Chapter 3

To model nachos from bhimkol blossom by using an artificial intelligence approach

3.1 Introduction

Nutrition and phytochemicals rich plants have been in great demand in the field of pharmaceuticals as food supplements. Almost every plant species that originated on earth has been studied for its different uses in day-to-day human life. Bhimkol blossoms (BB) have been used for the preparation of a special dish by the people living in the Assam Region (India) since antiquity [28]. A boiled blossom dish is used as a traditional medicine to treat fever, low to high blood pressure, nutritional deficiency, and anaemia. Though BB is known for high nutrition and phytochemicals, it is still veiled as underutilized food and is usually discarded as waste material after the harvesting of fruits [33].

Phytochemical contents have been reported to exhibit various health beneficial properties and antimicrobial activities over some food-borne pathogenic microorganisms by disrupting the microbial cell membranes [13]. Various phytochemical contents in banana blossom were reported in wide varieties *viz*., *Musa* sp. var. Nanjangud rasa bale, *Musa acuminata*, and *Musa sapientum*, *with* several secondary metabolites that exhibit potent antioxidant and antimicrobial properties [33]. Various pathogens like *Escherichia coli*, *Salmonella typhimurium*, *Campylobacter jejuni*, *Legionella pneumophila*, *Staphylococcus aureus*, and *Streptococci*) contaminate food and lead to various serious diseases [1]. The secondary metabolites such as polyphenols, steroids, and terpenoids are naturally present in the plants, which help them to protect themselves from plant predators and pathogens [28]. Some fatty acids like hexanoic acid, octanoic acid, and palmitic acid were reported to exhibit inhibitory effects against *Streptococcus mutans*, *Streptococcus gordonii*, and *Streptococcus sanguis* [15]. Prior research substantiates the belief that frequent use of antibiotics may lead to numerous side effects and may impose negative effects on health in many ways and therefore, consuming natural food is contemplated to be much healthier than consuming antibiotics [13]. In today's world, due to high urbanization, people are deficient in fresh farm grown goods with proper nutrition and health benefits. There is an upsurge in the escalation of demand for fresh farm foods and organic foods to avert the adverse effects of consuming foods grown using chemical fertilizers.

As BB is known for its richness in phytochemicals, the secondary metabolites present may have the potential activity to inhibit the growth of some common food-borne

bacteria. As the consumption level of the BB is comparatively low commercially, therefore the development of a nutrition-rich with high phytochemical content processed food product would be highly beneficial to the consumers. In developing any food product, proper formulation and optimization of product forming parameters are paramount to achieving consumer satisfaction and market demand [6]. There are several process optimization models and software such as response surface methodology (RSM), particle swarm optimization (PSO), artificial neural networking (ANN), and genetic algorithm (GA). Implementing these methods provides an optimized and desired product with the most desired qualitative and quantitative attributes. Optimization models are being used very frequently nowadays to obtain the best out of the best from a complicated set of problems. There is scant research on the BB, particularly its nutritional and phytochemical components showing antioxidant and antimicrobial activities. Using agricultural raw materials like BB for highly valued products is always a priority, which helps in environmental protection and economic growth. Chips and nachos are snack foods consumed regularly and in high demand globally. Nachos are the modified snack food originally from the Latin America, usually made from corn powder mixture cut into any thin sheet shape available in fried or baked form. They are mostly served with the side items such as sauces, mayonnaise and vegetables [26]. The present study concentrated on the study of nutritional profiling with antioxidant and antibacterial properties of BB. Thereafter optimized nachos have been formulated through ANN-PSO from the BB, and sensory analysis has been performed to obtain the best with the help of customer satisfaction through PCA analysis.

3.2 Materials and methods

3.2.1 Raw materials and chemical reagents

Inflorescence of BB was collected from the Tezpur University Campus, Assam (26.7003 ^{o}N, 92.8308^{o}E), India. Immediately after collection BB was washed under running tap water and then chopped the individual major parts (*viz*., whole blossom, bract, male flower, and spadics). Chopped parts were dried in a hot air oven (Advantage lab, AL01-05-100, Belgium) at 45^{o}C for 24 h and ground to powder, followed by sieving (300 µm) and stored in air-tight containers at 4^{o}C for further analysis.

All the reagents and chemicals used in the study were of analytical grades. All the chemicals (including standards) and bacterial cells such as *S. typhi* (*Salmonella typhi* -ATCC 6539), *B. cereus* (*Bacillus cereus* -ATCC 14579), *S. aureus* (*Staphylococcus aureus* -ATCC 25923), *E. coli* (*Escherichia coli* -ATCC 25922), *P. aeruginosa* (*Pseudomonas aeruginosa* -ATCC 35554) were purchased from Sigma Aldrich (USA) and Hi-Media Laboratories Pvt. Ltd. (India).

3.2.2 Nutritional and phytocomponent analysis

3.2.2.1 Proximate and other phytocomponents

The proximate and nutritional analyses were done following AOAC [4] methods. Some secondary metabolites, such as solanine and cyanogenic glycosides, were determined following AOAC [4] methods. Saponin and oxalate content was determined following the methods of Mahmood et al. [25] and Aina et al. [2], respectively.

3.2.2.2 Vitamins

Water-soluble vitamins (thiamine, riboflavin, nicotinamide, and pyridoxine) were determined by reverse phase-high performance liquid chromatography (RP-HPLC) [7]. Three concentrations of water-soluble standards from 0 to 1 mg/ml were prepared along with sample extract (1 mg/ml). A column (Symmetry 300^{TM}) C18-4.3 x 250 mm, 5 µm was used at isocratic mode with mobile phase methanol (A) and 0.023 M phosphoric acid (B) (pH 3.5), at ratio A:B (33:67) in 0.5 ml/min flow rate and each run was carried out for 25 min at 270 nm.

For determination of fat-soluble vitamins (D, E, K, A, and β-carotene), 2 ml of homogenized banana blossom powder (BBP) (10% W/V) was mixed with 2.5 ml of ethanolic solution of ascorbic acid (0.2%). Potassium oxide (1 ml of 50%) was added to the mixture and purged with nitrogen for 20 s and then the mixture was subjected to heat in the water bath at 80^{o}C for 10 min and then centrifuged (Eppendorf, 5430R, Germany) at 1702 g for 4 min. The supernatant was collected and subjected to further filtration by using a 0.45 µm syringe filter. For the HPLC determination of fat-soluble vitamins, standards and sample were run at mobile phase acetonitrile: methyl alcohol: ethyl acetate at ratio 80:10:2 in 1 ml/min flow rate. Each run was carried out for 25 min at 325 nm.

3.2.2.3 GC-MS analysis of volatile phytochemicals and fatty acids

The volatile phytochemicals and fatty acid profiling were performed by Gas Chromatography-Mass Spectrometry (GC-MS), and the BB extract was prepared at a concentration of 5 mg/ml in ethanol [31].

The volatile phytochemical screening was done using GC-MS (Bruker Scion 436-GC) with the column BR-5 MS (stationary phase: 5% diphenyl / 95% dimethyl polysiloxane and specifications 30 m x 0.25 mm ID x 0.25 µm). The carrier gas 'helium' was used at 1 ml/min flow rate at split 10:1 by the detector TQ Quadrupole MS with the software MS Workstation 8. The oven temperature was programmed at 110^{o}C hold for 3.50 min up to 200^{o}C at the rate of 10^{o}C /min. The injector temperature was 280^{o}C with a total GC running time of 40.50 min. Fatty acid profiling was done following AOCS [5] method. Fatty acid methyl esters (FAME) were prepared first by the direct transmethylation method to estimate the fatty acid content.

3.2.3 Antioxidant activities

3.2.3.1 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity was estimated by DPPH inhibition [28]. The colorimetric measurements were done using a spectrophotometer (AG22331, Eppendorf, Germany) at 517 nm. The DPPH radical scavenging activity (antioxidant activity) was evaluated as the ratio of the difference between absorbance of DPPH solution ($A_{control}$) and absorbance of the sample and DPPH mixture (A_{sample}) to the absorbance of the DPPH solution (Equation 1).

$$\% \text{ Antioxidant activity} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (1)$$

3.2.3.2 Ferric reducing ability of plasma (FRAP) assay

The FRAP assay was carried out using the method of Lv et al. [24] with slight modification. The colorimetric measurements were done using a spectrophotometer at 523 nm, and the ferric reducing property was expressed in percentage.

3.2.3.3 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The ABTS assay was carried out using the method of Lv et al. [24]. The colorimetric measurements were done by using a spectrophotometer at 734 nm. The ABTS scavenging property was expressed in percentage as given in Equation 2.

$$\%\ \text{Antioxidant activity} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \qquad 2$$

3.2.4 Antibacterial activities

3.2.4.1 Preparation of bacterial culture

The microbial cell culture preparation was done following Hudzicki [16]. The five pathogen strains *viz*., S. *typhi*, *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa* were cultured in 13% nutrient broth by introducing 10 µl of glycerol stock culture to 5 ml of nutrient broth in test tubes and then incubated in a shaking incubator at 37^{o}C (Excella-E24r, New Brunswick, Germany) up to 24 h.

3.2.4.2 Disc diffusion method

The disc diffusion method was performed using the protocol described by Hudzicki [16]. The aliquot of 100 µl of each bacterial culture was spread and plated separately on five nutrient agar plates (28%). Then three concentrations of BB extract (0.5 to 4.0 mg/ml) and kanamycin (10 to 30 µg/ml) were introduced to the wells prepared on every Petri plate containing cultures. Then each Petri plate was incubated in a shaking incubator (Excella-E24r, New Brunswick, Germany) at 37^{o}C for 12 h. The areas of every inhibition zones (diameter in mm) were calculated with the help of a measuring scale for all the Petri plates. The experiment was performed in triplicate and antibacterial activity was expressed in the IC_{50} of inhibition.

3.2.5 Formulation of nachos by optimal mixture design

Table 3.1 Experimental design by OMD for formulation of nachos

Run	A (g)	B (g)	C (g)	D (ml)	E (g)
1	0.20	5.85	2.95	0.50	0.50
2	0.59	5.93	2.38	0.78	0.31
3	0.57	4.74	3.84	0.75	0.10
4	0.71	4.04	4.35	0.50	0.40
5	0.20	6.00	2.80	0.90	0.10
6	0.20	4.39	4.41	0.90	0.10
7	0.22	5.12	3.16	1.00	0.50
8	0.94	3.63	3.93	1.00	0.50
9	0.71	4.04	4.35	0.50	0.40
10	0.20	4.53	3.77	1.00	0.50
11	1.00	4.43	3.47	0.82	0.28
12	0.59	5.93	2.38	0.78	0.31
13	1.00	5.70	2.17	0.63	0.50
14	1.00	6.00	2.40	0.50	0.10
15	1.00	3.20	5.00	0.69	0.11
16	0.26	3.70	4.81	0.73	0.50
17	1.00	5.26	3.05	0.50	0.19
18	0.97	6.00	1.93	1.00	0.10
19	1.00	4.43	3.47	0.82	0.28
20	0.75	4.15	4.00	1.00	0.10
21	0.20	4.11	5.00	0.50	0.19
22	0.20	5.52	3.68	0.50	0.10
23	0.57	4.74	3.84	0.75	0.10
24	0.59	5.93	2.38	0.78	0.31
25	0.33	3.44	5.00	1.00	0.23

The nachos' formulation was done using an experimental design set by the optimal mixture design (OMD) with the help of Design Expert 11 software [27]. Optimal mixture design was selected for the formulation because it gives non-negative set of input

variables or components to obtain proportionate amount of mixture. Each set of experiment have equal sum of ingredients or components for the analysis of effective mixture formulation [18].

In the formulation process of nachos, the amount of corn starch (0.2 to1 g) (coded A), wheat flour (3 to 6 g) (coded B), BB (1.5 to 5 g) (coded C), refined oil (0.5 to 1 ml) (coded D) and black pepper (0.1 to 0.5 g) (coded E) were taken as independent factors whereas, salt, water, ginger garlic paste, baking temperature and time were taken as fixed independent factors (Table 3.1). The experimental design was introduced by using quadratic model in OMD. A total of 25 experimental runs were generated and further optimized by artificial neural networking (ANN) and particle swarm optimization (PSO).

3.2.6 Process of making nachos

Nachos were prepared according to the 25 experimental runs obtained from OMD (Fig. 3.1) [17]. The dough was prepared by adding D (0.5 to 1 ml) and drinking water (10 ± 0.6 ml) in a mixture of A (0.2 to1 g), B (3 to 6 g), C (1.5 to 5 g), E (0.1 to 0.5 g), salt (0.2 g) and ginger garlic paste (0.1 g). The prepared dough was flattened into a sheet (0.25 cm thickness) separately and then cut into smaller triangular pieces (1.5 x 1.5 x 1.5 cm). Cut dough sheets were placed over a baking tray containing butter paper. A slight amount of refined oil was sprinkled above the cut pieces before baking. Trays containing cut dough pieces were put in the pre-heated deck oven (Sinmag electric oven, SM-502, China) and allowed to bake for 15 min at 180^{o}C. After the completion of baking, nachos were allowed to cool down and stored in airtight containers separately.

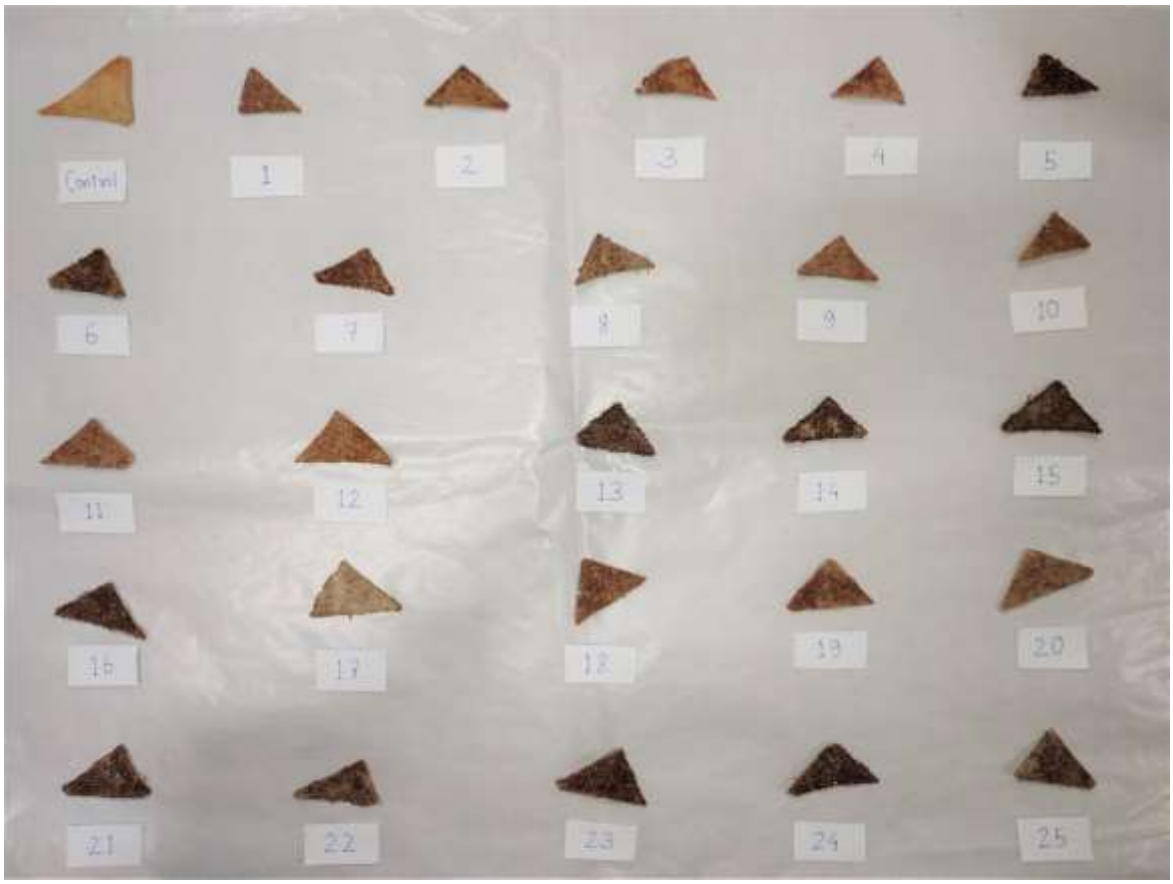

Fig. 3.1 Formulated nachos prepared from bhimkol blossom

3.2.7 Sensory analysis of nachos

Sensory analysis was carried out [29] based on the five parameters *viz*., taste, flavor, mouth feel, texture, and overall acceptability (OA) in the hedonic scale from 1 to 9, i.e., dislike extremely to like extremely. Thirty-five different panelists were taken for the sensory analysis to assume customer demand in the market. Average OA was taken for formulation and optimization of the development of nachos. At each run, the browning index was determined from the L*, a*, and b* values obtained from the Hunter colorimeter (HunterLab, UltraScan VIS, Virginia).

3.2.8 Texture analysis

The texture analysis [20] was conducted by the texture analyzer (Stable Micro System, TA-HD plus, UK), where the fracturability and hardness (N) were noted down by a P/0.25S ball probe. The analysis was performed at a pre-test speed of 1 mm/s, test speed of 1 mm/s, and distance of 10 mm. The hardness of the nachos was the maximum force

required to break the nachos. The numbers of positive peaks obtained during puncture were used to express the fracturability of the nachos.

3.2.9 ANN model

In this study, an artificial neural toolbox was used in a mathematical software (MATLAB-V9.2, R2017a) to predict the best OMD-formulation nachos from BB [21]. ANN can solve a non-linear and complicated set of problems by using computing systems through biological neural structures. It runs the operation by summing junction and transfer function with the help of connections; weights (*w*), neurons, and biases (*b*).

The summing junction of an operator of a neuron works on *w* and *b* to net input argument, which is to be processed. The hyperbolic tangent transfer function (Equation 3) was used to solve the non-linear problem, which receives the summing function and produces a scalar output of a neuron.

$$f(x) = \frac{e^{x}-e^{-x}}{e^{x}+e^{-x}} - 1 \leq f(x) \leq 1 \quad (3)$$

The input and output layer of a neuron is connected in topology, and each neuron in a neural network is structured in many layers involving several neurons. A feed-forward back-propagation algorithm was used by trainlm and trained the network according to Levenberg-Marquardt. The input layer comprised of five numbers of neurons *viz*., A, B, C, D, and E whereas the output layer was OA. Out of 25 runs, 70% was taken for data set training, 15% for the validation set, and the rest 15% for the testing set. An iterative optimization was given to train the ANN using the BP algorithm to obtain minimized performance functions by adjusting the weights. Some performance functions *viz*., root mean square error (RMSE), mean square error (MSE), coefficient of regression (R^2), mean absolute error (MAE), and mean absolute percentage error (MAPE) were introduced as shown in the following Equations 4, 5, 6 and 7.

$$\text{MAE} = \frac{\sum_{t=1}^{n}|A_t - F_t|}{n} \quad (4)$$

$$\text{MAPE} = \frac{\sum_{t=1}^{n}\left|\frac{A_t - F_t}{A_t}\right|}{n} \times 100 \quad (5)$$

$$\text{RMSE} = \sqrt{\frac{\sum_{t=1}^{n} (A_t - F_t)^2}{n}} \quad 6$$

$$R^2 = 1 - \frac{\sum(A_t - F_t)^2}{\sum(A_t - \bar{F}_t)^2} \quad 7$$

Where, n=25, A_t is experimental value, $F_t$is predicted value

3.2.10 Particle swarm optimization

Particle swarm optimization (PSO) is a metaheuristic approach known as swarm intelligence. The technique is used to obtain the best solution to complicated problems by using swarm intelligence inspired by natural bird flocking and schooling [8]. PSO was used to get an optimum set of inputs to obtain the best output (OA). After the model was developed in ANN, the optimization was initiated through the PSO algorithm. The position (x_i^j) and velocity (v_i^j) of each particle will be altered by the particles best value (x_i^{j*}) and global best value (x_i^j) [21]. Swarm intelligence used the following given Equations 8 and 9 to best solutions for the problem in an efficient way.

$$v_i^{j+1} = wv_i^j + \Phi_1 r_1\left(x_i^{j*} - x_i^j\right) + \Phi_2 r_2\left(x^j - x_i^j\right) \quad 8$$

$$x_i^{j+1} = x_i^j + v_i^{j+1} \quad 9$$

Where, the v_i^j, v_i^{j+1} are the velocities of particles *i* at the *j* and *j+1* iterations. Similarly, x_i^j, x_i^{j+1} are the sites of particle *i* at the *j* and *j+1* iterations. Φ_1 and $\Phi_2$are the cognition and social learning factors. Best solution obtained by the particle *i* until iteration *j* is denoted as x_i^{j*} and x_i^j is the best sites or position obtained from x_i^{j*} out of whole swarm population *i* at *j* iteration. Lastly, *w* is the weight and r_1 and $r_2$are the random numbers between 0 and 1 that are distributed in the algorithm.

3.2.11 Principal component analysis

Principal component analysis (PCA) was conducted in SPSS 11 [19]. A sensory evaluation was conducted for the nachos produced at an optimum set of input layers (obtained from ANN-PSO), control, and commercial nachos based on a hedonic scale.

The PCA was introduced to summarize the variations in datasets and reduced the variations between the sensory evaluation of nachos produced at an optimum set of input (obtained from ANN-PSO), control, and commercial nachos. The eigenvalue was set up to 1 in the extraction process of the dataset and the component plot at rotated space was analyzed with the varimax concept.

3.3 Results and discussions

3.3.1 Nutritional and phytocomponents

3.3.1.1 Proximate and phytocomponents

Many varieties of banana blossoms have received interestingly increasing attention for their potential medicinal role. High nutritional content in BB is uplifting its demand in various cuisines throughout the world. A significant amount of minerals (ash content 18.94±0.10% db) and high moisture content (92.5±0.50% wb) were found in BB. The content of minerals plays a major role in keeping our body in a healthy state. Abundant moisture content in the blossom holds many bioactive compounds. The crude protein, crude fat and carbohydrate content were found at 1.83±0.04%, 6.83±0.28%, and 66.87±0.20%, respectively and are in line with the results reported by Begum and Deka [9]. The crude fiber content in the blossom was estimated (12.12±0.28%) and revealed good amount, wherein soluble dietary fiber was 18.28 ± 0.16% and insoluble dietary fiber was 50.32±0.04%. Fibers in both soluble and insoluble forms are necessary for healthy bowel movement. Bound phytochemicals in fibers possess antioxidant activity throughout the gastrointestinal (GI) tract and potentially treat the GI inflammations and irritations [9]. Some other phytocomponents *viz*., reducing sugar, vitamin C, oxalic acid, saponin, solanine and cyanogic glycoside in BB were 0.41±0.01%, 6.24±0.31, 550±0.22, 5300±0.81, 1.29±0.00, and 1±0.00 mg/100g, respectively. Vitamin C contributes as a great antioxidant source and helps in preventing ulcers, inflammations etc. Saponin, mostly considered an alkaloid has various medicinal effects such as hypocholesterolemic, antioxidant, anticarcinogenic, anticoagulant, hypoglycemic, immunomodulatory, neuroprotective along with antimicrobial activity and also responsible for the bitterness of the blossom [11].

3.3.1.2 Vitamins

Some fat-soluble and water-soluble vitamins were assayed by HPLC are presented in Table 3.2 and Fig. 3.2. Fat-soluble vitamins: vitamin E and vitamin A were detected at concentrations of 8.73 and 0.32 mg/g, respectively.

Table 3.2 Fat and water-soluble vitamins in bhimkol blossom

Vitamins		RT[a] (min)	Bhimkol blossom (mg/g)
Fat-soluble	Vitamin D	2.60	ND[b]
	Vitamin E	2.68	8.73
	Vitamin K	6.85	ND[b]
	Vitamin A	3.30	0.32
Water-soluble	Thiamine	7.00	0.09
	Riboflavin	14.43	ND[b]
	Nicotinamide	5.87	ND[b]
	Pyridoxine	7.19	0.21

[a]RT; Retention time, [b]ND; Not detected

Water-soluble: thiamine and pyridoxine were detected at concentrations of 0.09 and 0.21 mg/g, respectively. Vitamin E is a strong antioxidant that has various uses in the pharmacological and food sectors for its numerous health beneficial effects such as gene regulator, immunomodulatory, antiallergic, anticarcinogenic, neuroprotective [12]. Vitamin A can prevent vision problems, cancer, and cell damage and have the ability to support healthy skin. Thiamine and pyridoxine have the property to prevent beriberi, and neurological manifestation dysfunction of the cardiovascular system [10]. Pyridoxine is essential for the maintenance of body cells and helps in the metabolism of amino acids, makes hemoglobin, transmits signals to the brain, normalizes the nerve system, creates antibodies and protects from illness etc. [22].

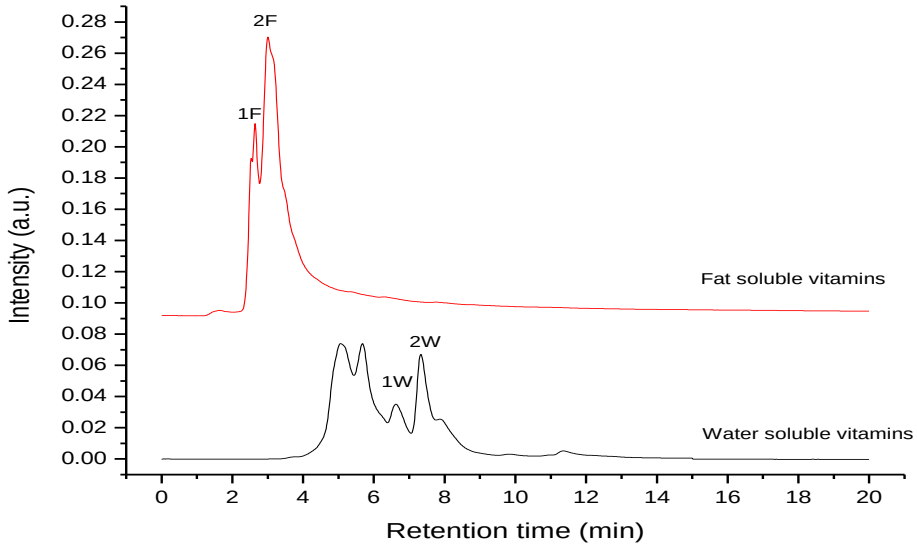

Fig. 3.2 HPLC chromatograms of vitamins in bhimkol blossom: vitamin E (1F), vitamin A (2F), thiamine (1W), and pyridoxine (2W)

3.3.1.3 GC-MS analysis of volatile phytochemicals and fatty acids

In the investigation of volatile phytochemicals in the BB extract, some volatile compounds *viz*., methoxyacetic acid, 2-tridecyl ester, ethanol 2-(octadecyloxy), octadecane, 3-ethyl-5-(2-ethylbutyl) and 1-Hexadecanol 2-methyl were seen in the maximum peak area. Identified volatile compounds in GC-MS (Table 3.3 and Fig. 2.3a) might be the volatile secondary metabolites that exhibit aromatic attributes of the blossom. Methoxyacetic acid and 2-tridecyl ester, 1-Hexadecanol 2-methyl- and 1-Hexadecanol 2-methyl- [3] are antimicrobial metabolites exhibits antioxidant activity and strong anti-inflammatory property [14]. Octadecane, 3-ethyl-5-(2-ethylbutyl) is a bacterial metabolite that tends to act as a defense mechanism along with antioxidant and possess anti-inflammatory effect [3].

Table 3.3 Volatile phytochemicals profiling in bhimkol blossom

Sl. No	RT (min)	Name of the compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area %
1.	4.96	l-Gala-l-ido-octose	$C_8H_{16}O_8$	240	0.06
2.	5.64	d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$	255	0.41
3.	9.98	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	0.17
4.	10.85	2H-Oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-, [4S-(4R*,5E,10S*)]-	$C_{10}H_{16}O_3$	184	0.30
5.	11.39	4a,8a-Butano[1,4]dioxino[2,3-b]-1,4-dioxin, tetrahydro-	$C_{10}H_{16}O_4$	200	0.59
6.	14.42	Thiazole, 2-amino-4-(p-aminophenyl)-	$C_9H_9N_3S$	191	0.70
7.	15.23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.40
8.	18.04	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	0.06
9.	19.82	Oleic Acid	$C_{18}H_{34}O_2$	282	3.94
10.	22.33	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	3.53
11.	22.40	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	5.34
12.	22.78	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	5.29
13.	22.93	Methyl 10,12-pentacosadiynoate	$C_{26}H_{44}O_2$	388	1.48
14.	25.18	Methoxyacetic acid, 2-tridecyl ester	$C_{16}H_{32}O_3$	272	21.59
15.	25.36	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	10.15
16.	26.47	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	400	0.90
17.	27.96	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	2.26
18.	30.99	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314	23.42
19.	33.40	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312	0.64
20.	37.00	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	15.76

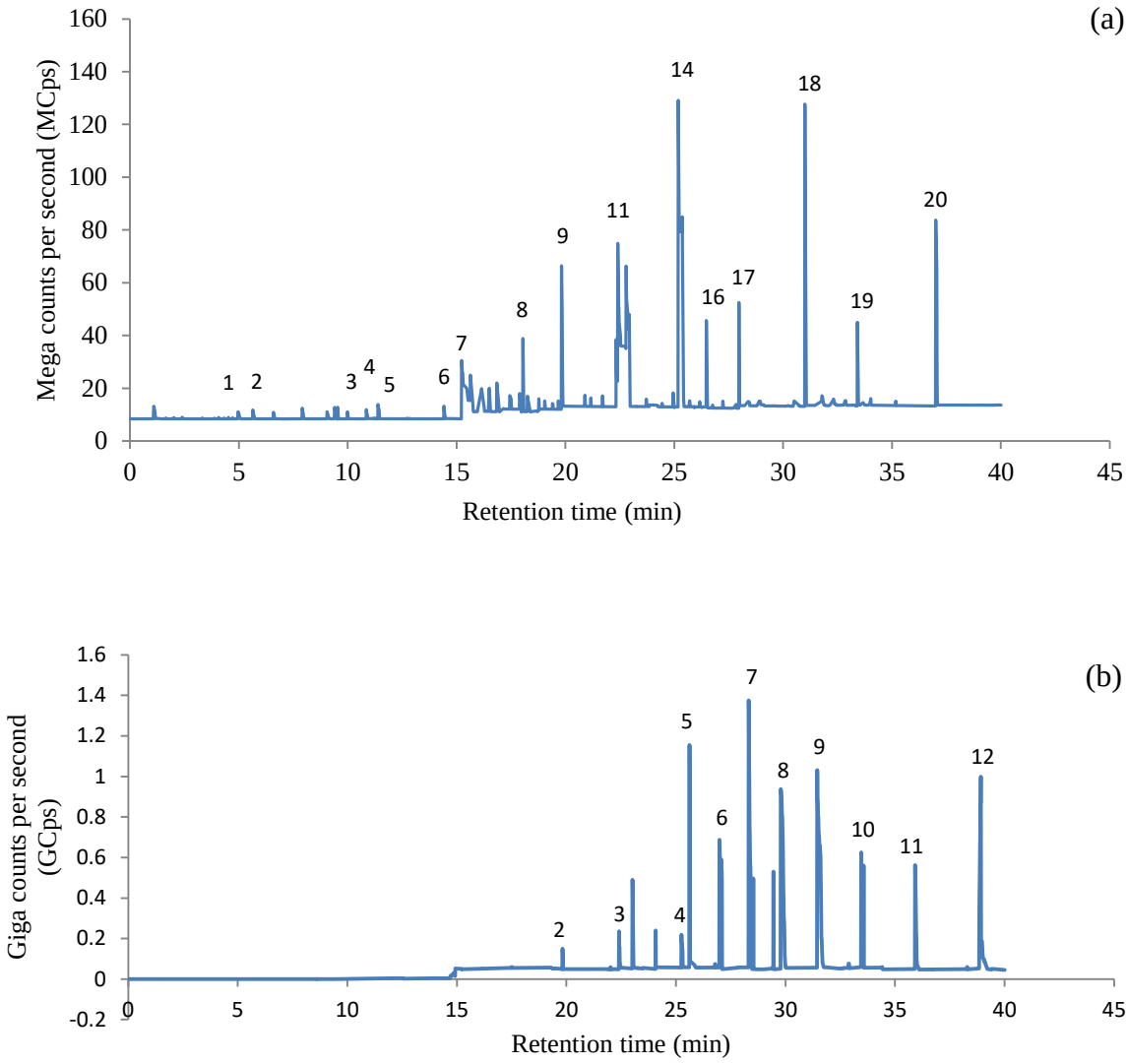

Fig. 3.3 GC chromatographs of volatile phytochemicals and fatty acids profiling; (a) volatile phytochemicals profiling in bhimkol blossom: (1) l-Gala-l-ido-octose; (2) d-Gala-l-ido-octonic amide; (3) β-D-Glucopyranose, 4-O-β-D-galactopyranosyl-; (4) 2H-Oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-, [4S-(4R*,5E,10S*)]-; (5) 4a,8a-Butano[1,4]dioxino[2,3-b]-1,4-dioxin, tetrahydro-; (6) Thiazole, 2-amino-4-(p-aminophenyl)-; (7) n-Hexadecanoic acid; (8) 17-Octadecynoic acid; (9) Oleic Acid; (11) Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate; (14) Methoxyacetic acid, 2-tridecyl ester; (16) Cholestan-3-ol, 2-methylene-, (3β,5α)-; (17) 12-Methyl-E,E-2,13-octadecadien-1-ol; (18) Ethanol, 2-(octadecyloxy)-; (19) Ethanol, 2-(9-octadecenyloxy)-, (Z)-; (20) Octadecane, 3-ethyl-5-(2-ethylbutyl)- (b) fatty acids profiling in bhimkol blossom: (2) Oleic Acid, 9,12-Octadecadienoic acid (Z,Z)-;(3) 9,12-Octadecadienoic acid (Z,Z)-; (4) cis-13-Eicosenoic acid; (5) 7-Methyl-Z-tetradecen-1-ol acetate; (6) 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; (7) 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-; (8) Linoleic acid ethyl ester; (9) Z-8-Methyl-9-tetradecenoic acid; (10) Erucic acid; (11) 9-Octadecenoic acid (Z)-, phenylmethyl ester and (12) 10-Undecenoic acid, octyl ester

Some of the fatty acids were detected in GC-MS analysis and listed in Table 3.4. Fatty acids *viz*., 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-, Z-8-Methyl-9-tetradecenoic acid, 7-Methyl-Z-tetradecen-1-ol acetate, Linoleic acid ethyl ester, Erucic acid and 9-Octadecenoic acid (Z)- were detected in majority peak area in GC chromatogram (Fig. 3.3b). A total of 66.67% of monounsaturated fatty acid (MUFA) and 33.33% of polyunsaturated fatty acid (PUFA) were found among twelve fatty acid detections.

Table 3.4 GC-MS fatty acids profiling in bhimkol blossom

Sl. No	Fatty acid	RT (min)	Name of the compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area %
1.	MUFA	15.22	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	0.30
2.	MUFA	19.82	Oleic Acid	$C_{18}H_{34}O_2$	282	1.26
3.	PUFA	22.40	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	4.11
4.	MUFA	25.25	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310	6.34
5.	MUFA	25.61	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	12.65
6.	PUFA	26.98	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	7.22
7.	PUFA	28.32	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	17.57
8.	PUFA	29.78	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	11.37
9.	MUFA	31.45	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240	14.10
10.	MUFA	33.45	Erucic acid	$C_{22}H_{42}O_2$	338	10.03
11.	MUFA	35.91	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372	9.49
12.	MUFA	38.95	10-Undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296	5.58

Moreover, MUFA and PUFA were reported for their inversely effect on coronary heart disease and have the tendency to lower total plasma cholesterol concentration. Some of the fatty acids like linoleic acid, oleic acid and fatty acids ethyl esters were reported for their strong antimicrobial activity against oral pathogens [15]. The presence of the double bond in MUFA and PUFA makes them different from any other fatty acids and is responsible for its various additional health beneficial properties such as protective mechanism in growth and development, antiallergic, anticancer, antidiabetic and has potential to treat Alzheimer's disease [30].

3.3.2 Antioxidant activities

Three different parts of the blossom (bract, male flower, and spadics) and whole blossom showed high antiradical scavenging activities (Fig. 3.4a). The results of the antiradical scavenging activities of blossom and its various parts by DPPH, ABTS, and FRAP assays revealed that bract showed the highest antioxidant activities. The antioxidant activities by standard trolox were 85.96±0.13, 78.00±1.63, and 81.89±0.46 % for DPPH, ABTS, and FRAP, respectively whereas the antioxidant activities by bract were 74.14±0.55, 64.67±0.47 and 75.21±0.91% for DPPH, ABTS, and FRAP, respectively. The high antioxidant property of plant extract depicts the potentiality of being health beneficial attributes that may treat cell damage, repair inflammation, can minimize the risk of some diseases such as diabetes, cancer, tumor, hyper-cholesterol [28].

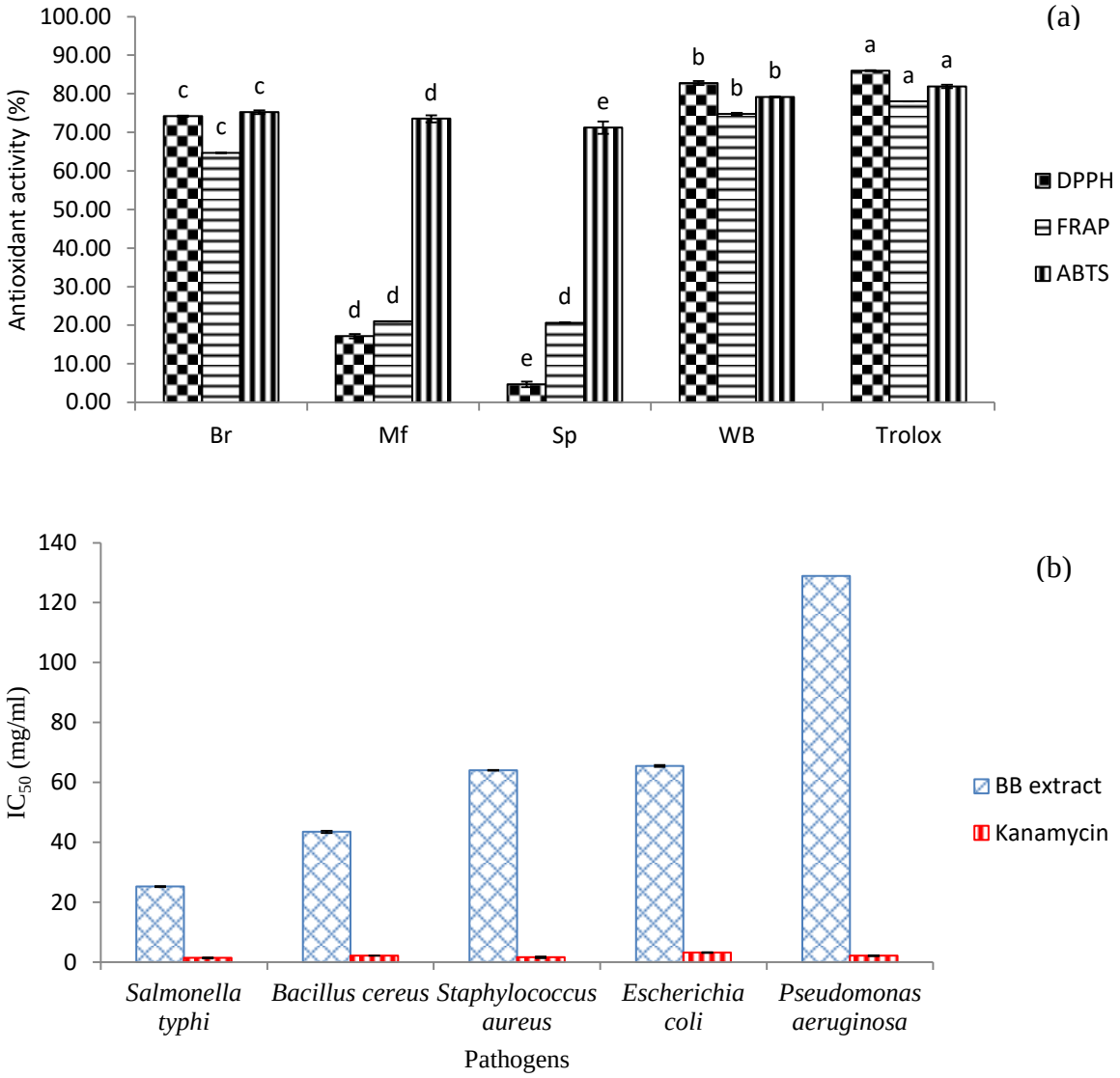

Fig. 3.4 Antioxidant activities and antibacterial activity of bhimkol blossom extract; (a) Antioxidant activities by various parts of bhimkol blossom: Br; bract, Mf; male flower, Sp; spadics WB; whole blossom. Error bar with different letters are significant different values (p<0.05), and (b) Antibacterial activity of bhimkol blossom extract and kanamycin

3.3.3 Antibacterial activities

The antibacterial activity was expressed in the IC_{50} based on the zone of inhibitions of BB extract and kanamycins against five foodborne pathogens (Fig. 3.4b). The maximum zone of inhibition over *S. typhi* was seen at 25.21±0.14 (mg/ml) as compared to other pathogens. Growth inhibitions by BB extract of *B. cereus*, *S. aureus*, *E. coli*, and *P.*

aeruginosa are presented. Inhibition areas were seen to be different over different pathogens, as every microorganism has a different level of susceptibility to different medium and environments. Similar result was reported by Gonelimali et al. [13] where with the increase in the concentration of plant extract, the antibacterial activity increased and found varied levels of inhibitions over different pathogens. Kanamycin, which was taken as the standard antibiotic in the study, evinced the maximum zone of inhibitions with 1.42±0.15 mg/ml IC_{50} against *S. typhi*, and the least inhibition effect was seen against *E. coli* with 3.20±0.09 mg/ml IC_{50} value and our results are corroborated by Ramu et al. [33] where the banana blossom variety *Musa* sp. var. Nanjangud rasa bale ethanol extract at 2.5 mg/ml was tested for its antimicrobial properties against *S. aureus*, *B. cereus*, and *E. coli*, and the area of inhibition shown by them was 15.24±0.28, 10.84±0.19, and 17.99±0.19 mm, respectively. Variation of inhibitions may be due to different combinations and constituents of bioactive molecules present in each variety of banana blossom.

3.3.4 Sensory analysis of Nachos

The formulation of nachos by optimal mixture design in Design Expert 11 was done and the average thicknesses of nachos were found at around 0.11±0.05 cm. The sensory analyses of 25 runs of nachos are presented in Fig. 3.5a. The browning index was found to be higher with an increasing amount of wheat flour (B) (in Fig. 3.5b). Moreover, in bakery products browning occurs when a protein and sugar is heated above around 120^{o}C which is also known as Maillard reaction. Maillard reaction up to a certain limit develops pleasant flavor and aroma, but after certain limit it may give off flavor and may lead to low quality sensory property. Therefore, quantity and duration of application of heat to the product majorly affects the browning index and sensory properties of product [32]. The fracturability was observed a bit higher in an increased amount of refined oil (D) and wheat flour (B) at a lower level of BB (C) ratio. Hardness increased with the lower content of water and fat. The acceptable hardness and crispiness depend on the product to product. The desirable textural attribute obtained might be due to the ratio of the input parameters producing the desired hardness and crispiness of nachos. Adequate amount of oil and water content before baking leads to rapid evaporation of moisture from the dough sheet and results in a more porous structure to crispiness [23].

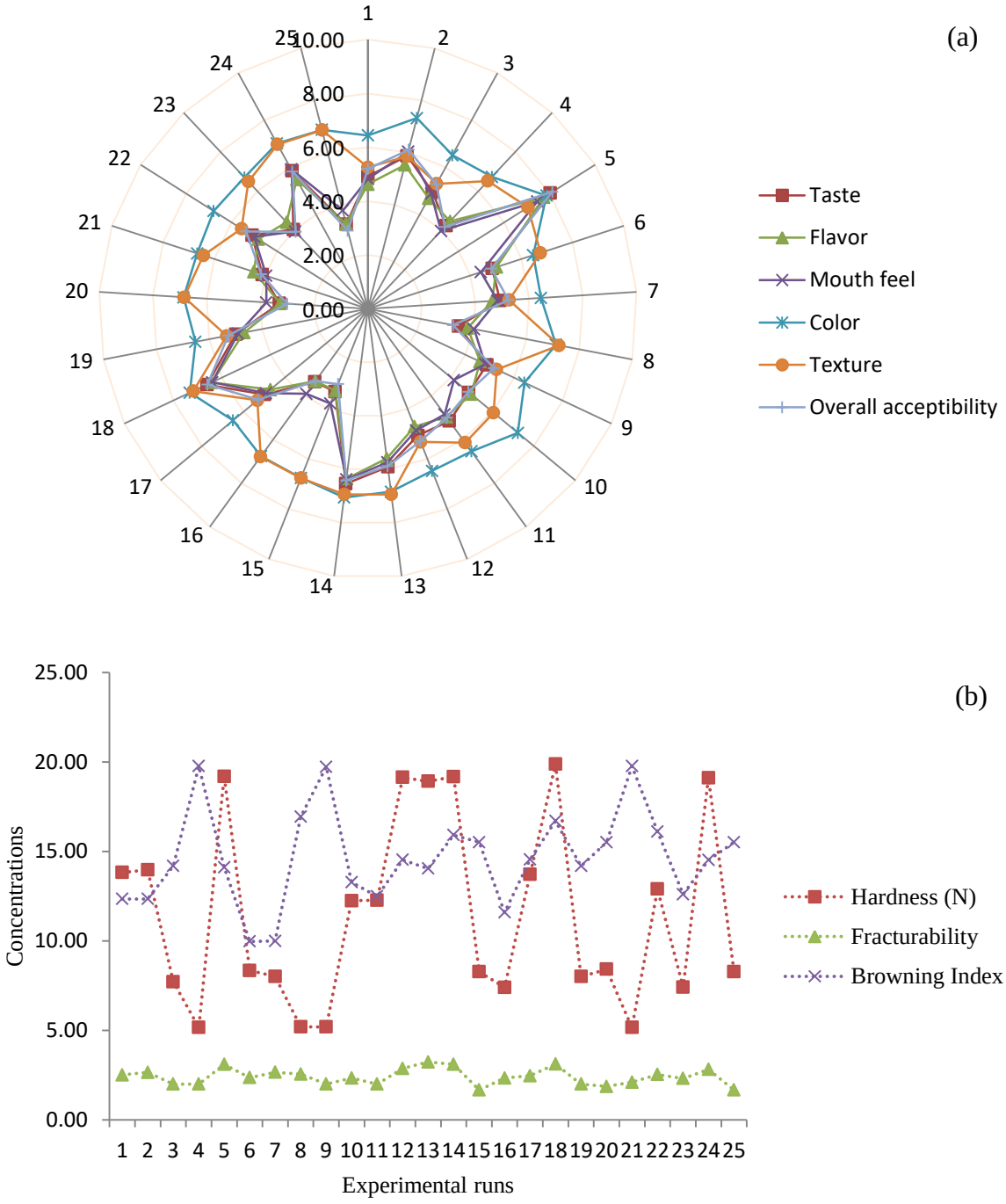

Fig. 3.5 Radar chart and graph of sensory evaluation for 25 experimental run nachos; (a) sensory evaluation and (b) sensory attributes

3.3.5 Optimization by ANN-PSO

The performance of ANN training was influenced by the training given to the experimental runs (Fig. 3.6). Experiment datasets were put randomly in the workspace for each subset. The coded level for input and output data ranged from 0 to 1 (0 at minimum and 1 at maximum). Input and output data were normalized between 0 to 1 and scaled network input, output was pondered to evade over-fitting of the training process. A regression analysis was performed for ANN outputs and the experimental datasets were 0.85, 0.78, 0.96, and 0.80 for training, validation, testing, and all data, correspondingly. The performance functions *viz*., RMSE =1.13, MSE =1.28, R^2 =0.96, MAE =0.12, MAPE =0.19 were recorded. The best validation performance was 0.306 at epoch 6. The fitness value was noted at 0.081. The training was controlled once the minimal MSE percentage was obtained. Minimum RMSE represents the minimal variance between the experimental and predicted set of output data and lesser error in the experiment [21].

Effective optimization of any process is mandatory to develop and formulate any product to obtain the best quality, cost-effective, high consumer acceptability, identification, and up-gradation of the problems, to reduce market loss and demand. In optimization of OA by ANN coupled with PSO, the optimum set of parameters obtained were the amount of A = 0.13 g, B = 0.39 g, C = 0.33 g, D = 0.66 g, and E = 0.26 g. The output (OA) of the nachos obtained at an optimum set of the parameter from ANN-PSO was 8.9. Inertia weight obtained was 7 at a maximum number of iterations for optimization which was set up to 40 iterations.

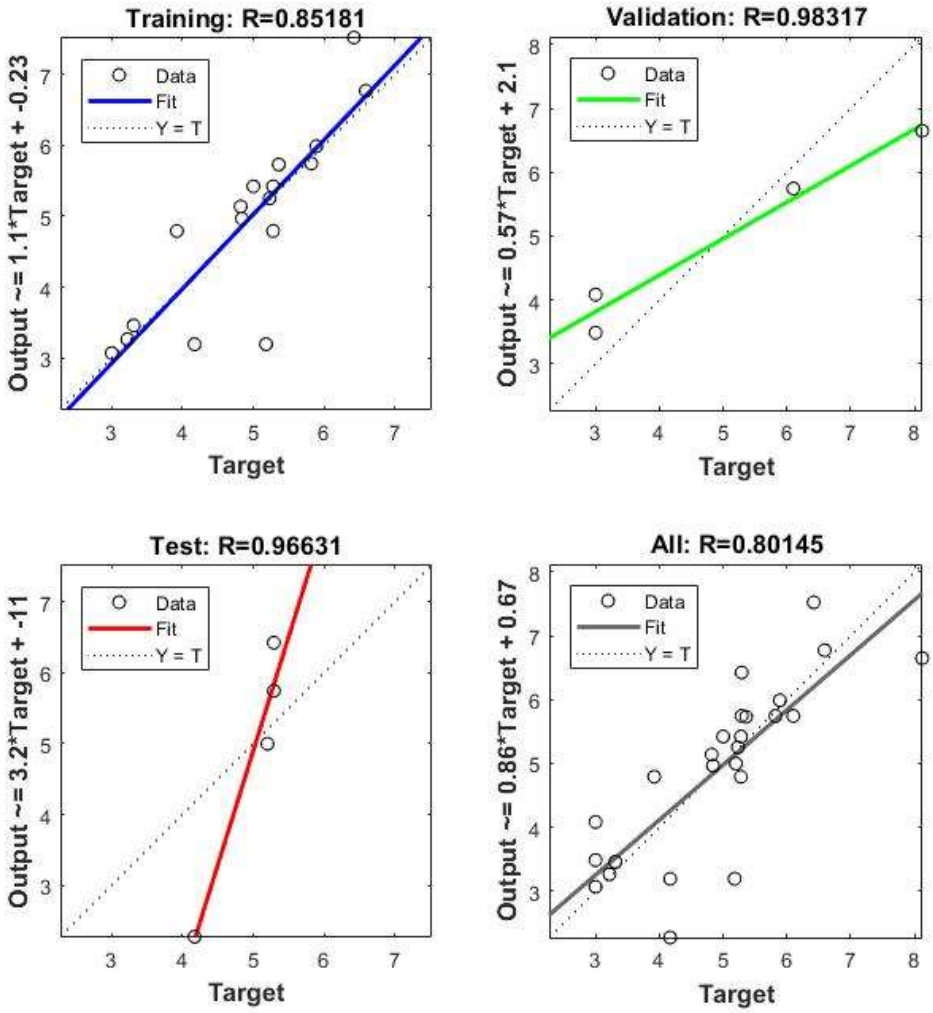

Fig. 3.6 Performance of ANN-PSO for training, validation, dataset and all data

In the output layer (OA), the sensory scale of the nachos was hugely affected by the input layer B used. By the increasing amount of B and D, the increased value of the output layer (OA) was recorded. The reason behind the increased OA might be due to the better consumer acceptability recorded as sensory analysis based on taste, texture, aroma, mouthfeel, and color. The OA was also decidedly affected as the C was higher, which might be due to the bitterness and more fibrous texture of the BBP. The obtained optimized parameter was the best imparting high consumer acceptability with 8.9 OA.

3.3.6 Principal component analysis of nachos

In the sensory analysis of commercial, control, and BB nachos, commercial nachos had 100% of 8 and above hedonic ratings in each sensory parameter. Control nachos had 91.42 and 68.57% of 8 and above rating for texture and color, respectively. On the other hand, BB nachos had 100% of 8 and above rating for texture and color with 8.43±0.39 rating of OA. The principal component analysis (PCA) of sensory properties showed the inter-relationship between the variables and detected similarities and differences (Fig. 3.7). In the component plot in rotated space, the first principal component depicts the variations in the data as much as possible. Then the second principal component defines the remaining variations orthogonally to the first. For the control and BB nachos, variations were identified in three principal components, as shown in Figures 5 b and c. The plot of a variable towards positive axes of components 1, 2, and 3 showed the positive correlation of variables.

In the PCA of commercial nachos, Fig. 3.7a OA, mouthfeel, and flavor weighted highest in component 1 and taste weighted more in component 2 with 0.555, 0.701, 0.720, and 0.879 eigenvalues, respectively. In Fig. 3.7b, taste and flavor weighted more towards component 1, whereas OA, mouthfeel weighted towards component 2, and texture weighted towards component 3 at eigenvalues 0.816, 0.693, 0.776, 0.768, and 0.821, respectively. In Fig. 3.7c, OA, flavor, and taste are weighted towards component 1, whereas mouthfeel was weighted towards component 2, and then texture towards component 3 at eigenvalues 0.690, 0.785, 0.607, 0.861, and 0.862, respectively.

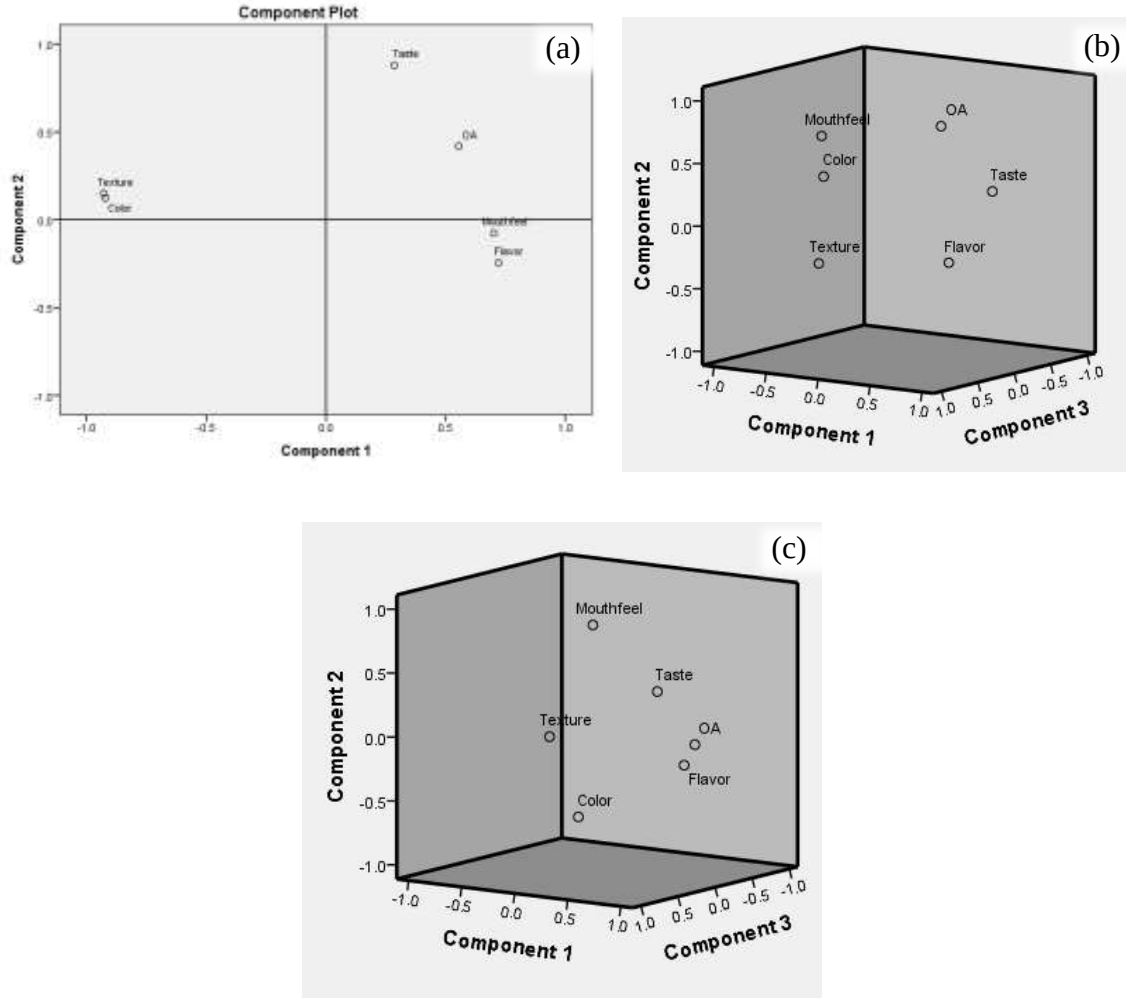

Fig. 3.7 Product component analysis (PCA) of sensory evaluation of nachos: (a) commercial nachos (b) control nachos (c) bhimkol blossom nachos

3.3.7Antioxidant activities of nachos

Antioxidant activities of nachos prepared from *Musa balbisiana* blossom, control and commercial nachos were found at 7.60±0.21, 1.19±0.09, and 2.17±0.22% (DPPH inhibition activity), respectively. Nachos prepared from bhimkol blossom showed higher antioxidant property than control nachos and commercial nachos, which might be because of its phytochemical constituents.

3.4 Conclusion

Bhimkol blossom displayed a good amount of phytochemicals and nutritional compounds, which have the potential to supply day-to-day human healthy nutrition diet.

Good antioxidant and antibacterial properties were observed that might contribute to various health-beneficial properties. Nachos prepared from BB with good overall acceptability from the sensory evaluation were obtained after optimizing the formulation of processing parameters using an effective metaheuristic approach (OMD-ANN-PSO). After the effective training of the network, optimized BB nachos were obtained from ANN-PSO with less RMSE, MAPE, and MAE. In contrast, a satisfactory reading of sensory quality was obtained for BB nachos to control and commercial nachos. The nutrition-rich nachos have immense potential for commercialization on a small to large profitable industrial scale.

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