Chapter 5

To study the effect of *Euryale ferox* seed shell extract addition on the *in-vitro* starch digestibility and predicted glycemic index of wheat-based bread

5.1 Introduction

Processed and convenience foods are an integral part of modern life and it is almost impossible for human to live without them. With a growing interest in health and nutrition, consumers are looking for food that has the potential to protect against lifestyle-related diseases besides the nutritional aspects of the food. As a result, food manufacturers and researchers have focused on improving the nutritional properties of starch, which is the primary ingredient of convenience foods [10]. Starch can be modified to enhance its health beneficial properties. Resistant starch, for example, is a type of starch which remains undigested in the small intestine. However, metabolized by colonic microflora, producing short chain fatty acids (SCFA's) and help in maintaining colon health [14]. Further, epidemiological studies have shown that regular consumption of resistant starch helps to lessen the chances of developing obesity, type 2 diabetes mellitus, and cardiovascular diseases owing to its lower glycemic index [14]. One of the promising ways to reduce the digestibility of starch is by adding natural plant extracts rich in polyphenols, as they can interact non-covalently with starch, thus modulating the glycemic responses of carbohydrates [12, 15]. Moreover, incorporating phenolic-rich fractions like cereal bran and phenolic extracts have increased the bio accessibility and bioavailability of phenolics in food matrices [28]. Bread is regarded as one of the best carriers for food fortification, supplementation, and enrichment due to its economical, availability, popularity, and extensive consumption [24]. Moreover, bread is a carbohydrate-rich, high glycemic index (GI) staple food consumed all over the world; hence reformulating bread to lower its glycemic load is of great interest [15]. The key enzymes responsible for converting carbohydrates to monosaccharides are α-amylase and α-glucosidase. So, inhibiting these main enzymes will slow down the breakdown of starch, which helps to manage post-prandial blood sugar level. For these reasons, plants extract that could hinder the function of α-amylase and α- glucosidase is frequently employed in developing antidiabetic drugs as they have fewer adverse effects than synthetic drugs [25].

Euryale ferox seed shell, which makes up roughly half of the seed, contains triterpenoids, flavonoids, saponins, and phenolics, making it an attractive raw material for polyphenol extraction and recovery. *Euryale ferox* seed shell extract has become increasingly popular in recent years as a natural food preservative [30] and has also revealed many pharmacological effects [16, 27].

Epidemiological studies have shown a correlation between the *Euryale ferox* seed shell extract and the prevention of several human chronic diseases, including type 2 diabetes and cardiovascular diseases. This suggests that it could be used as a natural source of phytochemicals in functional foods formulation owing to its numerous bioactivities of polyphenols, particularly their anti-oxidant, anti-inflammatory, anti-microbial activity, and modulation of signal transduction [16, 27]. *Euryale ferox* seed shell, an agro-industrial waste, can be exploited as a future source of bioactive components, leading to their bioprocessing into potentially marketable products.

In the present study, *Euryale ferox* seed shell extract was used in bread formulation to explore its impacts on sensory attributes and starch digestibility. Phytochemicals extract fortification generally imparts a remarkable effect on the color of the bread crumb as it depends solely on the color of the raw materials used for making dough [20]. However, for a novel food product development, color alteration is not always a negative element for consumer acceptability [31]. Starch digestion kinetics may be studied using various mathematical modeling approaches [2]. Based on the first-order reaction kinetics, digestion kinetics was formulated and then determined the predicted glycemic index (pGI). For more adequate and accurate predictions, neural network approaches can be applied. A neural network helps to model input and output variables based on the training, testing, and validation approaches. The neural network executes its prediction mapping in association with weight bias values and activation function. Swarm intelligence, or in generic terms particle swarm optimization (PSO), is an evolutionary approach for the optimization of process parameters. A neural network optimized based on PSO helps to develop a stronger prediction capacity for applying the black-box modeling approach [3]. This study applied a hybrid technique based on swarm intelligence and artificial neural network, known as swarm intelligence supervised neural network (SISNN), along with mathematical modeling approaches for starch digestion kinetics. Overall, the goal of the current investigation was to study the effect of EFSSE on the *in vitro* starch digestibility and pGI of the formulated bread.

5.2 Materials and methods

5.2.1 Preparation of bread

The bread making procedure described by Begum et al. [2] was used, for the control bread, 100 g of refined flour, 60 g of water, 4g of fat, 4 g of sugar, 1.5 g of salt, and 1.5 g of instant yeast were used. EFSSE at the levels of 0.25%, 0.5%, 1%, and 2% were used to replace wheat flour in the production of five batches of bread. The EFSSE levels were decided based on our preliminary studies. After combining all of the above ingredients, the dough was left for proofing at 30°C for 90 min. The dough was shaped and deposited in a mildly oiled pan for 45 min for final proofing and then baked for 15 min at 220°C (Sinmag oven, SM 502). Bread loaves were removed after baking and allowed to cool. The baked loaves were packed in plastic pouches for further investigation.

5.2.2 Specific volume of bread

The AACC [1] rapeseed dispersion technique was used to assessed the volume of the bread loaves and the specific volume of each sample was calculated using the following equation:

$$Specific\ volume\ (cm^3/g) = \frac{loaf\ volume\ (cm3)}{loaf\ weight\ (g)} \quad (1)$$

5.2.3 Texture profile analysis of 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. From the center of the bread slice, a sample of bread of 2 cm width and 10 mm radius was sliced out. At an assessment momentum of 1 mm s^{-1}, a cylindrical probe with a diameter of 25 mm was utilized to flatten the bread samples to 50% of their original thickness. Bread hardness or firmness was measured from the peak compression force. Springiness was defined as the proportion of the height of the sample which bounced back at the time of initial contraction to the highest distortion. The important attributes such as springiness, chewiness, gumminess, and cohesiveness were assessed using the following equation.

$$Gumminess = \frac{A\,2}{A1} \times Hardness \quad (2)$$

$$Springiness = \frac{L2}{L1} \quad (3)$$

$$Chewiness = \frac{L2}{L1} \times Gumminess \quad (4)$$

$$Cohesiveness = \frac{A2}{A1} \quad (5)$$

Where, A1 and A2 are the area of the initial and later compression cycle, respectively. L1 and L2 are the time variation between the first and second compression cycle, respectively.

5.2.4 Color properties of bread

Color of the crust and crumb of the bread sample was analyzed by Hunter colorimeter (Ultrascan VIS, Hunterlab, USA). The data was presented in the form of L*, a*, and b*, where L* indicates the lightness, positive and negative "a" signifies redness and greenness, respectively whereas positive "b" signifies yellowness and negative "b" indicates blueness.

5.2.5 *In vitro* starch digestibility, and predicted glycemic index (pGI)

5.2.5.1 Starch fraction

The *in vitro* starch digestibility method of Englyst et al. [8] was followed to determine starch hydrolysis during 20-180 min. Dried bread powder (50mg) was mixed with 4 mL of sodium acetate buffer (0.5 M, pH 5.2). In order to prepare working solution A, 12 g of α-amylase (porcine pancreatin) was thoroughly dissolved in 80 mL of deionized H_2O. After that, working solution B was prepared by mixing 3.85 mL of deionized water with 3.15 mL of amyloglucosidase (15 U/mL, Sigma Aldrich). Then, 54 mL of solution A and 6 mL of solution B were combined to create working solution. Following 10 min equilibration period at 37°C, 5 mL of the working solution of enzyme was added to the prepared sample suspensions. Thereafter, the samples were incubated in a water bath with continuous shaking (190 rpm) at 37°C. Then, 0.5 mL of the hydrolyzed solution were taken out at various periods (20, 40, 60, 80, 100, and 120, and 180 min) and blended with 20 mL of 66% ethanol to deactivate enzymes. For the total starch determination, the residue was suspended in 6 mL of 2M KOH and placed in a shaking water bath for 30 min. The mixed solutions were centrifuged at 3000*g* for 10 min and the supernatants were utilized for the determination of glucose content using GOPOD kit (GAGO-20). Absorption was detected at 505 nm, and a conversion factor of 0.9 was used to convert the amount of glucose to

starch. The rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were calculated using the following formulas:

$$TS = T_g \times 0.9 \quad (6)$$

$$RDS\ (\%) = \frac{(G_{20} - G_0) \times 0.9}{TS} \times 100 \quad (7)$$

$$SDS\ (\%) = \frac{(G_{120} - G_{20}) \times 0.9}{TS} \times 100 \quad (8)$$

$$RS\ (\%) = \frac{TS - (G_{120} \times 0.9)}{TS} \times 100 \quad (9)$$

Where, G_0, G_{20}, and G_{120} denote the mg of glucose released at 0, 20, and 120 min of hydrolysis, respectively.

5.2.5.2 Mathematical modeling approach for determination of predicted glycemic index (pGI)

The rate of starch digestion was expressed as the percentage of total starch (TS) hydrolyzed at different times (20, 40, 60, 80, 100, 120, and 180 min). According to the equation of Goni et al. [12], the kinetics of starch hydrolysis were calculated using the first order equation: $C = C\infty\ (1 - e^{-kt})$, where C is the percentage of starch hydrolyzed at time t (min), C ∞ is the extent of maximum hydrolysis, k is kinetic constant, and t is time (min). The following equation was used to calculate the area under the hydrolysis curve (AUC):

$$AUC = C\infty\ (t_f - t_o) - (C\infty / k)\ [\ 1 - \exp^{\{-k\ (t_f - t_o)\}}]. \quad (10)$$

Where C∞ is the percentage of starch hydrolyzed at 180 min, t_f is the final time (180 min), t_0 is the initial time (0 min) and k is the kinetic constant.

The ratio of AUC of each sample to the AUC of the control white bread was used to determine the hydrolysis index (HI). The HI value obtained for each sample was used in the equation given below for determining the pGI [12]:

$$\text{Hydrolysis Index} = \frac{\text{AUC of each sample}}{\text{AUC of reference white bread}} \times 100 \quad (11)$$

$$pGI = 39.71 + 0.549HI \quad (12)$$

5.2.5.3 Swarm intelligence supervised neural network (SISNN) approach

An innovative approach *viz.*, swarm intelligence supervised neural network (SISNN) technique, was implemented for the predictive simulation of digestion kinetics and pGI. SISNN architecture was formulated by considering sample concentration (SC), and digestion time (DT) as the input neurons and hydrolyzed starch concentration after digestion (CAD) as the output neuron. SISNN technique implements a modified neural network approach with the integration of swarm intelligence viz., particle swarm optimization (PSO), to select weight and bias values during selective iterations [4]. Information processing occurs in response to the interplay substantial number of simulated neurons in neural network (NN) modelling. Large numbers of processing components with variable weights are connected by links in artificial neural networks (ANN). The input and hidden layers, threshold function, summation function, and output layer are all crucial elements of NN. The total quantity of neurons in the hidden layer was identified by the hit and trial method. NN can identify and express a network of nonlinear interactions between input and output variables without using prior models.

The structural formulation of the SISNN model is similar to the general NN model, but in this case, weight and bias values were selected using particle swarm optimization (PSO) for each iteration. PSO is a highly sophisticated computational optimization method constructed on the evolutionary nature of the population, inspired by the preying manners of birds or fishes. The concept of this approach is determined by the social interaction of birds in a group, who use their personal and social awareness to look for food randomly in the region. This strategy can efficiently and effectively identify solutions among the population through correct cooperation and competition. The relative velocity and the best position of the swarms were estimated by using the following equations [5]:

$$V_i^{k+1} = W^k v_i^k + C_1 R_1^k \left(P_i^k - S_i^k\right) + C_2 R_2^k \left(P_g^k - S_g^k\right) \quad (13)$$

$$S_i^{k+1} = S_i^k + v_i^{k+1} \quad (14)$$

Where, W is the weight of inertia; C_1 and C_2 are cognitive and social parameters, respectively commonly known as positive constants; R_1 and R_2 are arbitrary numbers uniformly assigned in the array [0-1]; i = 1, 2, . . .N and N is the magnitude of the swarm, and k = 1, 2, . . . is the present iteration.

The total input (H_{ij}) to hidden units j is a linear function of outputs (x_i) of the units which are associated to j and of the weights w_{ij} on these association i.e.,

$$H_{ij} = \Sigma_i x_i w_{ij} \quad (15)$$

A hidden unit is a non-linear result of its overall input which has a real-value output (H_{oj}). Biases (θ_j) are established as an additional input to individual unit which has a value of one always [19].

$$H_{oj} = \frac{1}{1+e^{-\left(Hij+\theta_j\right)}} \quad (16)$$

The experimental data was arranged into three sections: 70%, 15%, and 15% for training, testing, and validation, respectively, and was fitted with the help of the produced MATLAB code. The final output was calculated by using the *tansig* activation function. As a result, the input and output layers each had two and one neurons, respectively. The program for the SISNN was performed in the platform of MATLAB R2015a. By considering a learning rate varying in between 0.5 to 1, each run of SISNN algorithm was executed. Total of 2000 iterations were examined for each run of this algorithm. The ANN algorithm was run in 2000 iterations with a learning rate ranging from 0.5 to 1. These 2000 iterations were repeated ten times for each ANN architecture, yielding 20,000 NNs. The neurons in the hidden layer were tuned with every run, so they had the highest coefficient of determination (R^2) and the smallest mean square error (MSE). The optimal architecture was chosen based on the lowest MSE value and highest R^2. The predicted GI was determined using the output and reaction rate constant. The statistical parameter shows the adequate predictability of the network. Based on the best SISNN structure, the pGI was predicted and validated.

5.2.6 Sensory evaluation of the bread samples

5.2.6.1 Collection of sensory scores

The formulated bread was brought to room temperature and were sliced into 2×3×5 cm, and its sensory attributes were evaluated. Samples were randomly coded and served individually. 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). The panel comprised 30 semi-trained personnel from the research scholars from the Tezpur University, Assam, India.

5.2.6.2 Analysis of the sensory scores using POM supervised PCA technique

The sensory scores were obtained for various bread products by applying the proportional odds modeling (POM) supervised principal component analysis (PCA) technique. The POM is a multifaceted extension of linear regression models that allows the modelling of response category probabilities under external factors such as the linear forecaster. The response variable consumer attitude can have values in the range of 1, 2, 3, 4, 5, 6, 7, 8, 9 such that 1<2<3<4<5<6<7<8<9. Thus, the response of the i^{th} panelist is symbolized by the vector yi= (Ii1, Ii2,..., Ii9), where I_{ij} represents response category index variables, with Iij= 1 if it is in the j^{th} category otherwise I_{ij}= 0. The data analysis methodology used in this study quantifies consumer attitudes probabilistically, taking into account the effect of covariables and the possibility of non-measurable elements (panelist effect). The logistic equations can be used to create proportional odds models for various attributes as mentioned below:

$$L1 = \alpha 1 - (\beta 1X1 + \beta 2X2 + \ldots + \beta 7X7) \quad (20)$$

$$L2 = \alpha 2 - (\beta 1X1 + \beta 2X2 + \ldots + \beta 7X7) \quad (21)$$

$$L3 = \alpha 3 - (\beta 1X1 + \beta 2X2 + \ldots + \beta 7X7) \quad (22)$$

$$L4 = \alpha 4 - (\beta 1X1 + \beta 2X2 + \ldots + \beta 7X7) \quad (23)$$

Here ‘α’ is intercept and ‘β’ is slope in this equation. The maximum likelihood theory is used to calculate these parameter estimations [9].

Principal component analysis (PCA) is an effective tool to decrease the number of dependent variables (i.e., characteristics) to a lesser number of underlying variables (called factors) based on the patterns of connection amongst original variables data, trying to extract the most important elements among the variables analyzed while maintaining the most original variable information. For the evaluation of 5 experimental bread samples, PCA and POM were accustomed to find the highest effective component or characteristic upon various quality charactersitics *viz*., appearance, color, taste, texture and overall

acceptance. The detailed analysis (POM supervised PCA) was performed using R and Origin software.

5.2.7 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.

5.3 Results and discussion

5.3.1 Specific volume of bread

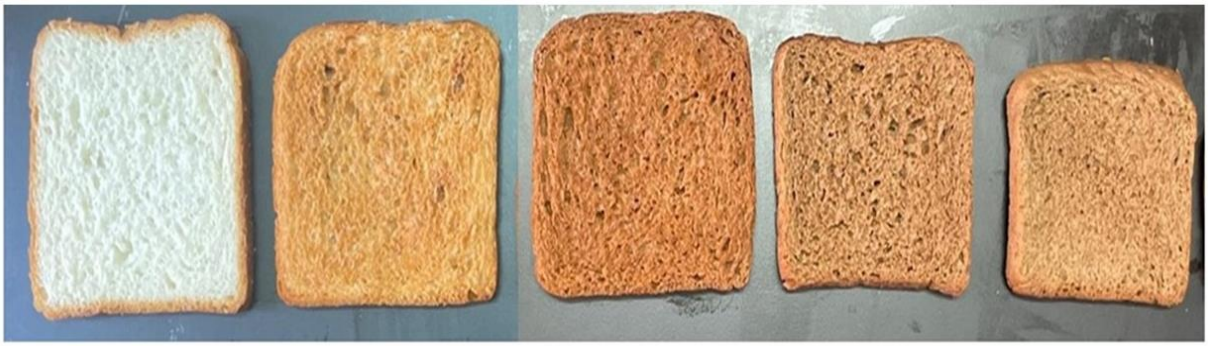

Fig. 5.1 Bread fortified with *Euryale ferox* seed shell extract

Table 5.1 Specific volume and textural properties of bread fortified with *Euryale ferox* seed shell extract

Sample	Specific volume (cm^3 /g)	Hardness (N)	Springiness	Chewiness (N)
Control	5.63±0.05^{a}	10.40±0.17 ^{a}	0.92±0.02^{a}	7.32±0.94^{a}
Product-A	5.51±0.06 ^{ab}	10.46±0.75^{ab}	0.89±0.01^{a}	7.53±0.45^{ab}
Product-B	5.48±0.11 ^{abc}	11.41±1.12^{abc}	0.88±0.03^{a}	8.28±0.47^{bc}
Product-C	5.45±0.08 ^{abcd}	11.64±0.99^{abcd}	0.87±0.02^{a}	10.20±0.72^{d}
Product-D	5.25±0.25 ^{c}	12.62±0.70^{bcd}	0.85±0.05^{b}	10.50±0.86^{d}

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

The specific volume of bread is a vital criterion to evaluate the quality of bread as it is correlated to dough expanding capability and oven bounciness [2]. Changes in specific volume for different bread samples are presented in Table 5.1. Breads containing 0%, 0.25%, 0.5%, 1% and 2% of EFSSE had specific volume values of 5.63, 5.51, 5.48, 5.45, 5.25 cm^3/g, respectively. The volume of EFSSE incorporated loaves was slightly lower than that of control bread. The specific volume of the bread loaves reduced as the level of EFSSE incorporation increased. However, up to the 1% level of incorporation, the difference in volume was statistically insignificant ($p>0.05$). During dough mixing, carbon dioxide produced by yeast is trapped in the dough created by inter- and intra-disulfide linkages, enabling bread volume to expand during fermentation [20]. Our results are in harmony with Chusak et al. [7], who added *Clitoria ternatea* flower extract to bread and reduced the volume and height of the loaf while increasing the compactness and also reducing visible air pockets due to the dense crumb structure [22, 31]. The physical and chemical interactions of polyphenols with water and gluten could reduce loaf volume [20]. Polyphenols break up the gluten network and elasticity of dough by forming covalent bonds with polysaccharides and hamper the ability of the dough to hold gas, resulting in a reduced loaf volume [26]. Another factor was the potential of polyphenol to reduce amylase activity in bread flour, thereby decreasing the substrate for yeast fermentation and resulting in reduced gas production [31].

5.3.2 Texture profile analysis of bread

The texture of the bread crumb, which is generally caused by starch gelatinization, is the most main factor in deciding the quality and acceptability of bread. The present study tested four parameters related to a bread crumb texture. The incorporation of EFSSE, however, had no noticeable impact on cohesiveness and springiness ($p > 0.05$). The textural qualities of bread crumbs are affected by EFSSE incorporation in bread flour (Table 5.2). There were insignificant differences in the hardness of the bread up to 1% incorporation of the extract. The hardness of the samples varied significantly among samples (10.40-12.80N) and the hardness of the control bread (10.40N) was the least. In our results, the hardness of the Product-D (2% EFSSE) was the highest (12.62 N). The increase in hardness for bread fortified with EFSSE was due to compromised gluten network development and resulting hardening of bread crumbs. The hygroscopic property of polyphenols extracts absorbs water more than the native wheat starch granules in the

dough and thereby increasing the hardness [20]. Hardness is related to bite force and is commonly used as an index to determine the acceptability of bread quality [17]. The findings of these studies, especially on the influence of polyphenols on bread hardness, could be attributed to: (i) the composition and concentration of extract added to bread and (ii) different bread making processes and ingredients [22].

Springiness ranged from 0.85-0.92 and control had the maximum springiness however the differences were insignificant up to 2% incorporation. The lowest springiness was found in the highest level of incorporation, which is attributed to the reduced loaf volume [26]. Chewiness is directly related to hardness and ranged from 7.32-10.50 N. Product-D (2% EFSSE) exhibited lowest cohesiveness (0.36), indicating a decline in the strength of the internal bond between bread ingredients [2]. Perhaps, the decrease in specific volume was interrelated with the changes in textural attributes. Various studies have reported that the fortification of bread with polyphenols alter the texture of bread crumbs which are in line with our results. Similar conclusions have also been reported for bread developed with the addition of grape seed extract [17] and vine tea extract [21]. However, Pasrija et al. [20] reported that plant extract polyphenols did not significantly affect the hardness of bread. In contradiction to these, some studies reported that polyphenols incorporation increases the hardness and chewiness of bread [18].

5.3.3 Color properties of bread

Table 5.2 Color properties of bread fortified with *Euryale ferox* seed shell extract

Sample	**Crumb**			**Crust**		
	L*	**a***	**b***	**L***	**a***	**b***
Control	70.44±0.94^{a}	-0.31±0.04^{a}	8.34±0.25^{a}	46.86±0.73^{a}	6.01±1.89 ^{a}	12.23±1.35 ^{a}
Product-A	67.23±0.88^{b}	1.05±0.10^{b}	18.59±0.97^{d}	45.37±0.24 ^{a}	6.98±0.71 ^{ab}	14.20±1.9 ^{a}
Product-B	64.11±0.58^{bc}	1.58±0.11^{bc}	15.97±0.68^{c}	44.50±0.42 ^{a}	6.68±1.11 ^{ab}	11.40±1.51 ^{a}
Product-C	58.47±0.30^{bc}	1.98±0.12^{bc}	15.08±0.77^{c}	42.44±0.10 ^{a}	11.50±1.82 ^{c}	13.85±1.07 ^{a}
Product-D	53.73±1.11^{d}	3.43±0.16^{d}	10.35±0.54^{b}	41.77±0.39 ^{a}	12.68±1.77 ^{c}	12.72±0.76 ^{a}
Values are mean of triplicates ±SD; Values are mean of triplicates ±SD; Values with the different letters in the same column differ significantly ($p<0.05$)						

EFSSE utilized in the present study was fine water-soluble brown color powder. As expected, bread fortified with EFSSE had produced a variable range of color as contrast

to the control. The comparison of color between control and fortified bread is shown in Table 5.2. With a higher percentage of EFSSE addition, the color of the crumb of bread exhibited a rise in redness (a* value) but a decrease in L* value. It is worth mentioning that the a* and b* value, did not display the same upward or downward trend as the L* value. The L*, a*, and b* values of the crumbs of the EFSSE incorporated bread were significantly different from the control bread. Results of various authors [17, 21, 26] corroborated our present findings and revealed that changes in a* and b* values are directly linked to the color of raw materials used for bread making. Bread with 2% EFSSE was acceptable by the sensory panelists, although the sensory scores were decreased. However, EFSSE incorporation did not produce a significant impact on the color of the crust. Similar findings have been reported for bread supplemented with tea extract [26, 31].

5.3.4 *In vitro* starch digestibility and predicted glycemic index

The percentages of TS, RDS, SDS, and RS in the developed bread products (%) are shown in Table 5.3. The prepared bread has lower total starch content (77.63, 76.39, 75.30, and 74.07% for Product-A, Product-B, Product-C, and Product-D, respectively) in comparison to the control sample (78.30%).

Table 5.3 Starch fractions of bread fortified with *Euryale ferox* seed shell extract

Sample	TS (%)	RDS (%)	SDS (%)	RS (%)
Control	78.30±1.08^{a}	49.59±2.57^{a}	47.47±3.84^{a}	2.94±1.28^{a}
Product-A	77.63±0.55^{ab}	36.12±4.04^{b}	55.15±4.48^{b}	8.73±2.65^{ab}
Product-B	76.39±0.87^{bc}	32.80±3.60^{bc}	56.20±3.10^{b}	10.96±3.62^{bc}
Product-C	75.30±0.15^{cd}	27.88±3.03^{c}	56.22±5.32^{b}	15.90±4.26^{cd}
Product-D	74.07±0.55^{d}	21.62±0.62^{d}	58.25±3.37^{b}	20.13±3.39^{d}

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

5.3.4 Starch digestion kinetics and prediction of GI

5.3.4.1 Mathematical modeling approach

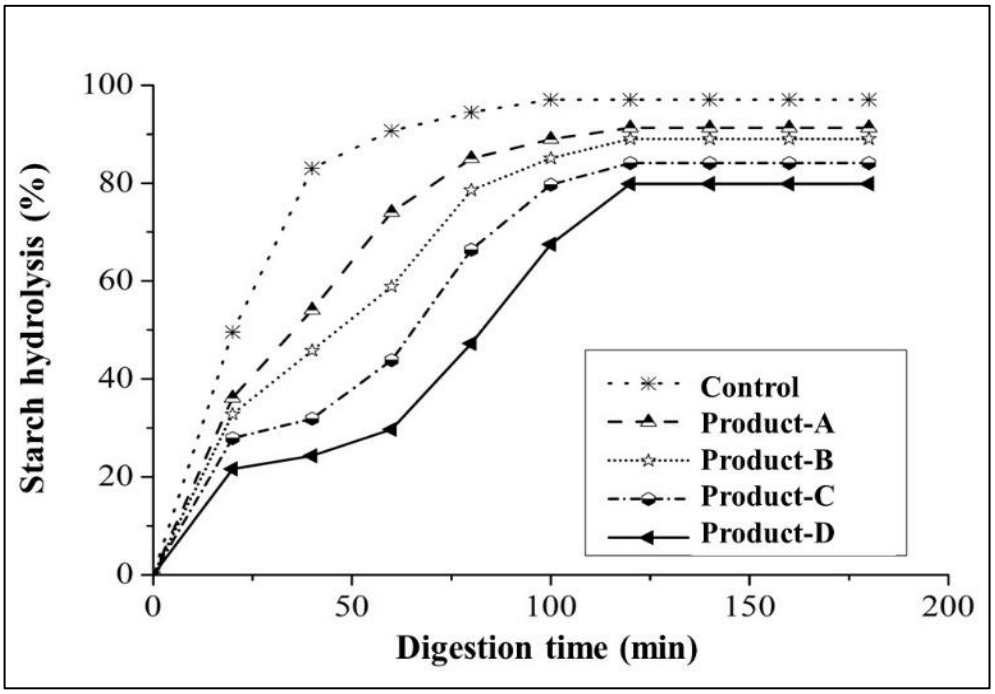

Fig. 5.2 (a) Starch hydrolysis (%) pattern of different bread samples fortified with *Euryale ferox* seed shell extract

The starch digestion pattern of the formulated bread samples is presented in Fig. 5.2. The rate of starch digestion differed among the bread samples. The fortification of EFSSE led to a decline in starch hydrolysis and was proportional to the level of EFSSE addition. The RDS portions of the formulated bread samples were reduced from 49.59% (control) to 21.62% (2% EFSSE). However, the SDS and RS fractions were increased from 47.47% (control) to 58.25% (2% EFSSE) and 2.94 (control) to 20.13% (2% EFSSE) respectively. Similar findings have been reported where polyphenol rich plant extracts have been utilized as a strategy to reduce the glycemic potential of bread [10, 12, 15]. Polyphenols are intended to bind to starch by occupying its hydrophobic helical region, preventing α-amylase from adhering to starch [28]. Furthermore, the starch-polyphenols interactions help to establish an ordered structure or crystallinity, which facilitates the formation of resistant starch [6, 23]. Thus, the phenolic compounds in EFSSE *viz.*, the catechin, gallic acid, rutin etc. [30] might be responsible for the delay in starch hydrolysis by hindering the action of carbohydrate digesting enzymes [13, 15].

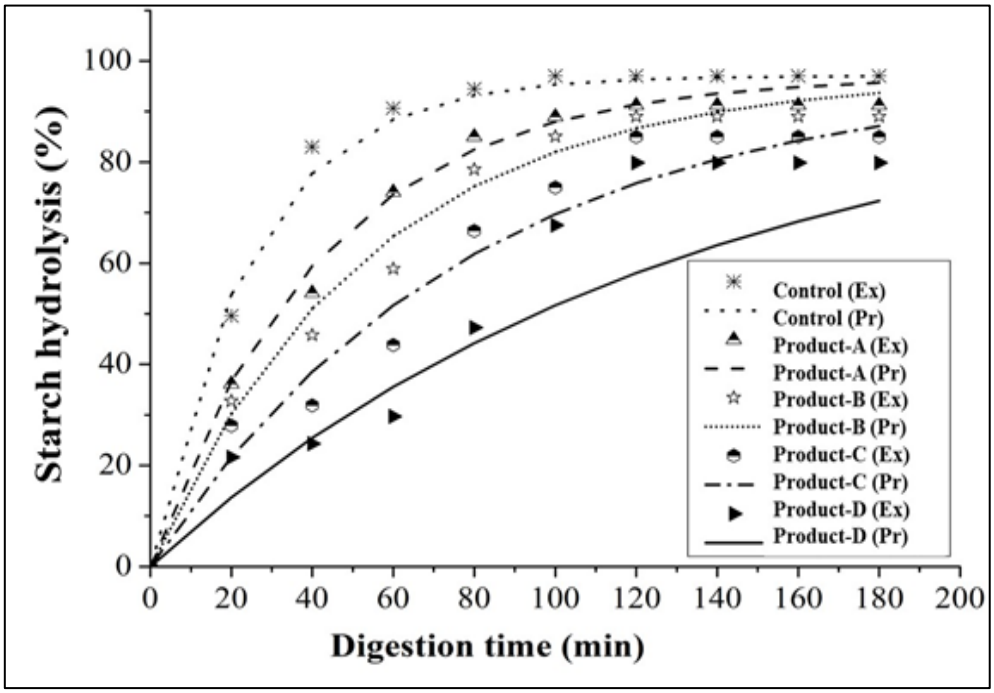

Fig. 5.2 (b) Fitting plots between experimental and predicted data for starch hydrolysis kinetics of different bread samples fortified with *Euryale ferox* seed shell extract on mathematical modeling

Table 5.4 Digestion kinetics of bread fortified with *Euryale ferox* seed shell extract

Sample	k(min^{-1})	R^2	RMSE	SSE
Control	0.04038	0.99424	2.47	55.20
Product-A	0.02369	0.99325	2.86	74.05
Product-B	0.01863	0.98372	3.84	133.05
Product-C	0.01266	0.95645	5.79	302.36
Product-D	0.00670	0.93791	11.97	1289.83

When white bread was fortified with varied quantities of EFSSE, there was a substantial drop in C∞ values. The degree of starch hydrolysis can be seen in Fig. 5.2 (a). The maximum hydrolysis extents (C∞) of fortified breads, which ranged between 91.27 and 79.87, were drastically lower than that of control bread (97.06). The reaction rate constant, i.e., k values for the *in vitro* digestion kinetics, were obtained by using the equation 12. The goodness of fit for the selected model was computed using the highest R^2, lowest

RMSE, and SSE values. The digestion kinetics of bread based on a mathematical modeling approach are presented in Table 5.4.

The fitting plots between experimental and predicted data for starch hydrolysis from different bread samples are illustrated in Fig 5.2 (b). The results revealed that k value for the digestion kinetics decreased as the EFSSE concentration increased from 0.25%-2%, indicating that the lower the 'k' value, the slower the starch digestion rate. The modelled digestion patterns of all bread samples are also presented. The low RMSE values (Table 5.4) depicting a minimal variation between the modelled and experimental results and implied that the developed model was of good quality, suggesting a significant agreement between the modelled and experimental results. The ability of simulation data to represent the digestive characteristics of all the bread samples was further supported by the high R^2 values.

Table 5.5 Determination of pGI of bread fortified with *Euryale ferox* seed shell extract

Sample	k value (min^{-1})	AUC	HI (%)	Predicted GI
Control	0.040^{a}	15068.29 ± 138.23^{a}	100.00 ± 2.00^{a}	94.61 ± 1.36^{a}
Product-A	0.023^{b}	12630.10 ± 122.23^{b}	83.82 ± 1.56^{b}	85.73 ± 1.25^{b}
Product-B	0.018^{c}	11414.92 ± 96.45^{c}	75.75 ± 1.23^{c}	81.28 ± 1.63^{c}
Product-C	0.012^{d}	9175.68 ± 76.44^{d}	60.89 ± 1.20^{d}	73.14 ± 1.73^{d}
Product-D	0.006^{e}	6024.98 ± 63.26^{e}	49.98 ± 1.25^{e}	68.66 ± 2.44^{e}

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

The predicted glycemic index (pGI) was determined based on the reaction rate constant data (Table 5.4) using equations 11 and 12. From the data presented in Table 5.5, it may be explained that the polyphenol fortified bread samples evinced a dose-dependent decrease in GI in comparison to the control bread. Control bread with the highest RDS content has the highest pGI (94.61) as compared to the other fortified bread. The pGI value was linked to the amount of digestible starch fractions, such as RDS and SDS. While, Product-A (85.73), Product-B (81.28), Product-C (73.14), and Product-D (61.67).

Therefore, adding EFSSE to bread can be considered as a better strategy to alter the digestibility and pGI of white bread.

Although there were substantial increased in the SDS and RS fractions in EFSSE fortified breads, Product-A, Product-B were still under the high GI foods (GI > 70). Product-D will be considered as an intermediate GI breads. Several authors have also reported that bread fortified with polyphenol-rich plant extract *viz*., tea polyphenols [10, 12, 31, 26]; *Clitoria ternatea* extract [7]; green coffee bean phenolics [24] could successfully reduce starch digestibility alters the glycemic index of foods. Low GI and high RS diets have been shown to prevent and manage diseases associated with glucose metabolism [10]. Thus, foods with a low GI are favored by health-conscious consumers [6].

5.3.4.2 SISNN based modeling approach

Weight and bias values were optimized based on the integrated PSO algorithm in SISNN. Fig. 5.3 illustrates the selection of the best network based on minimum MSE. For architecture 2-4-1, the best results were obtained with R^2 of 0.99 and MSE of 0.015 (Table 5.6). The 2-4-1 architecture for the predictive modeling of *in vitro* digestion kinetics is illustrated in Fig. 5.4. The best architecture of the SISNN i.e., 2-4-1 was implemented to determine the reaction rate constant (k) and predicted glycemic index (pGI). The plots of experimental and estimated digestion kinetics based on the SISNN are shown in Fig. 5.5. The plots show adequate fitting of the SISNN-based kinetics for all the bread samples, including the control samples. The weight and bias values of the best SISNN architecture are shown in Table 5.6. Weight and bias values were selected for the 2-4-1 architecture and was used for executing the above parameters. The methodology for determining SISNN-based reaction rate constant (k) is presented in Fig. 5.3. Zaied et al. [29] reported a similar approach of swarm intelligence based neural network simulation for the digestion process in a solar bioreactor.

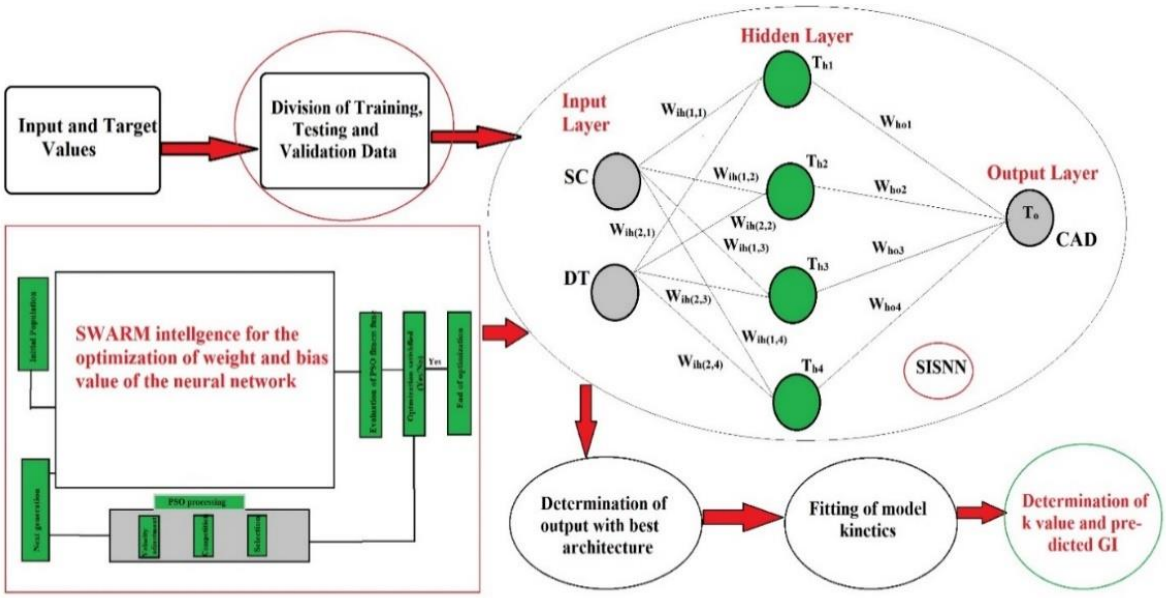

Fig. 5.3 Methodology for the determination of swarm intelligence supervised neural network (SISNN) based reaction rate constant (k) and pGI

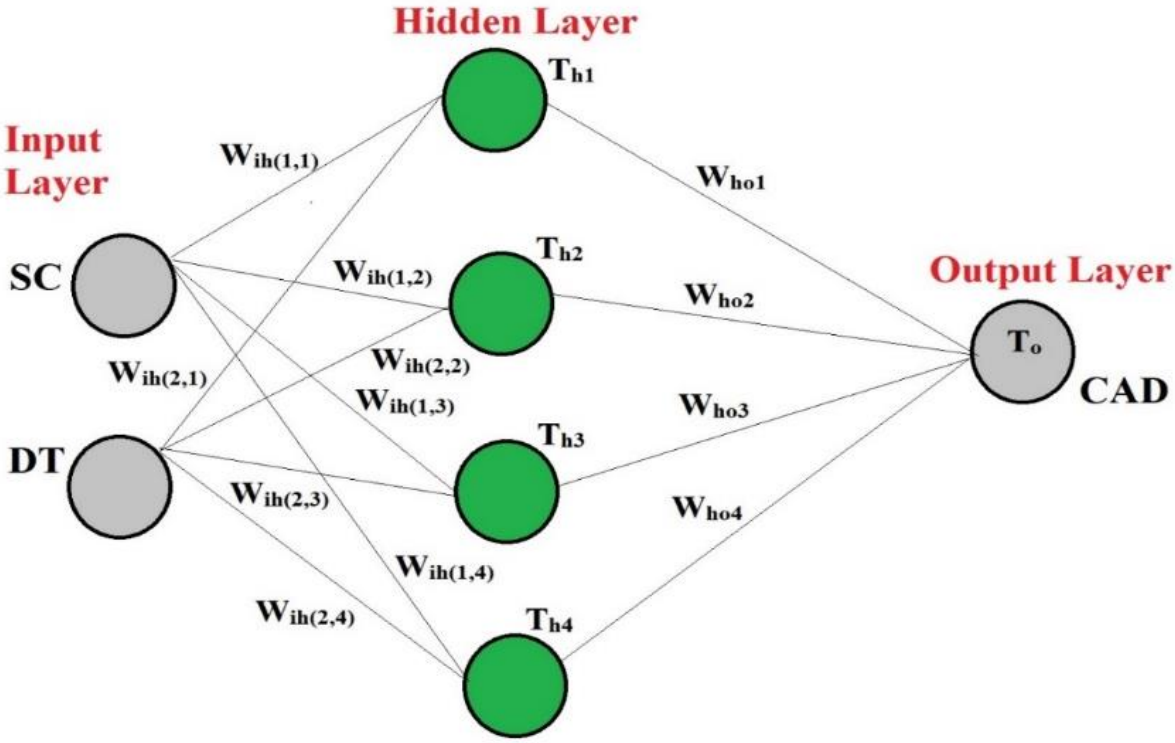

Fig. 5.4 Architecture for the predictive modeling *in vitro* digestion kinetics of bread fortified with *Euryale ferox* seed shell extract

Table 5.6 Selection of swarm intelligence supervised neural network (SISNN) best architecture

Network Architecture	MSE	R^2
2-2-1	0.070	0.92
2-3-1	0.016	0.98
2-4-1	0.015	0.99
2-5-1	0.011	0.98
2-6-1	0.012	0.97

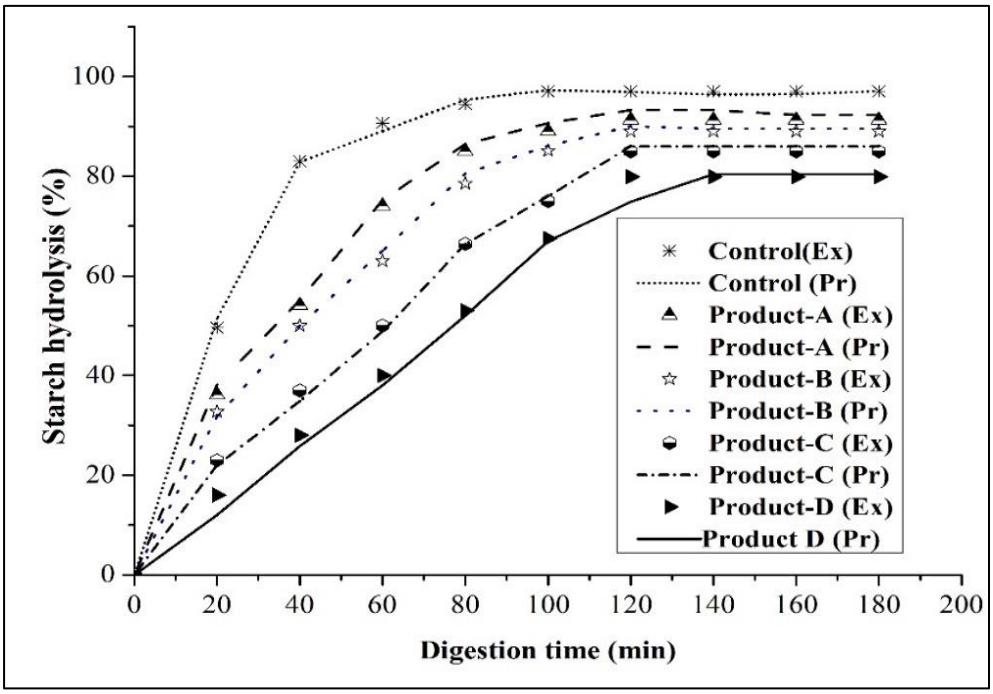

Fig. 5.5 Plots of experimental and estimated starch hydrolysis kinetics of bread fortified with *Euryale ferox* seed shell extract based on Swarm Intelligence Supervised Neural Network (SISNN)

Table 5.7 Determination of reaction rate and predicted GI of bread fortified with *Euryale ferox* seed shell extract based on swarm intelligence supervised neural network (SISNN) predicted data

Samples	k (min^{-1})	R^2	RMSE	SSE	Predicted GI
Control	0.04101	0.995	2.26	46.25	95.41
Product-A	0.02592	0.990	1.52	21.03	86.89
Product-B	0.02026	0.983	2.23	45.11	82.32
Product-C	0.01347	0.994	2.18	43.01	74.74
Product-D	0.00497	0.959	2.26	46.25	66.22

The results of SISNN based reaction rate kinetics are displayed in Table 5.7. The pGI values were determined on the basis of SISNN predicted k values. Better performance was observed in SISNN-based kinetics compared to mathematical modeling-based digestion kinetics (Table 5.7). The coefficient of determination (R^2) value was 0.99 for SISNN-based prediction whereas in the case of mathematical based predictions, it was <0.99 in most of the samples excluding the control sample. Hence, in this study SISNN could be claimed to be the better method for mapping *in vitro* digestion kinetics.

5.3.4.3 Sensitivity analysis

To check adequate predictability of SISNN-based digestion network under randomized conditions, sensitivity analysis was also performed for the 2-4-1 architecture. For these three unknown EFSSE concentrations viz., 0.15, 0.30, and 0.60% were used and the digestion time varied from 0-180 min (Fig. 5.6). These concentrations were considered by taking the middle value of the concentration of EFSSE varying from products A and D. This data was taken as input in the 2-4-1 architecture, and the corresponding output was calculated. The reaction rate constant and predicted GI associated with SISNN sensitivity analysis-based data are shown in Table 5.8. The present results revealed that an adequate trend of pGI was observed, which was in line with the predicted trends based on the mathematical modeling approach. Therefore, SISNN-based reaction kinetics can be claimed to be a proper modeling technique to analyze the starch hydrolysis behavior.

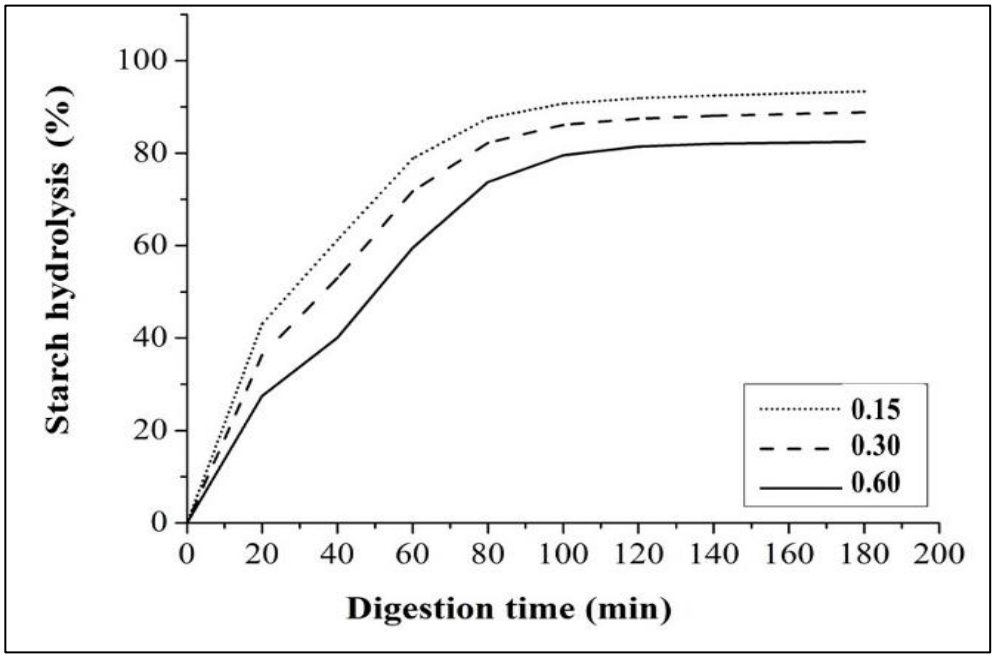

Fig. 5.6 Sensitivity analysis of starch hydrolysis and predicted GI associated with swarm intelligence supervised neural network (SISNN)

Table 5.8 Reaction rate constant and predicted GI associated with swarm intelligence supervised neural network (SISNN) sensitivity analysis-based data

Concentrations (g/100g)	k value (min^{-1})	R^2	Predicted GI
0.15	0.0287	0.996	88.81
0.30	0.0224	0.993	84.21
0.60	0.0183	0.983	80.44

3.4 Sensory evaluation by using proportional odd modelling (POM) supervised PCA (principal component analysis) technique

The sensory scores for 5 experimental breads for various quality characteristics *viz*., appearance, color, taste, texture, and overall acceptability, are represented with the help of a radar plot [Fig. 5.7 (a)]. Thirty (30) numbers of judges were considered for the scoring of the bread products based on these five attributes. Principal component analysis (PCA) is a valuable approach for reducing data and extracting the most important aspects among the variables being researched. The PCA tool was applied for selecting the most effective component or attribute for the evaluation of five types of breads. The principal component

was selected based on the Eigen values and a significant break could be observed after point 3 [Fig. 5.7 (b)]. Hence, the first three components were chosen as the principal components (PC-1, PC-2 and PC-3). The details of extracted Eigen vectors are presented in Table 5.9.

Among the PC-1 values, the Product-B was found as the highest, whereas Product-A and control were found in the case of PC-2 and PC-3, respectively. The biplots for these three principal components are illustrated in Fig. 5.7 (c), Fig. 5.7 (d) and Fig 5.7 (e). From these plots, similar results could be observed for PC-1, PC-2 and PC-3. Ghosh & Chattopadhyay [9] also reported the application of PCA for the sensory evaluation of food products. To ensure the ranking of the different bread products, sensory scores were also analyzed using the proportional odds modeling (POM) approach. By considering control as the reference, the ranking of the bread samples could be placed in the order of control>Product A>Product B>Product C>Product D. The ranking was done based on the logistic values (1 for reference sample (control) and other values as mentioned in the Table 5.10. Therefore, from the above-mentioned ranking, it can be concluded that among the EFSSE added bread samples, Product-A and Product-B showed better results.

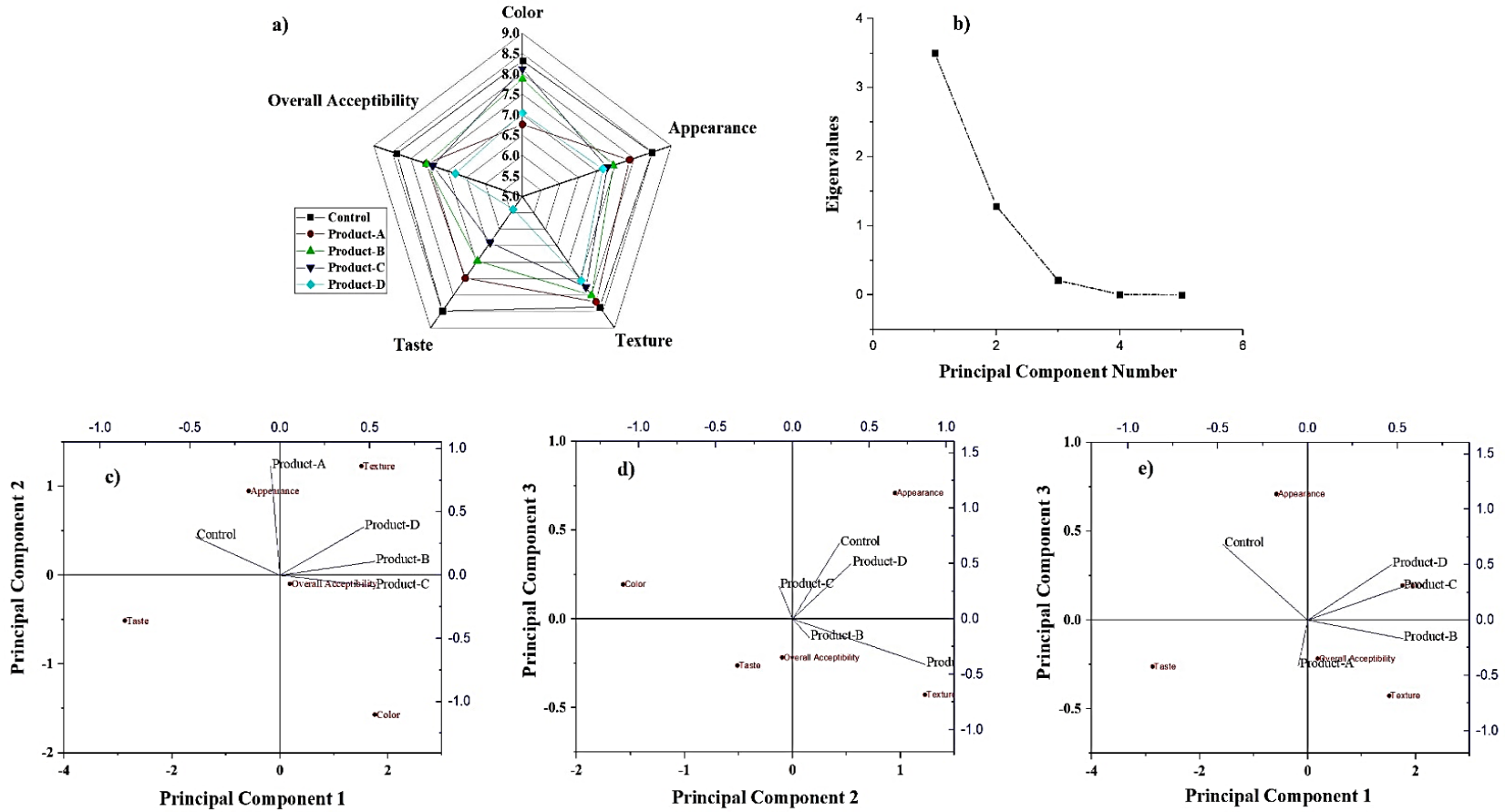

Fig. 5.7 Selection of the principal component based on the Eigen values for sensory evaluation of bread fortified with *Euryale ferox* seed shell extract

(a) Radar plot of sensory scores; **(b)** Principal Component Analysis; **(c)** Principal Component 1

(d) Principal Component 2; **(e)** Principal Component 3

Table 5.9 Determination of extracted Eigen vectors of principal component analysis (PCA)

Samples	Coefficients PC1	Coefficients PC2	Coefficients PC3
Control	-0.47285	0.30367	0.68299
Product-A	-0.05171	0.86235	-0.40896
Product-B	0.528	0.10867	-0.16719
Product-C	0.52688	-0.08815	0.29872
Product-D	0.46621	0.3802	0.4991

Table 5.10 Selection of proportional odds modeling (POM) coefficients

Coefficients:	**Value**	**Standard Error**
Product-A	0.037	0.3941
Product-B	-0.03941	0.3967
Product-C	-0.08275	0.4002
Product-D	-0.08646	0.4081
Intercepts:	Value	Standard Error
1|2	-1.4064	0.302
2|3	-0.4182	0.283
3|4	0.441	0.2829
4|5	1.3549	0.3005

5.4 Conclusion

The effects of EFSSE on the *in vitro* starch digestibility of formulated bread were significant and noticeable. EFSSE fortified bread evinced lower glycemic index. The results of the present investigation also exhibited that with appropriate levels of EFSSE incorporation, it is possible to develop functional bread without causing significant changes in other sensory properties, except the color change. The various interactions between the bioactive constituents of EFSSE with α-amylase and α-glucosidase could reduce *in vitro* starch digestibility. Therefore, the results of the present investigation have shown that EFSSE may be utilized as a curative ingredient that can be used to produce lower glycemic index functional bread.

Bibliography

1. A.A.C.C. Approved Methods of the AACC, 10th ed., *American Association of Cereal Chemist*, St. Paul, 2000.
2. Begum, Y. A., Chakraborty, S., & 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, 2020.
3. Chakraborty, S., Gautam, S. P., Bordoloi, T., & Hazarika, M. K. Neural network and computational fluid dynamics modeling for the gelatinization kinetics of instant controlled pressure drop treated parboiled rice. *Journal of Food Process Engineering*, 43(11): e13534, 2020.
4. Chakraborty, S., Gautam, S. P., Sarma, M., & Hazarika, M. K. Adaptive neuro-fuzzy interface system and neural network modeling for the drying kinetics of instant controlled pressure drop treated parboiled rice. *Food Science and Technology International*, 27(8): 746-763, 2021.
5. Chakraborty, S., Sarma, M., Bora, J., Faisal, S., & Hazarika, M. K. Generalization of drying kinetics during thin layer drying of paddy. *Agricultural Engineering International: CIGR Journal*, 18(4): 177-189, 2016.
6. Chi, C., Li, X., Zhang, Y., Chen, L., Xie, F., Li, L., & Bai, G. Modulating the *in vitro* digestibility and predicted glycemic index of rice starch gels by complexation with gallic acid. *Food Hydrocolloids*, 89: 821-828, 2019.
7. Chusak, C., Henry, C. J., Chantarasinlapin, P., Techasukthavorn, V., & Adisakwattana, S. Influence of *Clitoria ternatea* flower extract on the *in vitro* enzymatic digestibility of starch and its application in bread. *Foods*, 7(7):102, 2018.
8. Englyst, H. N., Kingman, S. M., & Cummings, J. H. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46: S33-50, 1992.
9. Ghosh, D., & Chattopadhyay, P. Application of principal component analysis (PCA) as a sensory assessment tool for fermented food products. *Journal of Food Science and Technology*, 49: 328-334, 2012.

10. Goh, R., Gao, J., Ananingsih, V. K., Ranawana, V., Henry, C. J., & Zhou, W. Green tea catechins reduced the glycemic potential of bread: An *in vitro* digestibility study. *Food Chemistry*, 180: 203-210, 2015.
11. Goni, I., Garcia-Alonso, A., & Saura-Calixto, F. A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, 17(3): 427-437, 1997.
12. Kan, L., Capuano, E., Fogliano, V., Oliviero, T., & Verkerk, R. Tea polyphenols as a strategy to control starch digestion in bread: the effects of polyphenol type and gluten. *Food &Function*, 11(7): 5933-5943, 2020.
13. Lemlioglu-Austin, D., Turner, N. D., McDonough, C. M., & Rooney, L. W. Effects of sorghum [*Sorghum bicolor* (L.) Moench] crude extracts on starch digestibility, estimated glycemic index (EGI), and resistant starch (RS) contents of porridges. *Molecules*, 17(9): 11124-11138, 2012.
14. Li, S., Ward, R., & Gao, Q. Effect of heat-moisture treatment on the formation and physicochemical properties of resistant starch from mung bean (*Phaseolus radiatus*) starch. *Food Hydrocolloid*, 25(7): 1702-1709, 2011.
15. Lin, J., Teo, L. M., Leong, L. P., & Zhou, W. *In vitro* bioaccessibility and bioavailability of quercetin from the quercetin-fortified bread products with reduced glycemic potential. *Food Chemistry*, 286: 629-635, 2019.
16. Liu, Y., Wei, S., & Liao, M. Optimization of ultrasonic extraction of phenolic compounds from *Euryale ferox* seed shells using response surface methodology. *Industrial Crops and Products*, 49: 837-843, 2013.
17. Ma, Q., Cai, S., Jia, Y., Sun, X., Yi, J., & Du, J. Effects of hot water extract from vine tea (*Ampelopsis grossedentata*) on acrylamide formation, quality and consumer acceptability of bread. *Foods*, 9(3): 373, 2020.
18. Miao, M., Jiang, B., Jiang, H., Zhang, T., & Li, X. Interaction mechanism between green tea extract and human α-amylase for reducing starch digestion. *Food Chem*istry, 186: 20-25, 2015.
19. Onipe, O. O., Beswa, D., & Jideani, A. I. *In vitro* starch digestibility and glycaemic index of fried dough and batter enriched with wheat and oat bran. *Foods*, 9(10):1374, 2020.
20. Pasrija, D., Ezhilarasi, P. N., Indrani, D., & Anandharamakrishnan, C. Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. *LWT - Food Science and Technology*, 64(1): 289-296, 2015.

21. Peng, X., Ma, J., Cheng, K. W., Jiang, Y., Chen, F., & Wang, M. The effects of grape seed extract fortification on the antioxidant activity and quality attributes of bread. *Food Chemistry*, 119(1): 49-53, 2010.

22. Reshmi, S. K., Sudha, M. L., & Shashirekha, M. N. Starch digestibility and predicted glycemic index in the bread fortified with pomelo (*Citrus maxima*) fruit segments. *Food Chemistry*, 237: 957-965, 2017.

23. Sun, L., & Miao, M. Dietary polyphenols modulate starch digestion and glycaemic level: A review. *Critical Reviews in Food Science and Nutrition*, 60(4): 541-555, 2020.

24. Świeca, M., Gawlik-Dziki, U., Sęczyk, Ł., Dziki, D., & Sikora, M. Interactions of green coffee bean phenolics with wheat bread matrix in a model of simulated *in vitro* digestion. *Food Chemistry*, 258: 301-307, 2018.

25. Tlili, N., Kirkan, B., & Sarikurkcu, C. LC–ESI–MS/MS characterization, antioxidant power and inhibitory effects on α-amylase and tyrosinase of bioactive compounds from hulls of *Amygdalus communis*: The influence of the extracting solvents. *Industrial Crops and Products*, 128: 147-152, 2019.

26. Wang, R., Zhou, W., & Isabelle, M. Comparison study of the effect of green tea extract (GTE) on the quality of bread by instrumental analysis and sensory evaluation. *Food Research International*, 40(4): 470-479, 2007.

27. Wu, C., Chen, R., Wang, X. S., Shen, B., Yue, W., & Wu, Q. Antioxidant and anti-fatigue activities of phenolic extract from the seed coat of *Euryale ferox* Salisb. and identification of three phenolic compounds by LC-ESI-MS/MS. *Molecules*, 18(9):11003-11021, 2013.

28. Xu, J., Wang, W., & Li, Y. Dough properties, bread quality, and associated interactions with added phenolic compounds: A review. *Journal of Functional Foods*, 52: 629-639, 2019.

29. Zaied, B. K., Rashid, M., Nasrullah, M., Bari, B. S., Zularisam, A. W., Singh, L., Kumar, D., & Krishnan, S. Prediction and optimization of biogas production from POME co-digestion in solar bioreactor using artificial neural network coupled with particle swarm optimization (ANN-PSO). *Biomass Conversion and Biorefinery*, 1-16, 2020.

30. Zhang, C. *In vitro* antioxidant properties of *Euryale ferox* seed shell extracts and their preservation effects on pork sausages. *Journal of Food Processing and Preservation*, 39(6): 1172-1182, 2015.

31. Zhu, F., Sakulnak, R., & Wang, S. Effect of black tea on antioxidant, textural, and sensory properties of Chinese steamed bread. *Food Chemistry*, 194: 1217-1223, 2016.