are presented in mg gallic acid equivalent (mg GAE/g) of dry extract.

6.2.8 Total flavonoid content (TFC)

The TFC of EFSSE was analyzed using the aluminum chloride (AlCl₃) colorimetric method of Singelton et al. [21]. The results are presented in mg quercetin equivalents (mg QE/g) of dry extract and were determined in triplicate.

6.2.9 Determination of Antioxidant Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging ability of each extract was assessed by following the method of Singelton et al. [21] with minor modification as described in Section 3.2.6.1 (Chapter 3).

% inhibition =
$$\frac{A_B - A_E}{A_B} \times 100$$

where A_B: absorbance of the blank, and A_E: absorbance of the extract

6.2.10 Sensory evaluation of the bread samples

The formulated bread was brought to room temperature and were sliced into $2\times3\times5$ cm, and its sensory attributes were evaluated. The visual appearance, color, flavor, texture, and overall acceptability of bread were assessed using a 9-point hedonic scale (9-1: Like extremely to dislike extremely). [Explained in 5.2.6.1]

6.2.11 Statistical analysis

Three repetitions of each experiment were carried out. The experimental results were subjected to analysis of variance (ANOVA). Significant differences between the data were obtained by Tukey's tests using SPSS 24 (IBM Analytics, USA) and p value < 0.05 was considered statistically significant.

6.3 Results and discussion

6.3.1.1 Specific volume of gluten free bread

The specific volume of breads prepared with *Euryale ferox* starch-polyphenol complex is shown in Table 6.1. The specific volumes of the gluten free bread formulation containing pre-EFKS-P complexes were lower than the control bread. However, up to 30% level of incorporation, the difference in volume was statistically non-significant (p>0.05). However, at 40-50% replacement, the poor crumb structure exhibited larger and irregular gas pockets, and reduced specific volume.

The addition of pregelatinized tapioca starch to gluten free bread leads to reduced air pockets due to the denser crumb structure and reduced specific volume [18]. However, Ziobro et al. [25] reported that substitutions with 10-15% of pregelatinized starch enhanced volume of gluten free bread. It is because pre-gelatinized starch disperses easily, water absorption and swelling capacity is more, form gel, which can create a produce a covering layer on the surface of yeast cells providing carbon source for yeast fermentation [12, 17]. Another factor was the potential of polyphenol to reduce amylase activity in bread flour, thereby decreasing the substrate for yeast fermentation, resulting reduced CO₂ production [3]. As a result of the above two contradicting probable phenomenon, there were non-significant change in the specific volume of the samples. Specific volume, is regarded as the most reliable measure for acceptability of bread. Since consumers are often attracted to larger bread loaves, thus breads with higher specific volumes are desirable [20]. Therefore, adding *Euryale ferox* starch-polyphenols complex to gluten free bread seem to be a favorable modification.

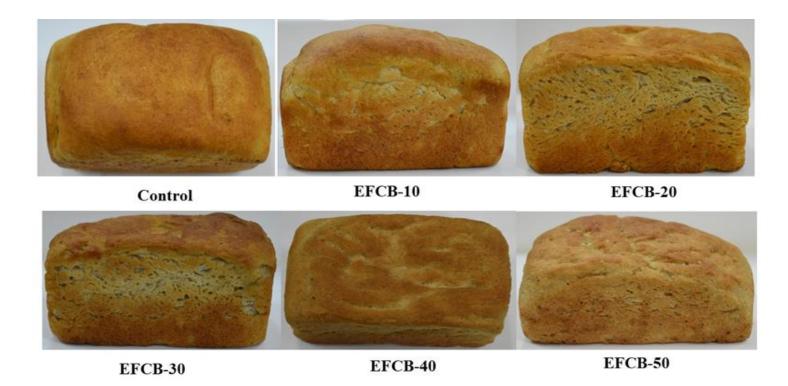


Fig. 6.1 Euryale ferox kernel starch-Euryale ferox seed shell extract complex incorporated gluten free bread

6.3.2 Moisture retention and baking loss of gluten-free bread

The freshly baked breads had a moisture content ranged from 75-87%, their baking loss was minimal as shown in Table 6.1. The moisture content increased significantly when 10-50% of gluten free flour mix was replaced by starch-polyphenol complex. It is likely that the higher water binding capacity of starch-polyphenols complex help to retain the moisture in the crumb. Since starch-polyphenol complex exhibited higher water absorption capacity than native flour or starch because pre-gelatinization creates numerous pores on the surface of the granules, which makes it effortless for moisture to penetrate to the interior of starch granules and increases moisture content. Furthermore, gelatinization disrupts granular crystal structure, increases viscosity, strengthens the bonds between water and starch molecules thereby resulting higher moisture content in final products. In similar case, where the addition of pre-gelatinized starch could retain the moisture content of bread [16].

6.3.3 Textural attributes of gluten-free bread

The textural properties of gluten-free bread crumbs were affected by the incorporation of starch-polyphenol complex (Table 6.1). The commonly used criteria to describe bread quality is hardness, as change in hardness typically indicates decrease in resilience. The hardness of the samples varied among samples (10.15-15.95 N) and the hardness of the control bread was the least. Chewiness is directly related to hardness and ranged from 8.31-14.68 N. Springiness ranged decreased from 0.87-0.76, there were significant differences from 10% replacement onwards. The lower springiness is responsible for the reduced loaf volume [46]. Further, the incorporation of complex, produce significant impact on cohesiveness (p > 0.05). The lower specific volume, denser crumb, as well as reduced cohesiveness due to the presence of lesser air bubbles, are factors that significantly affect the textural attributes. Due to restricted swelling, the dough did not stretch, restrict the gas cells to expand [16]. Moreover, gluten is regarded as a structural protein for bakery [12]. Owing to the lack of gluten, the adhesion between the components is reduced and cannot form a viscoelastic system, resulting in poor texture quality [20]. From the texture profile analysis, it was observed that adding starch-polyphenol complex at the optimum amounts (10-20 g/100 g bread flour) did not result in any unfavorable changes to the hardness.

Sample code	Moisture content	Baking loss	Specific volume (cm ³ /g)	Hardness (N)	Springiness	Chewiness (N)	Cohesiveness
Control	36.51±0.80 ^a	25.33±0.62 ^a	4.46±0.02 ^a	10.15±0.56°	0.87±0.01 ^e	8.31±0.12 ^d	0.68±0.04 ^c
EFCB10	37.55±0.80 ^a	24.09±0.74 ^{ab}	4.40±0.01 ^a	11.57±1.52°	0.84±0.01 ^{cd}	9.55±0.20 ^{cd}	0.70±0.04 ^b
EFCB20	37.83±1.47 ^a	23.23±0.44 ^{bc}	4.43±0.01 ^a	13.22±1.74 ^{bc}	0.82 ± 0.0^{bc}	10.75±0.71 ^{bc}	0.71 ± 0.05^{ab}
EFCB30	38.35±0.81 ^a	22.73±0.23 ^{bc}	4.46±0.07 ^a	14.18±1.69 ^{ab}	0.81 ± 0.01^{bc}	12.34±0.40 ^{bc}	0.73±0.01 ^a
EFCB40	40.00±0.73 ^b	21.78±0.88 ^{cd}	3.90±0.01 ^b	15.55±0.67ª	0.80±0.01 ^{ab}	13.89±0.83 ^{ab}	0.74±0.03 ^a
EFCB50	41.72±1.41 ^b	20.08±0.33 ^d	3.78±0.01 ^b	15.95±0.65ª	0.76±0.01 ^a	14.68±0.47 ^a	0.75±0.03 ^a

Table 6.1 Moisture content, specific volume, and textural properties of gluten free bread incorporated with starch-polyphenol complex

Results are mean of triplicates ±SD; Values with different letters in the same column differ significantly (p<0.05)

6.3.4 Color attributes of gluten free bread

Table 6.2 Color parameters of gluten free bread incorporated with *Euryale ferox* kernel

 starch-*Euryale ferox* seed shell extract complex

Sample	Crumb			Cru		
	L*	a *	b*	L*	a*	b*
Control	68.87±0.54 ^a	-0.65±0.15ª	14.79±0.26 ^a	47.29±0.62 ^a	1.17±0.28ª	8.47±0.46 ^a
EFCB10	64.59±0.59 ^b	1.19±0.27 ^b	11.62±0.33 ^b	45.52±0.42ª	1.29±0.17ª	8.60±0.34ª
EFCB20	61.09±0.79 ^{bc}	1.63±0.25 ^b	10.63±0.31 ^{bc}	44.67±0.28 ^{ab}	2.79±0.15ª	8.36±0.56ª
EFCB30	59.46±0.68°	2.19±0.27 ^{bc}	10.57±0.36 ^{bc}	43.63±0.32 ^b	2.67±0.27 ^a	8.12±0.28 ^a
EFCB40	58.58±0.35 ^{cd}	3.32±0.10 ^{bc}	9.34±0.22 ^{bc}	42.95±0.13 ^b	2.29±0.16 ^a	8.25±0.36ª
EFCB50	57.84±0.13 ^d	3.38±0.07°	8.23±0.36°	42.12±0.13 ^b	2.70±0.18ª	8.86±0.45ª

Results are mean of triplicates \pm SD; Values with different letters in the same column differ significantly (p<0.05)

The color characteristics of the gluten free breads formulated by incorporating starchpolyphenol complex are presented in Table 6.2. These analyses have revealed the L*, a*, and b* values of the crumbs of the incorporated bread were significantly different from the control bread. It is worth mentioning that the a* and b* value, did not display the same upward or downward trend as the L* value. The crumb lightness changed significantly even at 10% level of starch-polyphenol complex incorporation. When gluten-free flour was partially replaced by starch-polyphenol complexes, the lightness and yellowness indices decreased while the redness value increased. The oxidized polyphenols that were produced in starch-polyphenols complex might be responsible for the reddish-brown color of the fortified bread. Whereas, the crusts of the six experimental bread samples did not significantly differ in terms of redness (a*). The characteristic brown crust is result of the Maillard process, a non-enzymatic browning process that takes place amid proteins and reducing sugars. The Maillard reaction may slow down as the proportion of starchpolyphenol complex incorporation increased because the flour composition contains less protein. However, the brown color of complexes compensates the decreased Maillard reaction products.

6.3.5 In vitro starch digestibility (IVSD) of gluten free bread samples

6.3.5.1 Starch fractions

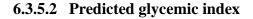
 Table 6.3
 Starch fractions of gluten free bread incorporated with starch-polyphenol

 complex

Sample	TS (%)	RDS (%)	SDS (%)	RS (%)
Control	78.06±1.23ª	91.33±0.79 ^a	5.20±1.06 ^a	3.45±1.50 ^a
EFCB10	77.40±1.63 ^a	82.40±2.30 ^b	7.58±3.30 ^{ab}	10.01 ± 1.00^{b}
EFCB20	$76.80{\pm}1.80^{a}$	74.70±2.10 ^c	9.76±2.50 ^{bc}	15.53±0.50 ^c
EFCB30	76.17±0.85 ^a	66.75±1.89 ^d	11.86±3.50 ^{cd}	21.38±1.51 ^d
EFCB40	75.60±1.47 ^a	59.16±2.25 ^e	13.93±1.28 ^d	26.90±1.01e
EFCB50	75.00±2.00 ^a	51.20±2.30 ^f	15.38±1.75 ^d	33.41±1.42 ^f

Results are mean of triplicates \pm SD; Values with different letters in the same column differ significantly (p<0.05).

There was a linear decrease in RDS and a linear increase in SDS and RS fractions (Table 6.3). The RDS portions of the formulated bread samples were reduced from 91.33% (control) to 51.20% (50%). However, the SDS and RS proportion increased from 5.20% (control) to 15.38% (50%) and 3.45 (control) to 33.41% (50%) respectively. The starch-polyphenol complex could withstand the harsh temperature of baking and the SDS and RS fraction was partially retained. It can be concluded that, it is now possible to adjust different RDS, SDS, and RS levels to cater various industrial demands.



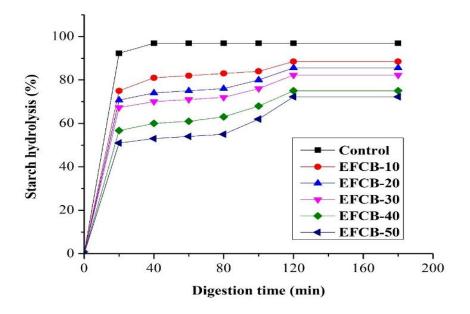


Fig. 6.2 Starch hydrolysis pattern of gluten free bread incorporated with starch-polyphenol complex

Table 6.4 Determination of predicted glycemic index (pGI) of gluten free bread

 incorporated with starch-polyphenol complex

Sample	C∞	K value	AUC	HI	pGI
Control	96.53±1.50 ^a	0.0531ª	15680.06±112.19 ^a	103.83±0.76 ^a	96.32±0.88 ^a
EFCB10	89.98±1.00 ^b	0.0315 ^b	13449.84±85.50 ^b	88.53±0.98 ^b	88.31±0.54 ^b
EFCB20	84.46±0.50°	0.0219 ^c	11468.08±40.48 ^c	75.78±0.44 ^c	81.31±0.24 ^c
EFCB30	78.61±1.51 ^d	0.0181 ^d	10082.06±87.02 ^d	66.30±0.91 ^d	75.98±0.69 ^d
EFCB40	73.10±1.01 ^e	0.0121 ^e	7847.14±53.80 ^e	51.80±0.48 ^e	68.05±0.38 ^e
EFCB50	66.58 ± 1.42^{f}	0.0081 ^f	5762.35 ± 68.90^{f}	37.81 ± 0.21^{f}	60.31±0.38 ^f

Results are mean of triplicates \pm SD; Values with different letters in the same column differ significantly (p<0.05)

There was a dose dependent decline in starch hydrolysis with the increase level of starchpolyphenol complex. The starch digestion pattern of the gluten-free bread specimen is presented in Fig. 6.2, and the rate of starch digestion was governed by first-order kinetics. The digestion kinetics of the samples are presented in Table 6.4. When gluten free bread flour was replaced by starch-polyphenol complex, there was a significant decline in C ∞ values, 96.53% (control) to 66.58% (50%). The bread samples exhibited a dose-dependent decrease in pGI (Table 6.4). Control, being the highest pGI value (96.32) while pGI of EFCB-10 (88.31), EFCB-20 (81.31), EFCB-20 (75.98), and EFCB-10 (68.05), and EFCB-50 (60.31) was observed. Therefore, substituting gluten free bread four by starch-polyphenol complex may be regarded as a better strategy to lower the pGI of bread. Foods with low GI are favored by consumers because of its potential to prevent and manage glucose metabolism related diseases [1, 30]. The glycemic index of the samples was influenced by the resistant starch fraction of the complex, the inhibition of α -amylase, α -glucosidase by the polyphenols release from the complex.

6.3.6 Total phenol and flavonoid content

The total phenolic and flavonoid content of gluten-free bread significantly increased as a result of the incorporation of starch-polyphenol complex. The increase was dose dependent, highest phenolic were observed in EFCB-50 followed by EFCB-40, EFCB-30, EFCB-20, EFCB-10, and control bread exhibited the lowest phenolic content [Table 6.5]. Similar trend was observed in total flavonoid content as well [Table 6.6]. However, replacing more than 40% of the bread flour by starch-polyphenol complex did not increase the total phenolic and flavonoid content significantly as the flour being used is already rich in polyphenols. Therefore, it may be concluded that starch-polyphenol complex incorporation can enhances the health beneficial properties of gluten-free bread. Further, the release of polyphenols from the starch-polyphenol complex may impair the starch digestibility.

Starch-polyphenols complex addition in gluten free bread

Solvent	Control	EFCB-10	EFCB-20	EFCB-30	EFCB-40	EFCB-50
Water	20.03±1.19 ^{aA}	36.37±2.59 ^{bA}	41.13±1.96 ^{cA}	47.15±1.03 ^{dA}	70.71±1.62 ^{eA}	83.12±2.43 ^{fA}
25% Ethanol	21.41±0.53 ^{aA}	37.06±0.35 ^{bAB}	40.16±0.52 ^{cA}	47.16±1.18 ^{dA}	75.78 ± 0.58^{eB}	82.19±2.77 ^{fA}
50% Ethanol	23.55±0.77 ^{aB}	39.08±0.84 ^{bB}	42.19±0.73 ^{cAB}	50.13±1.74 ^{dB}	74.68±1.67 ^{eBC}	83.87±0.79 ^{fA}
100% Ethanol	24.82±0.74 ^{aB}	40.19 ± 0.64^{bB}	44.08±0.87 ^{cB}	53.63±0.96 ^{dC}	77.04 ± 0.90^{eC}	89.42±1.25 ^{fB}

Table 6.5 Total phenolic content (mg GAE/100 g) of gluten free bread incorporated with starch-polyphenol complex

Results are mean of triplicates \pm SD; Values with different letters in the same column differ significantly (p<0.05); Values with the different letters in the same row differ significantly (p<0.05)

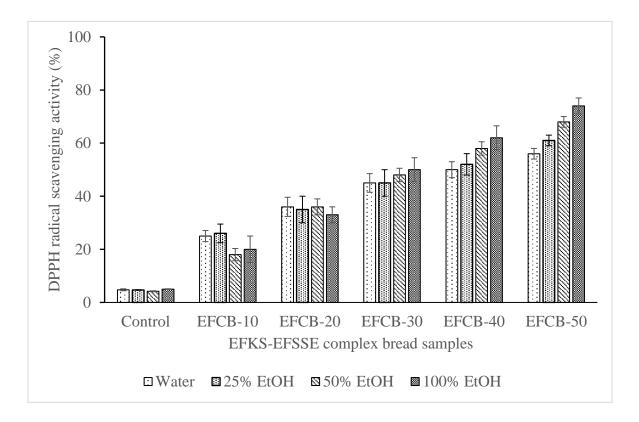
Table 6.6 Total flavonoid content (mg QE/100 g) of gluten free bread incorporated with starch-polyphenol complex

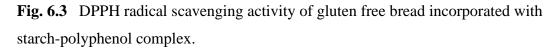
Solvent	Control	EFCB-10	EFCB-20	EFCB-30	EFCB-40	EFCB-50
Water	2.25±0.25 ^{aA}	5.72±0.54 ^{bA}	8.48±0.50 ^{cA}	11.85±0.13 ^{dA}	15.23±0.87 ^{eA}	18.64 ± 18^{fA}
25% Ethanol	2.43±0.27 ^{aA}	7.81±0.59 ^{bA}	10.71±0.86 ^{cB}	12.77±0.23 ^{dA}	16.14±0.65 ^{dA}	20.78±0.20 ^{eAB}
50% Ethanol	2.60±0.34 ^{aA}	9.92 ± 0.87^{bAB}	13.96±0.53 ^{cBC}	16.92±0.10 ^{dB}	19.83±1.24 ^{eB}	23.08±0.87 ^{fB}
100% Ethanol	2.88±0.01 ^{aA}	13.85±0.87 ^{bC}	17.49±0.16 ^{cD}	21.52±0.14 ^{dC}	24.79±0.78 ^{eC}	29.95±0.08 ^{fC}

Results are mean of triplicates \pm SD; Values with the different letters in the same column differ significantly (p<0.05); Values with the different letters in the same row differ significantly (p<0.05)

6.3.7 DPPH radical scavenging activity

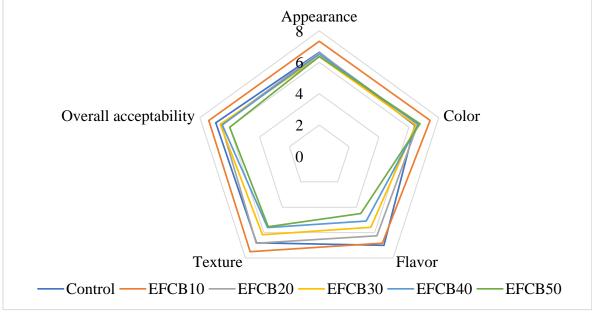
The supplementation of starch-polyphenol complex in gluten-free bread demonstrated a substantial increase in the antioxidant potential [Fig. 6.3]. The bread samples supplemented with starch-polyphenol complex exhibited remarkable antioxidant potential whereas the control bread exhibited negligible antioxidant capacity. Furthermore, the process of baking can indeed result in the synthesis of compounds having antioxidant characteristics, such as some Maillard reaction products that occur in the bread crust. These syntheses may conceal any actual loss in overall phenolic and flavonoid content as well as in antioxidant potential due to harsh baking conditions due to their ability to react with the Folin-Ciocalteau reagent [7]. Therefore, starch-polyphenol complex may be utilized to formulate gluten-free bread with high antioxidant potential.





6.3.8 Sensory evaluation

The sensory scores of the formulated bread samples for their quality characteristics *viz.*, appearance, taste, color, texture, and overall acceptability, are represented in Fig. 6.4. The radar chart revealed variation in the taste, appearance, color, and texture among the samples. The results of the colorimetric measurement were in agreement with the visual interpretation of color change. The control bread received the highest sensory scores. Out of the fortified samples, the experimental bread containing the 20% starch-polyphenol complex obtained the highest score in terms of overall acceptability. This outcome was expected because this sample had the best appearance, texture, and taste scores. The flavor qualities were dramatically reduced with the increased amounts of replacement. The dissatisfaction of the consumers with the taste seemed to be the main problem of the current study given that the panelists were accustomed with the conventional wheat-based bread. According to the results of the sensory analysis, a partial substitution of up to 30% results in satisfactory overall consumer acceptability. The acceptability of the samples containing more than 40% starch-polyphenol complex, was sharply reduced, which may be related to the presence of high amount of polyphenol that adversely affected the



product's flavor, taste, and texture of the bread.

Fig. 6.4 Radar plot of sensory scores of gluten-free breads incorporated with starch-polyphenol complex

6.4 Conclusion

Breads made with starch-polyphenol complex had a darker color than control bread, and more denser crumb structures. The crumb structure and appearance of the *Euryale ferox* starch-polyphenol complex loaves were similar to a control bread loaf. However, when the level of incorporation rises, the flavor score reduces. The findings of the study showed that, when combined with conventional gluten free bread flour, starch-polyphenols complex could be used to make bread with improved functional qualities without causing significant changes in other sensory properties. Supplementation of starch-polyphenol complex in the formulation of gluten-free bread improved the TPC, antioxidant activity, and reduce starch digestibility of bread samples. Therefore, in addition to nutritional improvement, but also organoleptic properties have been attained by employing optimum level in the manufacture of gluten-free bread. Moreover, starch-polyphenols complex is devoid of gluten. Therefore, people with celiac disease can also have this bread. The high resistant starch content of bread enriched with *Euryale ferox* starch-polyphenols complex may have a number of beneficial effects, such as enhancing post-meal satiety, and finally acting as prebiotics. From the findings of this research, we propose a promising functional novel starch in combination with plant polyphenols, which can be used to produce low GI gluten-free bread.

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