Chapter 6

To develop phytochemical enriched food product by incorporation of encapsulated phytochemical compounds and its properties

6.1 Introduction

Value addition is a term where the original value of a product is maximized by employing additional value on it [11]. In general, agricultural wastes can be converted into something very useful (*viz.,* fertilizers, extraction of bioactive compounds, production of food products, etc.) by adopting some processing steps [9]. In food models, functional properties can be enriched by the incorporation of some bioactive compounds. Many phytochemicals having numerous functional properties and health beneficial properties have been incorporated which increased the specialty of an original food model [1]. In food processing, there are several processing steps which include, cutting, washing, heat treatment, shock treatments, etc. where, the nutritional and phytochemical loss is normal, due to which, a product losses its original value [7]. Phytochemicals and bioactive compounds can be directly incorporated into any food model, but they should be administered under a proper investigation of their possible toxicity [4, 5]. Some bioactive compounds like antinutrients and alkaloids may possess both medicinal and toxic behavior at different levels of dose. Therefore, every food should be consumed within a limit and medicinal compounds should be regulated for their strict daily dose limit [2]. Functional ingredients are incorporated by adopting many techniques according to their aim of the incorporation and target of bioactivity [4]. For many drug delivery and target delivery, the bioactive compounds are delivered in a protective medium. Mostly, bioactive compounds are encapsulated and complexed with some coating hydrocolloids with regulated pH and temperature tolerance levels to deliver to the target specified effectively. Bioactivity in intestinal linings can be obtained by encapsulation of bioactive compounds with low pH resistance to survive stomach digestion [17]. Incorporation is meaningless if there is no bioactivity in the target location [3]. Bhimkol blossom contains numerous medicinal phytochemicals which are mostly consumed to treat hypertension and diabetes in a few worldwide regions [10] but moreover, they lose most bioactivities during processing and cooking. This study includes the incorporation of microencapsules containing isolated quercetin rich fraction in ready to cook soup mix prepared from bhimkol blossom. The developed food product with enriched phytochemicals will provide enhanced health beneficial properties possibly by exhibiting bioactivities in the intestinal wall.

6.2 Materials and methods

6.2.1 Chemicals and reagents

Chemicals and reagents used in the present study were of high purity analytical grade and the HPLC grade analytical standards and solvents were purchased from Sigma-Aldrich (USA).

6.2.2 Sample preparation

Bhimkol blossom extract (BBE) was obtained by ultrasonication as described in previous Chapters. Isolated quercetin rich fraction (BBQ) obtained from BBE by HPLC was microencapsulated in the polyelectrolyte complex chitosan-alginate in calcium chloride, detailed in Chapter 5. Microbeads obtained by microencapsulation of BBQ (BBQM) were stored at 4^{o}C in airtight plastic vials till further analysis.

6.2.3 Cytotoxicity assessment by MTT assay

The cytotoxicity assessment of BBE by MTT assay based on cell viability percentage was performed by following Ramu et al. [16] with slight modification. The cytotoxicity assessment was conducted in the cell line THP-1 (ATCC TIB-202, HiMedia, India). The cells were treated with seven concentrations of BBE (0 to 400 µg/ml in DMSO) and lipopolysaccharide (LPS) as a positive control in 96-well microplates. Cells were treated for 24 h at 37^{o}C in an incubator (BOD incubator, Optics Tech, 5634, India). After that, absorbance was observed under a multiplate reader (GloMax, Promega, India) at 540 nm for the assessment of cell viability.

6.2.4 *In vivo* acute toxicity study

6.2.4.1 Oral administration of BBE

An acute toxicity study of bhimkol blossom extract (BBE) on Wister rats was performed according to the Organization of Economic Co-operation and Development (OECD-423) guideline [15]. *In vivo* study was conducted in compliance with an ethical committee, CPCSEA in Defence Research Laboratory, Tezpur, India with Registration No.: 1227/GO/Rbi/S/08/CPCSEA and Protocol No.: 17/IAEC/DRL/25/2/2022. Random and equal ratio of male and female Wister rats (*Rattus norvegicus*) (Fig. 6.1) weighing 130–

190 g, 6-8 weeks old were selected for the study (n=3) and kept for acclimatization for 7 days in an animal house separately. The animal house was maintained at 22-24^{o}C, 12 h daily at light/dark cycle at RH (50±3^{o}). Rats were given a free standard pellet diet and water at the libitum. Selected rats were fasted overnight and dosed with 500 mg/Kg BBE at a rate of 20 ml/Kg. All rats were free to access water and food. After 14 days of dose, visual mortality, appearance, salivation, lethargy, and any injury or illness were investigated and studied for toxicity.

Fig. 6.1 Acclimatized Wister rats (*Rattus norvegicus*)

6.2.4.2 Biochemical, histopathology, and hematological study

After 14 days of acute toxicity study of BBE dose in rats, their weights were noted down and anesthetized partially by the inhalation of petroleum ether. Blood was collected by cardiac puncture and stored in the EDTA-containing tubes for further hematological study. After sacrificing rats, body organs viz., liver, spleen, heart, kidney, brain, lungs, stomach, and pancreas were removed and stored carefully for the study of relative body organ weights (Equation 1), biochemical and histopathological study [15].

$$\text{Relative organ weight} = \frac{\text{Organ weight (g)}}{\text{Body weight of the animal on sacrifice day (g)}} \times 100 \qquad 1$$

6.2.5 Preparation of ready to cook soup mix (RTC-SM)

Bhimkol blossom, the inflorescence part was washed in tap water and then chopped into smaller pieces. Chopped parts were dehydrated in a hot air oven at 50^{o}C for 24 h. Dried parts were ground to powder by using an electric mixer grinder. The ground powder of bhimkol blossom was sieved (300 µm) (BBP) to sort out the most uneven parts. A ready to cook soup mix was prepared by the addition of 0.5 g cardamom and 0.2 g salt in 2 g of BBP and coded as BBP RTC-SM.

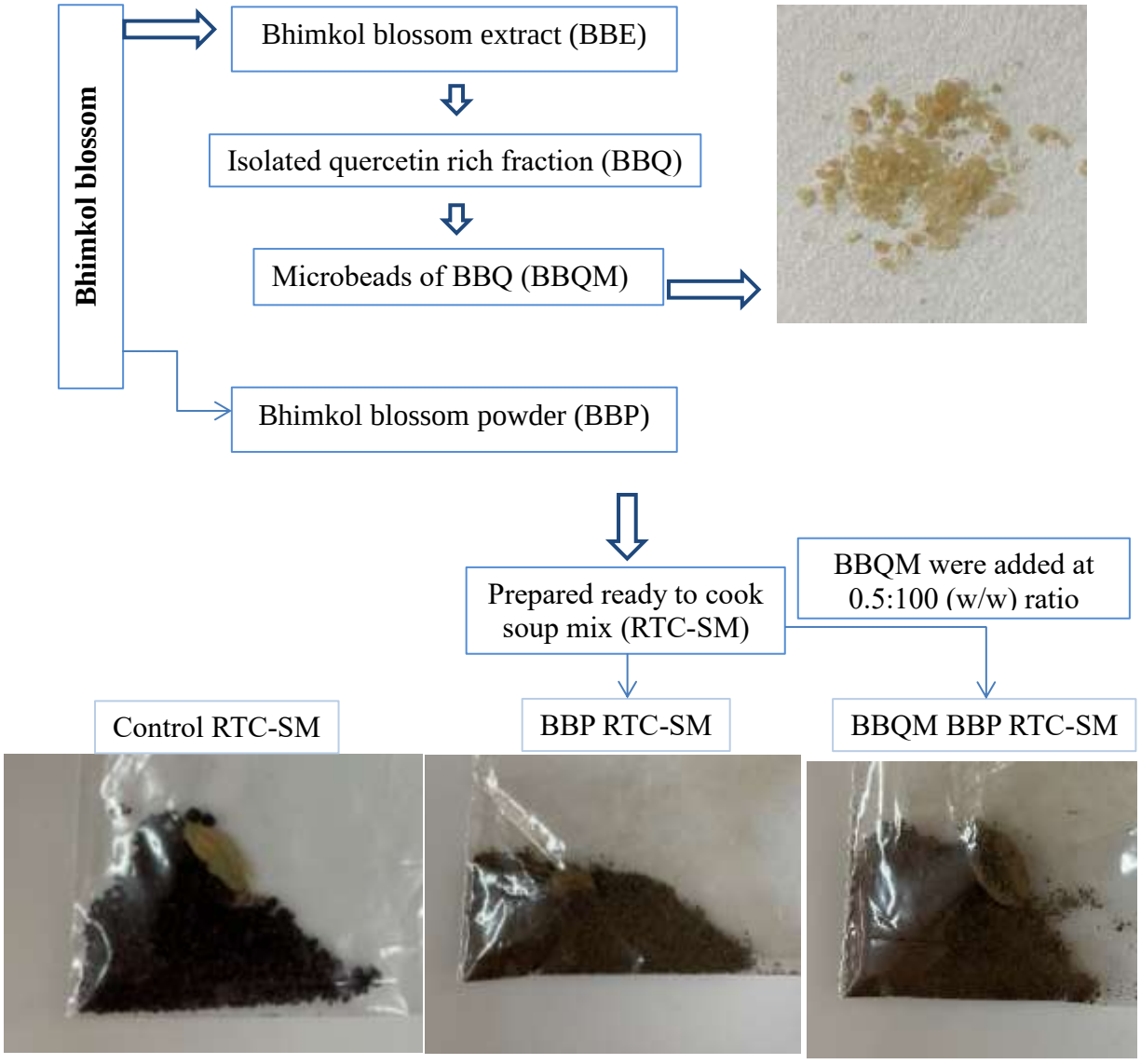

Fig. 6.2 Incorporation of BBQM in ready to cook soup mixes (RTC-SM)

6.2.6 Incorporation of microbeads containing isolated quercetin rich fraction (BBQM) in bhimkol blossom powder (BBP) RTC-SM

The microbeads, BBQM containing isolated quercetin rich fraction (10 mg) was incorporated in BBP RTC-SM (2 g) and coded as BBQM BBP RTC-SM (Fig. 6.2) by following Pasrija et al. [14]. The concentration of incorporation of BBQM was decided according to the acute toxicity result of BBE.

6.2.7 Effect of BBQM incorporation in sensory parameters of BBP RTC-SM soup

The soup was prepared by adding 1 cup of drinking water in each pack of RTC-SM and boiled at 120^{o}C for 1 min. Soup can be served directly without straining. For the comparison, similarly, a control soup was prepared from tea leaves (CTC).

6.2.8 Sensory analysis

A sensory analysis was performed on soup prepared from each RTC-SM (control, BBP, and BBQM BBP). The sensory parameters (viz., taste, flavor, mouth feel, texture, and overall acceptability (OA)) were tasted by twenty random food panelists and rated on the hedonic scale from 1 to 9 scale denoting extremely dislike to extremely like, respectively as detailed in Chapter 3.

6.2.9 Product component analysis

Principal component analysis (PCA) was conducted in SPSS 11. The sensory parameters of RTC-SM obtained in the sensory evaluation were analyzed. The PCA was introduced to summarize the variations in datasets and reduced the variations between the sensory parameters of each product. 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.

6.2.10 Determination of the antioxidant activity of RTC-SM

The antioxidant activity was estimated by DPPH inhibition assay as described in previous Chapter 2. The sample extract was prepared by ultrasonicating BBP RTC-SM (0.05 g/ml in 70% ethanol) at a frequency of 20 kHz, temperature (60^{o}C), extraction amplitude (35%), extraction time (20 min), and at 75% duty cycle (15 s pulse on and 5 s pulse off). For the sample preparation of BBQM BBP RTC-SM, a solvent mixture of

ethanol and phosphate buffer (pH 6.8) was used. Each solution was then filtered to collect supernatant and evaporated in a hot air oven at 45^{o}C for 12 h. After that, collected solid extracts were dissolved in methanol at 1 mg/ml in a test tube for the determination of DPPH scavenging activity. Blank solutions were prepared according to the solvent used for dissolving. DPPH solution of 10^{-4} M (24 mg in 100 ml methanol) was prepared and absorbance was noted at 517 nm. Then 3 ml of DPPH solution was added to 100 µl of the sample solution and absorbance was again read at 517 nm. DPPH radical scavenging activity (antioxidant activity) was calculated as the ratio of the difference between the absorbance of DPPH solution ($A_{control}$) and absorbance of sample and DPPH mixture (A_{sample}) to the absorbance of DPPH solution.

6.3 Results and discussions

6.3.1 Cytotoxicity assessment by MTT assay

Cell viability of the THP-1 cell line was decreased gradually by increasing the concentration of BBE (Fig. 6.3). Cell viability (IC_{50}) of BBE treated cell was obtained at 145 µg/ml. This indicates that cells treated with BBE below the 145 µg/ml range will exhibit better bioactivity, whereas the 145 to 250 µg/ml range may show good bioactivity. But beyond 250 µg/ml of BBE, it may exhibit a lesser positive effect. Hence, up to 250 µg/ml of BBE concentration has the high possibility to exhibit functional properties most effectively per 24 h in cells.

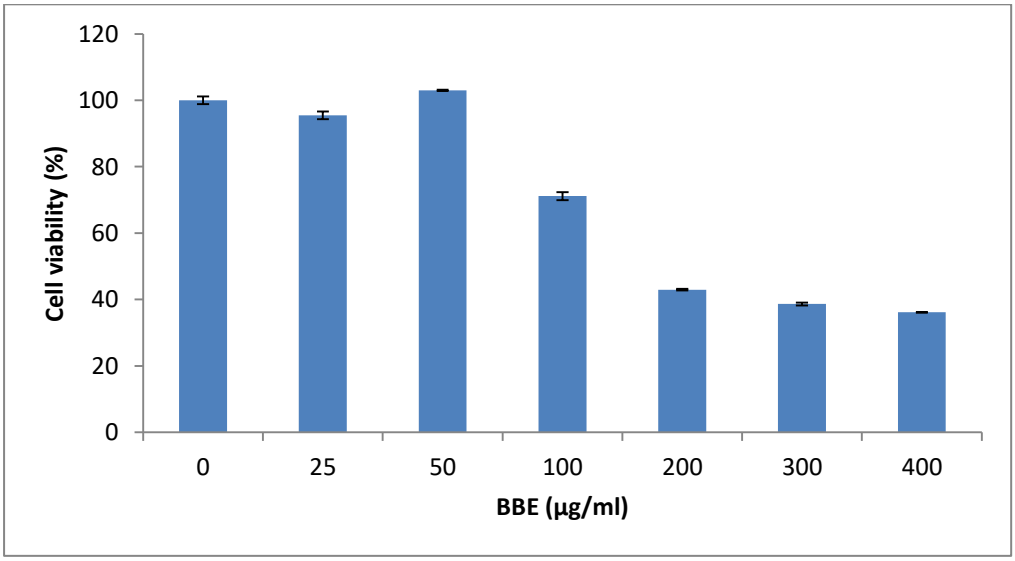

Fig. 6.3 Cell viabilities of BBE treated THP-1 cells

6.3.2 *In vivo* acute toxicity study

After 14 days of dose of BBE to Wister rats, the body weights of rats were compared with control rats (Table 6.1) and no toxicity symptoms were observed. No abnormal symptoms such as physical appearance, behaviors (salivation, lethargy, and excess sleep), any injury, and illness were observed. Neither significant weight change nor mortality was observed during the study. The relative body organ weights (Fig. 6.4), biochemical parameters (Table 6.2), and hematological parameters (Table 6.3) were found in the normal range. During the histopathology study of the liver and kidney by staining with hematoxylin and eosin, no injury, and no unusual glomerulus, and ovules were observed (Fig. 6.5) as a similar study reported by Kalita et al. [8]. In the kidney, the tubular structure, capillary tufts, mesangial cells, and Bowman's space were observed to be normal as the control. Generally in damaged or abnormality in the kidney reveals lesions, abnormal thickening, and thinning of glomerulus wall leading to leakage of fluids through the urinary tract. In the histopathology of the liver, no abnormal changes or damages were observed in hepatocytes and Kupffer cells. Central veins and sinusoids were seen without any effect as the control [13].

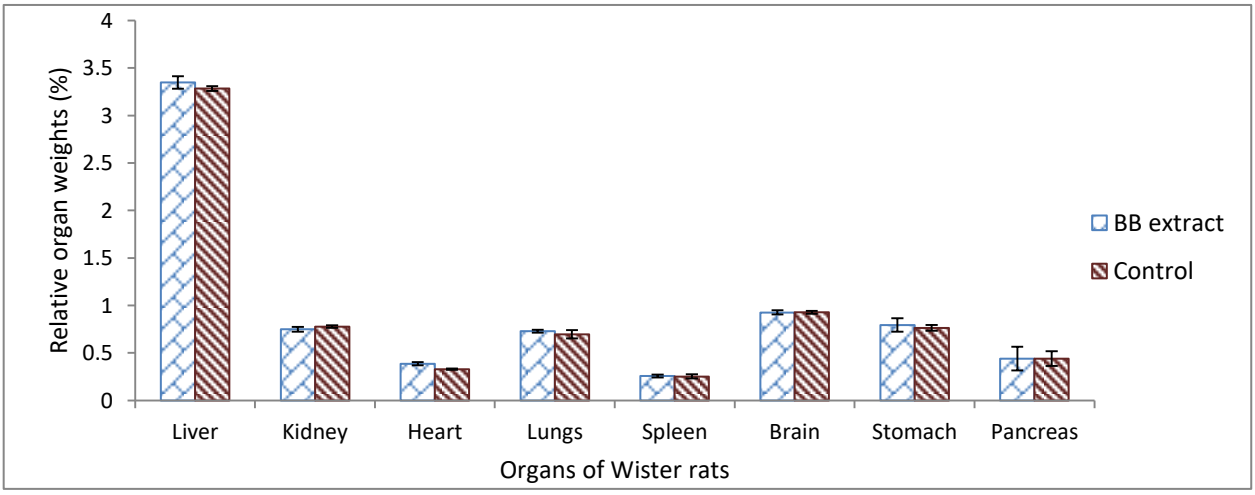

Fig. 6.4 Relative organ weights of control and rats after 14 days dose of BBE

Table 6.1 Body weight and weight of organs of control and rats after 14 days dose of BBE

Sample	Dose of BBE (mg/kg)	Body weight (after 14 days dose)	Body organs	Body organ weights (g) (after 14 days dose)
Rats dosed with BBE	500	170.16 ± 10.12	Liver	5.69 ± 0.26
			Kidney	1.28 ± 0.07
			Heart	0.66 ± 0.04
			Lungs	1.24± 0.10
			Spleen	0.44± 0.04
			Brain	1.58± 0.10
			Stomach	1.30± 0.11
			Pancreas	0.76± 0.27
Control	0	175.83 ± 5.84	Liver	5.78± 0.19
			Kidney	1.37± 0.02
			Heart	0.58± 0.02
			Lungs	1.22± 0.05
			Spleen	0.45± 0.05
			Brain	1.63± 0.03
			Stomach	1.34±0.10
			Pancreas	0.78± 0.16

Data presented as mean ± standard deviation (n=3)

Table 6.2 Biochemical parameters of body organs of rats after 14 days dose of BBE

SL No.	Parameter	Unit	BBE	Control
1	Urea	mg/dL	29.90 ± 2.29	27.83 ± 1.73
2	Creatinine	mg/dL	0.37 ± 0.05	0.47 ± 0.05
3	Total Protein	g/dL	6.10 ± 0.14	5.90 ± 0.24
4	Albumin	g/dL	3.00 ± 0.14	2.93 ± 0.12
5	Albumin/globulin (A/G) Ratio	-	0.97± 0.05	1.03 ± 0.05
6	Aspartate Aminotransferase (AST)	U/L	110.33± 6.34	137.00 ± 2.16
7	ALTV	U/L	43.67± 5.25	51.67 ± 0.94
8	ALKP	U/L	129.67± 0.47	129.00 ± 0.82
9	Total Bilirubin	mg/dL	0.10± 0.00	0.10 ± 0.00
10	Globulin	g/dL	3.07± 0.05	2.80 ± 0.08

Data presented as mean ± standard deviation (n=3)

Table 6.3 Hematological study of body organs of rats after 14 days dose of BBE

Sl. No	Parameter	Unit	BBE	Control
1	Total white blood cells (WBC's)	10^9 /L	8.27 ± 1.02	8.51 ± 0.99
2	Lymphocytes	10^9 /L	5.08 ± 1.86	5.36 ± 1.18
3	Monocyte	10^9 /L	0.27 ± 0.05	0.28 ± 0.04
4	Granulocyte	10^9 /L	2.92 ± 0.71	3.09 ± 0.25
5	Lymphocyte	%	60.15 ± 4.61	60.82 ± 2.76
6	Monocyte	%	3.32 ± 0.64	3.02 ± 0.23
7	Granulocyte	%	36.53 ± 4.13	34.05 ± 4.34
8	Total red blood cells (RBC's)	10^{12}/L	8.12 ± 0.45	7.28 ± 0.70
9	Hemoglobin	g/L	141.83 ± 8.25	138.17 ± 1.07
10	Hematocrit (HCT)	%	42.02 ± 1.87	40.83 ± 0.83
11	Mean Corpuscular volume (MCV)	IL	51.85 ± 0.93	51.58 ± 0.98
12	Mean Corpuscular hemoglobin (MCH)	pg	17.40 ± 0.06	17.33 ± 0.07
13	Mean Corpuscular hemoglobin concentration (MCHC)	g/L	337.00 ± 6.11	339.83 ± 7.60
14	Red cells distribution width (RDW)	%	13.47 ± 0.39	13.33 ± 0.51
15	Platelet count	10^9 /L	523.83 ± 0.47	512.67 ± 0.48
16	Mean platelet volume	IL	4.97 ± 0.05	4.95 ± 0.05
17	Platelet distribution width	%	15.80 ± 0.06	15.82 ± 0.04
18	Procalcitonin	%	0.26 ± 0.06	0.23 ± 0.01

Data presented as mean ± standard deviation (n=3)

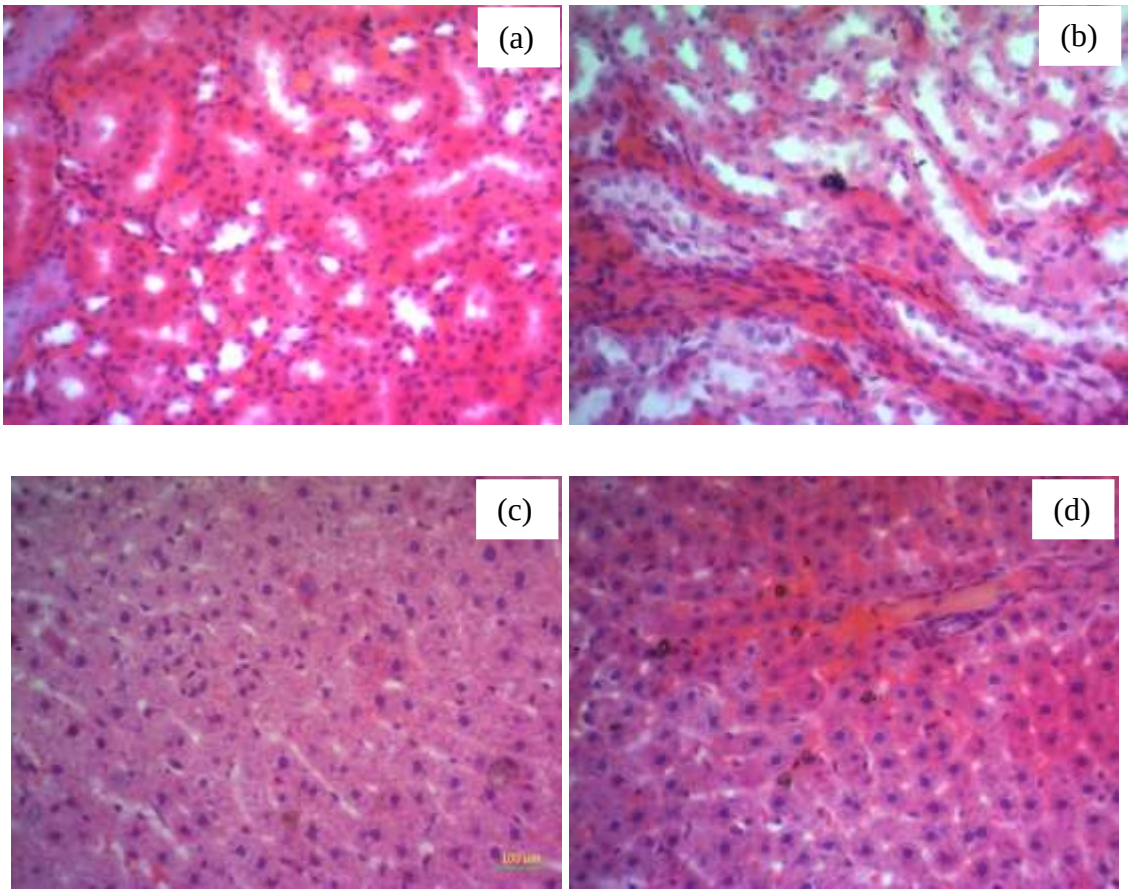

Fig. 6.5 Histopathology of kidney and liver of control and BBE dosed rats; (a) kidney of BBE dosed rat, (b) kidney of control rat, (c) liver of BBE dosed rat, and (d) liver of control rat

6.3.3 Incorporation of BBQM in BBP RTC-SM

As per in vivo acute toxicity investigation of BBE on rats, up to 500 mg dose was observed to be safe for consumption. Evaporated BBE (9.8% yield) was obtained from BBP and this suggests that consumption of BBP up to 510.2 g in 24 h might be harmless without any toxic effect on the body. As previously discussed in Chapter 2, BBE contained 2.85 µg/mg of quercetin, and BBQM contained 0.11 µg/mg of quercetin. From the cell viability study, concentration up to 250 µg/ml of BBE was concluded to be productively active in functional activities. Hence, 10 mg of BBQM (containing 3 mg BBQ per microbeads) was decided to incorporate in 2 g of BBP RTC-SM, with high possibilities of effective functional properties on cells in the intestine.

6.3.4 Effect of BBQM incorporation in sensory parameters of BBP RTC-SM soup

The sensory analysis of soup prepared from RTC-SM (control, BBP, and BBQM BBP) (Fig. 6.6) had average hedonic scores for sensory parameters all above 7 (like moderately) (Fig. 6.7). This suggests that the product developed had good customer acceptability (OA). There was no significant difference in sensory parameters between BBP RTC-SM and BBQM BBP RTC-SM, which indicates that there was no significant effect of BBQM incorporation on sensory parameters (Fig. 6.8). In both BBP RTC-SM and BBQM BBP RTC-SM soup, the taste was scored a bit lower than the other parameters and in compared to the control RTC-SM, which might be due to the natural astringency flavor of the bhimkol blossom [6].

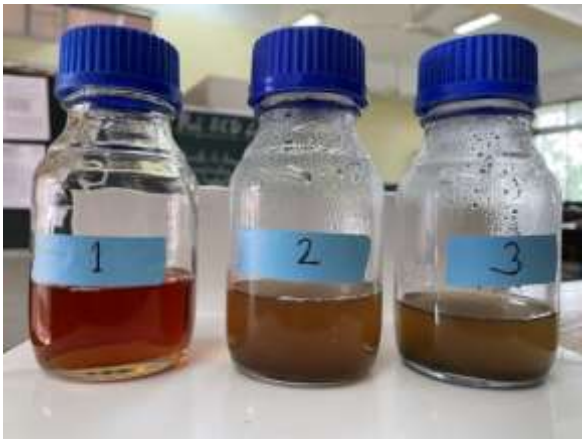

Fig. 6.6 Soup prepared from RTC-SM: (1) control, (2) BBP, and (3) BBQM + BBP

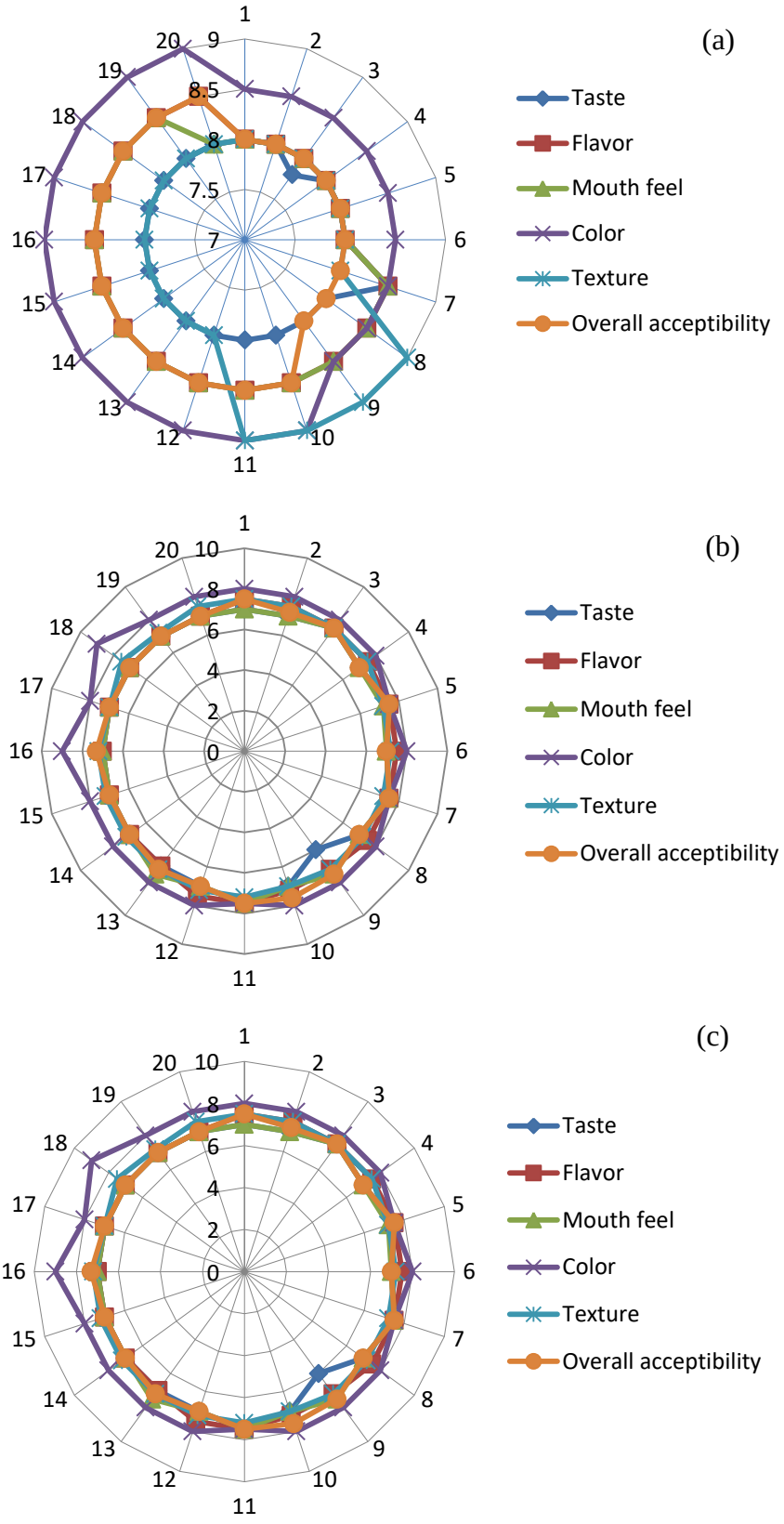

Fig. 6.7 Sensory analysis of soup prepared from RTC-SM soups; (a) Control, (b) BBP, and (c) BBQM + BBP

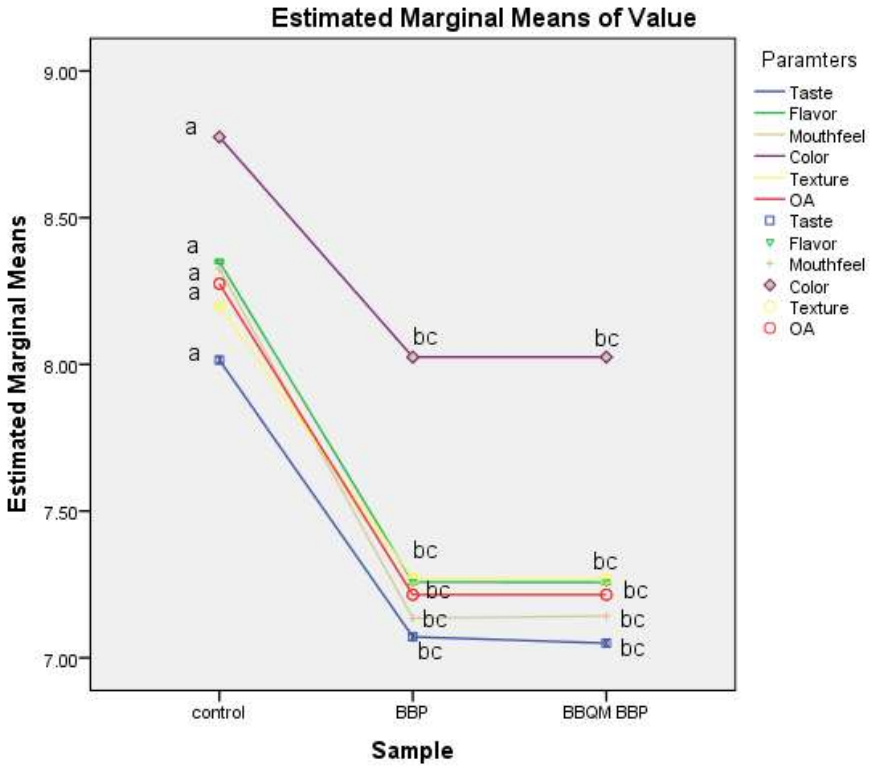

Fig. 6.8 Significant difference between overall acceptability of BBP and BBQM BBP RTC-SM, different letters indicates significant difference ($p \leq 0.05$).

6.3.5 Product component analysis

For the scree plot for the control RTC-SM (Fig. 6.9a), components 1, 2, and 3 were extracted as they were scored above 1 eigenvalue. For both BBP (Fig. 6.10a) and BBQM BBP RTC-SM soup (Fig. 6.11a), components 1 and 2 were extracted as they were scored above 1 eigenvalue. In the control RTC-SM soup, a positive eigenvalue of taste was scored highest in component 3 (Fig. 6.9b, Table 6.4) with 0.975 eigenvalues of OA towards component 1. In BBP RTC-SM soup, a positive eigenvalue of mouthfeel and OA scored highest in component 1 with 0.784 and 0.767 eigenvalues, respectively (Fig. 6.10b, Table 6.5). In BBQM BBP RTC-SM soup, a positive eigenvalue of mouthfeel, OA, and texture scored highest in component 1 (Fig. 6.11b, Table 6.6).

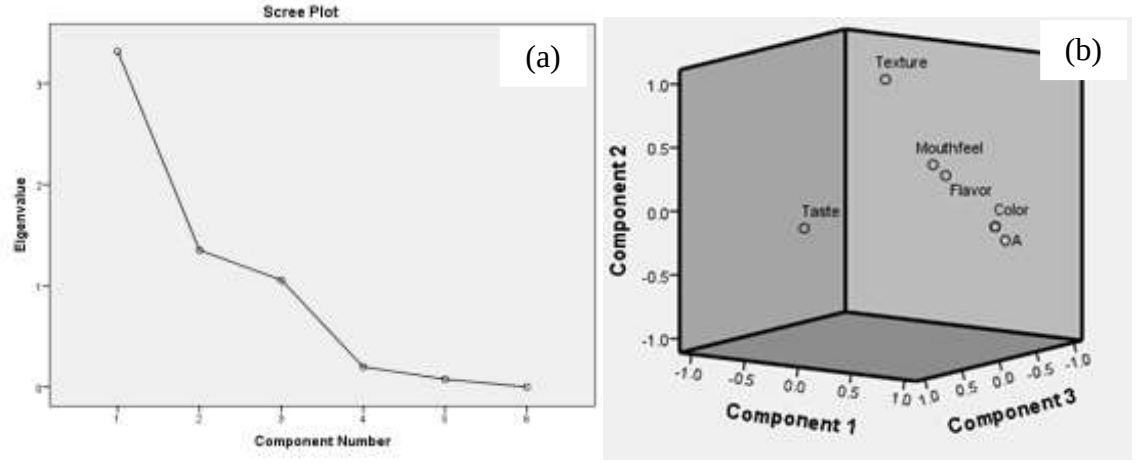

Fig. 6.9 Scree plot and rotated component plot of control RTC-SM soup; (a) Scree plot and (b) Rotated component plot

Table 6.4 Rotated component matrix of sensory parameters of control RTC-SM soup

Rotated Component Matrix[a]			
	Component		
	1	2	3
Taste			0.982
Flavor	0.840	0.365	0.330
Mouthfeel	0.746	0.446	0.365
Color	0.975		-0.140
OA	0.975		-0.140
Texture		0.980	

[a]Rotation converged in 6 iterations.

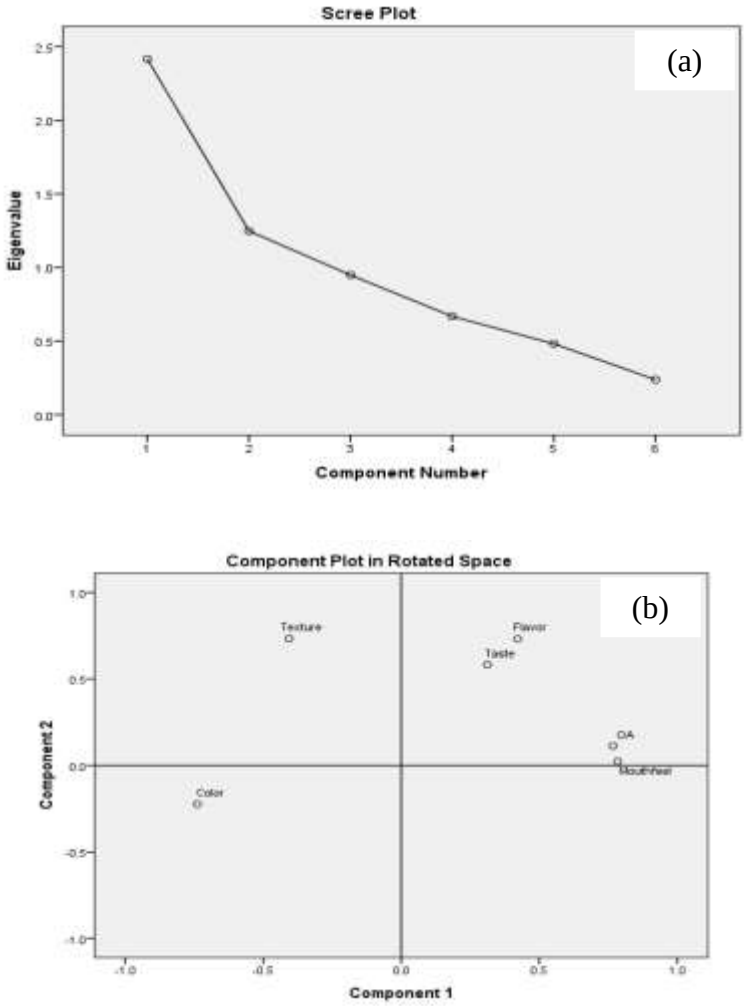

Fig. 6.10 Scree plot and rotated component plot of BBP RTC-SM soup; (a) Scree plot and (b) Rotated component plot

Table 6.5 Rotated component matrix of sensory parameters of BBP RTC-SM soup

Rotated Component Matrix[a]		
	Component	
	1	2
Taste	0.312	0.582
Flavor	0.422	0.732
Mouthfeel	0.784	
Color	-0.739	-0.224
OA	0.767	0.113
Texture	-0.406	0.733

[a]Rotation converged in 3 iterations

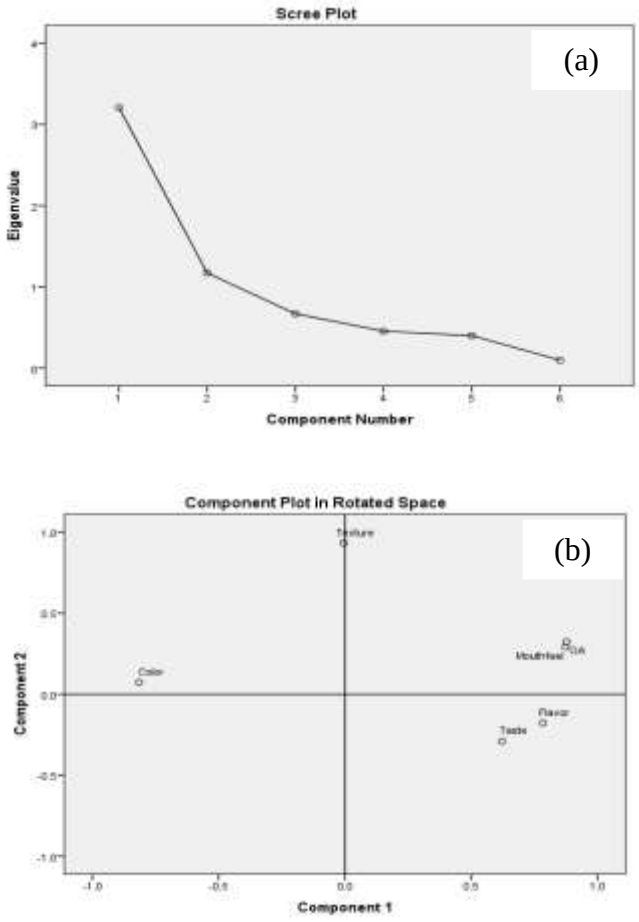

Fig. 6.11 Scree plot and rotated component plot of BBQM BBP RTC-SM soup; (a) Scree plot and (b) Rotated component plot

Table 6.6 Rotated component matrix of sensory parameters of BBQM BBP RTC-SM soup

Rotated Component Matrix[a]		
	Component	
	1	2
Taste	0.622	-0.291
Flavor	0.784	-0.178
Mouthfeel	0.873	0.292
Color	-0.815	
OA	0.878	0.323
Texture		0.933

[a]Rotation converged in 3 iterations

6.3.6 Antioxidant activity of RTC-SM

The DPPH scavenging activity (antioxidant activity) of soup prepared from BBQM BBP RTC-SM was higher than the BBP RTC-SM soup (Fig. 6.12). The antioxidant activity of BBQM BBP RTC-SM was hiked due to the incorporation of BBQ. A similar study by Neri-Numa et al. [12] and Dias et al. [5] also reported that the incorporation of bioactive ingredients increased the original potential antioxidant activity. As detailed in chapter 5, BBQ contains 53.12±0.31% of quercetin content along with catechin and some other phytochemicals, which might be responsible for the ability to scavenge more free radicals.

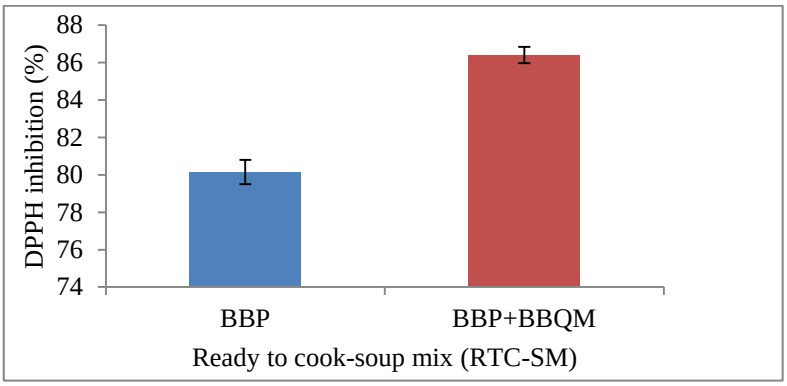

Fig. 6.12 DPPH inhibition activity of RTC-SM

6.4 Conclusion

Bhimkol blossom extract had no toxicity signs on Wister rats at 500 mg/kg body weight. Therefore, a ready to cook soup mix from bhimkol blossom powder (BBP RTC-SM) was concluded to be prepared under a safe dose. Soup prepared from developed RTC-SM showed good customer satisfaction, which indicates the good scope of market demand. The incorporation of microcapsules had no significant effects on the sensory parameters of the soup. Both soups prepared from bhimkol blossom with or without the incorporation of microcapsules containing quercetin rich extract exhibited good antioxidant activities whereas, the incorporation of microcapsules in BBP RTC-SM enhanced the antioxidant activity of BBP RTC-SM. After consumption of soup prepared from BBP RTC-SM with microcapsules, entrapped quercetin will mostly survive in stomach digestion and release its health beneficial bioactivities and functional activities in intestinal cells. The incorporation of microcapsules containing quercetin rich extract is

a highly effective approach to the value addition of underutilized agricultural by-products.

References

[1] Celli, G. B., Ghanem, A., and Brooks, M. S. L. Haskap berries (*Lonicera caerulea* L.)-A critical review of antioxidant capacity and health-related studies for potential value-added products. *Food and Bioprocess Technology*, *7*(6), 1541-1554, 2014.

[2] Chiva-Blanch, G. and Visioli, F. Polyphenols and health: Moving beyond antioxidants. *Journal of Berry Research*, *2*(2), 63-71, 2012.

[3] da Silva, B. V., Barreira, J. C., and Oliveira, M. B. P. Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies. *Trends in Food Science & Technology*, *50*, 144-158, 2016.

[4] Day, L., Seymour, R. B., Pitts, K. F., Konczak, I., and Lundin, L. Incorporation of functional ingredients into foods. *Trends in Food Science & Technology*, *20*(9), 388-395, 2009.

[5] Dias, M. I., Ferreira, I. C., and Barreiro, M. F. Microencapsulation of bioactives for food applications. *Food & Function*, *6*(4), 1035-1052, 2015.

[6] Fania, F. *Research and development final project kim ba-som (banana blossom kimchi)*. PhD Thesis, Ottimmo International, Indonesia, 2021.

[7] Henry, C. J. K. and Heppell, N. Nutritional losses and gains during processing: future problems and issues. *Proceedings of the Nutrition Society*, *61*(1), 145-148, 2002.

[8] Kalita, H., Boruah, D. C., Deori, M., Hazarika, A., Sarma, R., Kumari, S., Kandimalla, R., Kotoki, J., and Devi, R. Antidiabetic and Antilipidemic Effect of *Musa balbisiana* Root Extract: A Potent Agent for Glucose Homeostasis in Streptozotocin-Induced Diabetic Rat. *Frontiers in Pharmacology*, *7*, 102, 2016.

[9] Milea, A. Ș., Vasile, A. M., Cîrciumaru, A., Dumitrașcu, L., Barbu, V., Râpeanu, G., Bahrim, G. E., and Stănciuc, N. Valorizations of sweet cherries skins phytochemicals by extraction, microencapsulation and development of value-added food products. *Foods*, *8*(6), 188, 2019.

[10] Mondal, I. H., Rangan, L., and Uppaluri, R. V. S. Parametric optimality of tray dried *Musa balbisiana* Colla blossom. *Journal of Food Science and Technology*, *57*(12), 4599–4612, 2020.

[11] Murthy, P. S. and Naidu, M. M. Sustainable management of coffee industry by-products and value addition-A review. *Resources, Conservation and Recycling*, *66*, 45-58, 2012.

[12] Neri-Numa, I. A., Arruda, H. S., Geraldi, M. V., Júnior, M. R. M., and Pastore, G. M. Natural prebiotic carbohydrates, carotenoids and flavonoids as ingredients in food systems. *Current Opinion in Food Science*, *33*, 98-107, 2020.

[13] Nigatu, T. A., Afework, M., Urga, K., Ergete, W., and Makonnen, E. Toxicological investigation of acute and chronic treatment with *Gnidia stenophylla* Gilg root extract on some blood parameters and histopathology of spleen, liver and kidney in mice. *BMC Research Notes*, *10*(1), 625, 2017.

[14] Pasrija, D., Ezhilarasi, P. N., Indrani, D., and Anandharamakrishnan, C. Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. *LWT-Food Science and Technology*, *64*(1), 289-296, 2015.

[15] Porwal, M., Khan, N. A., and Maheshwari, K. K. Evaluation of Acute and Subacute Oral Toxicity Induced by Ethanolic Extract of *Marsdenia tenacissima* Leaves in Experimental Rats. *Scientia Pharmaceutica*, *85*(3), 29, 2017.

[16] Ramu, R. A. M. I. T. H., Shirahatti, P. S., Zameer, F. A. R. H. A. N., Lakkapa, D. B., and Nagendra, M. N. Evaluation of banana (Musa sp. var. Nanjangud rasa bale) flower and pseudostem extracts on antimicrobial, cytotoxicity and thrombolytic activities. *International Journal of Pharmacy and Pharmaceutical Science*, *7*(1), 136-140, 2015.

[17] Ribeiro, A. M., Estevinho, B. N., and Rocha, F. Preparation and incorporation of functional ingredients in edible films and coatings. *Food and Bioprocess Technology*, *14*(2), 209-231, 2021.