CHAPTER 10

CONCLUSION AND FUTURE SCOPE OF WORK

10.1. Conclusion

The present work includes studies on the phytochemical and antioxidant properties of selected fresh fruits of Assam, India. It also reports studies on the effect of cooking methods (steaming, boiling and microwave cooking) on the phytochemical and antioxidant properties of fourteen vegetables. The present study also determined the effect of different pasteurisation methods and spray drying on the total phenolic and flavonoid content and antioxidant properties of selected fruit juices. Optimisation of phenolic extraction from carambola pomace was carried out by response surface methodology (RSM) using a composite central rotatable design (CCRD) and the extract that was microencapsulated with maltodextrin were studied for its physicochemical properties. The dietary fibre obtained from by-products of six different fruits was studied for proximate content, phytochemical content, physicochemical and functional properties. From the studied fibre samples, carambola pomace fibre was selected to develop a functional beverage powder. Optimisation by response surface methodology using CCRD was used to obtain the optimum conditions for development of functional beverage powder. The health promoting properties of the developed functional beverage powder were evaluated in an animal model using Sprague dawley rat. The experimental results of the work have been detailed from chapters 3 - 9 in this thesis. The major conclusions drawn from the experimental work reported in the previous chapters (chapters 3 - 9) are presented.

10.1.1. Chapter 3

In this chapter the total phenolic content, flavonoid content, and antioxidant activities, ascorbic acid and major phenolic acids present in the thirteen fresh fruit samples from Tezpur, Assam were reported. Further, work on the effect of boiling, steaming and microwave cooking on the antioxidant activity of the phytochemicals of fourteen vegetables are presented.

1. Among the raw fruits studied, highest TPC was observed in black jamun followed by litchi, *bogi jamun*, amla, hog plum, pani jamun and carambola. Similarly, the above fruits showed good ferric reducing antioxidant potential and radical scavenging activities compared to the rest of the studied fruits. High

antioxidant activity could be due to high phenolic content but in some cases this varied.

2. On comparison across the thirteen fruit samples, pineapple, olive, Khasi mandarin and watermelon were low in total phenolic content; *poniol* and *leteku* had medium total phenolic content and rest of the samples viz. carambola, *pani jamun,* hogplum, amla, *bogi jamun*, litchi and black jamun were high in total phenolic content. Certain phenolics have a higher redox potential than other phenolics and therefore can exhibit independent results irrespective of their TPC.
3. Carambola showed good amounts of gallic acid, chlorogenic acid, caffeic acid, syringic acid, ferulic acid and quercetin. Amla and hog plum showed high content of gallic acid, catechin and ferulic acid. Khasi mandarin, litchi, *pani jamun,* amla, hog plum, watermelon showed good ascorbic acid content.
4. In case of vegetable samples, cooking by the methods of boiling, steaming and microwaving changed the phenolic content and antioxidant activity which can be attributed to variety of effects like destruction, release and transformation of the phytochemicals.
5. Cooking enhanced the antioxidant activity of most of vegetables studied. Overall, steaming was the most preferred method for cooking.
6. Among the vegetables, banana blossom, beetroot, teasel gourd, black eyed pea, *kharua* brinjal and roselle leaves were found to be rich in TPC and antioxidant properties. But, in case of cooked banana blossom and black eyed pea, a decrease in flavonoid content was observed.
7. In banana blossom, gallic acid was the predominant phenolic acid. The other phenolic acids present are syringic acid, rutin and quercetin. Steaming and boiling treatments to blossom released catechin from its bound form into the extracting medium. Catechin, ferulic acid and rutin were predominantly present in the roselle leaves. Processing had detrimental effect on these three phenolics. In some cases, disappearance of a particular phenolic acid was observed while in others, newer phenolic acid peaks were identified in the processed samples.
8. It can, therefore, be inferred that cooking had both positive and negative impact on the phytochemicals and antioxidant activities in the vegetables. In most cases, cooking increased the release of phenolics into the extraction medium and among the three cooking methods employed steaming emerged as the most suitable method followed by microwave cooking.

10.1.2. Chapter 4

The effect of spray drying on the physicochemical, phytochemical and antioxidant properties of the juices of four fruits viz. carambola (*Avorrhoa carambola*), watermelon (*Citrullus lanatus*), Khasi mandarin orange (*Citrus reticulata*) and pineapple (*Anona sativas*) were reported.

1. Highest yield was observed in Khasi mandarin spray dried juice powder.
2. Increased '*L*' and '*b*' values were observed in reconstituted samples with an exception in '*b*' value of pineapple sample. While a decrease was observed in the '*a*' values. The overall colour change (△E) was much more prominent in Khasi mandarin.
3. Particle size analysis of watermelon (0.1-4μM) and pineapple (0.2-7.2 μM) were less compared to that of carambola (53-104 μM) and Khasi mandarin (18-30 μM) samples.
4. The Hausner's ratio ranged between 1.22-1.57 while Carr index range was between 18.49-35.73 %.
5. The pineapple and Khasi mandarin powders had low cohesiveness compared to watermelon and carambola powders. As result of which, the flowability as per Carr index in watermelon and carambola is bad while in Khasi mandarin it is fair and is good for pineapple.
6. Watermelon and pineapple powders were more soluble compared to Khasi mandarin and carambola powders.
7. Hygroscopicity of Khasi mandarin and pineapple powders was significantly different from that of watermelon and carambola powders.
8. The phytochemical content and antioxidant activities also showed variations. In watermelon and pineapple, decrease in TPC and TFC was observed. However, spray dried carambola juice powder showed no significant changes in TPC and TFC values but exhibited increased FRAP and DPPH values.
9. Therefore, it can be inferred that depending on the fruit juice type the increase or decrease in phytochemicals and their antioxidant activities differed. Spray drying was found to be a convenient method to process and dry fruit juice into powder that has convenience of reconstitution just prior to consumption and also provides heath benefitting compounds.

10.1.3. Chapter 5

A comparative study on the effect of conventional thermal pasteurisation, microwave and ultrasound treatments on the phytochemical and antioxidant activities of juices from carambola (*Averrhoa carambola L.)*, black jamun *(Syzygium cumuni* L.Skeels.), watermelon (*Citrullus lanatus* var *lanatus),* pineapple *(Ananas comosus L. Merr),* and litchi *(Litchi* chinensis Sonn.) were reported.

1. The processing techniques used were effective in inactivating the microorganisms in almost all the processed juice samples. All the processed juices showed no growth in McConkey agar plates and thus, it can be inferred that no pathogenic gram negative bacteria was present in the samples.
2. In most cases, compared to the conventional thermal pasteurisation, microwaved and sonicated sample showed more positive effect on the phytochemical content. Depending on the type of fruit sample and treatment, increase or decrease in phytochemical values was observed.
3. In carambola juice, increase in gallic acid, catechin, chlorogenic acid, syringic acid and ferulic acid content was observed upon processing. However, in microwave treated juice at 600W, chlorogenic acid was not detected. Fresh (FR) and sonicated (SN) carambola juices showed the presence of gallic acid, catechin, chlorogenic acid, syringic acid and ferulic acid.
4. The litchi juice showed a decrease in quercetin and rutin. On the other hand, sonicated pineapple juice had a negative impact on the gallic acid, chlorogenic acid and quercetin, while it showed release and subsequent detection of catechin, syringic acid, ferulic acid and kaempferol, although in very small quantity.
5. In microwave treatment exposure time to temperature is less, while sonication involves application of low temperature and hence, both microwaved and sonicated samples were found to have positive effect on the phenolic content and antioxidant activity with exceptions in some cases.
6. Overall, in most cases, compared to conventional thermal pasteurisation, microwave treated and sonicated sample showed more positive effect on the phytochemical content.

10.1.4. Chapter 6

This chapter included the study on optimization of conditions for the extraction of polyphenols from pomace of sour variety carambola or star fruit (*Averrhoa carambola)* using response surface methodology and microencapsulation of the extracted polyphenols with maltodextrin by spray and freeze drying methods.

1. It was observed that variation in ethanol concentration and temperature had both positive and adverse effects on TPC, FRAP and DPPH activity in the ethanol extract of carambola pomace.
2. However, their combined interaction had a positive effect on the antioxidant activities. At ethanol concentration of 65% and temperature of 40°C for extraction, TPC, FRAP and DPPH activity were optimum.
3. RP-HPLC study of the extract showed presence of 9 different phenolic acids and ascorbic acid. Compared to the carambola juice, the polyphenol extract contained good amount of gallic acid, catechin and caffeic acid as well as moderate amount of ferulic acid, chlorogenic acid and coumaric acid.
4. The RP-HPLC results showed absence of caffeic acid, coumaric acid, rutin hydrate and quercetin in carambola juice when compared to the pomace extract.
5. In microencapsulation study, variation in the physical properties between the two variants of microencapsulates was observed. Highest encapsulating efficiency was obtained in freeze dried samples.
6. *In vitro* gastrointestinal simulation study showed greater release of phenolic compounds in the gastric fluid at pH 1.2.
7. Lastly, it can be concluded that, the proposed extraction model of the CCRD through RSM can be used for the polyphenol extraction of carambola pomace and the extracted polyphenols can be used in different food models as such or in encapsulated form depending on the requirements of the end products.

10.1.5. Chapter 7

This chapter included the comparative study of the health promoting and functional properties of the fibres obtained as by-products taken from six sources viz., pomace of carambola (*Averrhoa carambola* L.) and pineapple (*Ananas comosus* L. *Merr*), peels of watermelon *(Citrullus lanatus)*, Burmese grape (*Baccurea sapida* Muell. Arg) and Khasi mandarin orange (*Citrus reticulate* Blanco*)*, and blossom of seeded banana (*Musa balbisiana* Colla., *ABB*)

1. The six fibre samples showed varied proximate, physicochemical, phytochemical and functional properties.
2. Watermelon peel (WMPL) and banana blossom (BB) powder were rich in crude protein and ash content.
3. The pineapple pomace (PNPM) had highest amount of dietary fibre, however, other samples showed comparatively better results for all the parameters tested.
4. All the samples showed good water and oil holding capacity as well as swelling capacity.
5. The fibre samples contained good phytochemical content and antioxidant activity. Fibre derived from carambola pomace (CMPM) showed highest TPC and FRAP values.
6. Gallic acid was predominantly present in all the six samples. The highest content was reported in BB sample (27.00± 0.10 mg/100g).
7. Catechin was present in CMPM, BGPL and WMPL. Chlorogenic acid was present only in WMPL. Similarly, KMPL showed highest caffeic acid content (53.09± 0.12 mg/100g) followed by PNPM (11.09± 0.09 mg/100g).
8. In rest of the samples caffeic acid was not detected. Likewise, syringic acid was highest in KMPL (22.94±0.09 mg/100g) while CMPM and BGPL showed no detection. Ferulic acid was present in all the samples except in WMPL and KMPL. Similarly, coumaric acid was present only in BGPL and WMPL. The rutin hydrate content was highest in KMPL sample. BB and CMPM samples also showed the presence of rutin hydrate.
9. All the samples exhibited good glucose adsorption capacity, amylase inhibition, glucose diffusion rate and glucose diffusion rate index GDRI. Among the six samples, Burmese grape peel (BGPL), BB, CMPM showed high GDRI values.

10.1.6. Chapter 8

This chapter deals with the development of a beverage powder by spray drying technique consisting of three fruit juices viz., carambola, pineapple and watermelon mixed together and fortified with fibre rich fractions derived from carambola pomace powder. The concentrations of maltodextrin and inlet temperature of spray drier were optimised using response surface methodology for spray drying of the juice and fibre mixture. The response variables were moisture content, yield, bulk density, solubility and hygroscopicity.

1. The optimum conditions obtained from the proposed model for inlet temperature was 175°C and maltodextrin concentration was 25%. The actual values for the response variables for the optimum conditions were in tandem with that of the predicted values.
2. The final product contained 9.29% of total dietary fibre.
3. The time taken for dissolution of the beverage powder in distilled water was 48.52±0.31s.
4. Beverage powder size was distributed in the range of 0.90-1.50 μM and 43.50-102.00 μM. The Span value for the sample was 1.55±0.09. The beverage powder had intermediate cohesiveness whereas flowability property as per Carr index value was relatively good.
5. The color comparison for '*L*' and '*b*' values between feed and reconstituted samples showed significant difference ($p \leq 0.05$) during paired t-test. Overall color change ($\triangle E$) value was 6.47±0.
6. The sensory analysis by 9 point hedonic scale showed overall acceptability of the product at 7 (liked moderately).

10.1.7. Chapter 9

The efficacy of the health promoting properties of carambola pomace fortified spray dried mix fruit juice beverage powder in Sprague Dawley rat model is reported. The fibre rich functional beverage powder was developed as discussed in chapter 7.

1. The test diet showed positive results under the given conditions in both the test groups.
2. Decrease in cholesterol, HDL, LDL, TGL, SGPT and SGOT values were obtained. Maximum decrease was obtained in Group 2 (diet containing no added fat). Therefore, the test diet worked more effectively in absence of fat compared to Group 1 where $1/3^{rd}$ of the diet contained fat.
3. Similarly, decrease in SGPT and SGOT in rats fed with test diets showed normal liver functions which indicated that the test diet had a positive impact on the functioning of the liver by helping in lowering the serum glucose and cholesterol levels.
4. No significant increase in body weight was observed after 6^{th} week.
5. The histopathology of the heart and aorta tissues showed no tearing or major deviation from that of the healthy control rat tissue.

6. The analysis of the caecal matter showed presence of short chain fatty acids which are desirable for maintaining colon health.
7. The health promoting properties of the developed beverage powder suggests its future scope in the functional foods segment.

10.2. Future scope of work

The following scopes for future study can be considered from the findings of the present work.

1. The changes in phytochemical content and antioxidant activity of other processing methods like frying, blanching, high pressure processing and canning in vegetables; different drying treatments like osmotic drying, vacuum drying, foam mat drying on fruits can be analysed.
2. Storage study of the polyphenol rich maltodextrin encapsulates can be carried out.
3. The effect of different encapsulating agents on the property of the carambola pomace derived polyphenol extract and their subsequent application in development of some functional products can be studied.
4. A clinical study on the cholesterol and glucose lowering effects may be carried out on human subjects.
5. Further, the other five fibre rich sources that were studied along with the carambola pomace may be used in development of newer functional products and their effectiveness could be studied in detail both under *in vitro* and *in vivo* conditions.

List of publications

1. Saikia, S. and Mahanta, C.L. Effect of steaming, boiling and microwave cooking on the total phenolics, flavonoids and antioxidant properties of different vegetables of Assam, India, *Int. J. Food and Nutr.* Sci. **2**(3), 47-53, 2013.
2. Saikia, S., Mahnot, N.K. and Mahanta, C.L. Effect of spray drying of four fruit juices on physicochemical, phytochemical and antioxidant properties. *J. Food Process. Preserv.* **(MINOR REVISION FOR ACCEPTANCE)**
3. Saikia, S., Mahnot, N.K. and Mahanta, C.L. Optimisation of phenolic extraction from *Averrhoa carambola* pomace by response surface methodology and its microencapsulation by spray and freeze drying. *Food Chem.* **(MINOR REVISION FOR ACCEPTANCE)**