Chapter V

Summary and Conclusions

Chapter-V

SUMMARY AND CONCLUSIONS

5.1 Summary

Germination conditions (temperature and time) influenced the malting potential of both LAR and IAR. The embryo development was observed only in two germination temperatures i.e. 30 and 35°C. A high germination capacity and the rate were observed in IAR at 30- 35°C when compared to LAR leading to a higher decrease in malting yield. The amylose-amylopectin ratio (A-AR) also exhibited significant effect under different germination conditions. The enzyme activity which mainly remains incipient in the native rice was observed to increase exponentially during germination. Moreover, the enzyme activity of IAR malted rice was significantly higher than LAR in both germination temperature which may be due to the difference in production of gibberellic acid in different cultivars. The hydrolytic enzymes caused higher starch degradation in the IAR malted rice in comparison to LAR. The morphology of the malted flour from SEM revealed pin holes and eroded surface due to the action of hydrolytic enzymes. The structural study (XRD and FTIR) of both malted LAR and IAR revealed faster degradation of amylose as compared to amylopectin under all germination conditions which may be due to the complex nature of amylopectin. IAR paddy was found more suitable at both 30 and 35°C for malting based on the outcome of the present study.

The modelling of the enzyme reaction rate during the process of germination was evaluated. Germination being a complex process, the enzyme kinetics did not follow the Michaelis-Menten equation. Therefore, the reaction rate for both the paddy during the process of germination was estimated by Boltzmann function and the results showed that the R^2 values of the all predicted model equations were more than 0.85. The obtained reaction rate was employed in Michaelis-Menten equation to determine the model parameters (K_m and V_{max}) and results revealed that the statistical parameters were also not found in acceptable range. A stochastic model was developed to modify the enzyme reaction kinetics. The reaction order was determined and observed that the enzyme followed the zeroth order reaction kinetics while the change in the substrate concentration followed the second order for LAR rice and the first order for IAR rice for both temperatures. The different reaction order for LAR and IAR might be due to the variation in substrate concentration. Further, the process modeling of starch hydrolysis during the process of germination was performed by the artificial neural network (ANN). The statistical performance parameters for both developed

models i.e. the enzyme reaction kinetics and ANN based germination process model were found to be acceptable.

Low amylose (LAR) and intermediate amylose (IAR) paddy at different temperatures and times of germination showed the different biochemical changes in the endosperm. Hydrolysis of amylose and amylopectin occurred by cleaving the glycosidic linkages. The amylopectin content in both the paddy samples decreased at a faster rate with germination time as compared to amylose. A high extent of starch hydrolysis was observed in IAR germinated paddy than that of LAR to yield small size oligosaccharides. The two complementary vibrational spectroscopic techniques were employed to study the structural changes of starch hydrolysis; Raman spectroscopy provided various information related to homonuclear functional groups. With the onset of germination, the amylase acted on the pyranose ring structure of glucose units present in starch; a decrease in the intensity of the band at 948 and 938 cm-1 was observed. The degradation of the starch molecule resulted in the formation of a high level of hydroxyl groups as evidenced by the increase of 1054 cm^{-1} band. The formation of several new peaks associated with α and β glucose was also noticed. The various changes in the paddy along with the spectral data during germination offered a mechanism of starch hydrolysis. The biochemical and structural changes were correlated with the help of the method of principal component analysis (PCA). The PCA of LAR and IAR showed that time of germination, amylopectin, starch and Raman spectra bands were related to skeletal vibrations of the pyranose ring. The model developed employing partial least squares (PLS) for quantification of amylose, amylopectin, total starch and reducing sugar showed that the application of vibrational spectroscopy along with multivariate calibration regression has the potential to quantify these parameters.

The pasting properties and thermal behaviour of two malted rice having different amylose and amylopectin contents were evaluated. The germination time had a significant effect on pasting properties as they decreased during the germination process. Two pasting peak viscosities were observed in malted LAR samples. Higher extent of decrease in pasting properties implied the role of amylose-amylopectin content on the germination process for malted IRA variety. The thermal behaviour of malted LAR and IAR samples was significantly affected by the germination time, temperature, and amylose-amylopectin content. The values of T_O, T_P and T_C increased for the malted rice with germination time. A second-order kinetic change was observed for the pasting properties and the change in the thermal behaviour followed a rational function with respect to germination time. The correlation matrix showed a positive correlation between PT and T_O in both malted rice samples revealing the swelling of starch granules during hydrothermal treatment. PT also

showed a positive correlation with other pasting properties. A good correlation was also observed between amylose-amylopectin content, pasting properties and thermal behaviour. PCA also exhibited similar inter-relationship among the thermal and pasting properties in both LAR and IAR as both the groups showed similar behaviour too. Thus, this study showed the existence of an inter-relationship between the pasting properties and thermal behaviour of malted rice which was affected by germination conditions and amylose-amylopectin content.

A weaning formula was developed based on cereals and fruits available in Assam. The ingredients selected were whole wheat flour, rice flour, bengal gram flour, whole milk powder, Bhim kol and malted rice flour. Some of the ingredients were modified by the process of dry heat treatment i.e. roasting which was observed to improve the organoleptic properties and the pasting properties (PT, PV, HPV, BD, SB and CPV) decreased significantly after roasting. The optimization was performed by using D-optimal mixture design and a total number of 31 mixtures were obtained and the responses taken into consideration were energy content, protein content, WAI and WSI. A linear model was employed for the responses and the model was observed to be significant for all the responses as the coefficient of determination (R^2) was more than 80 %. The ingredients were observed to have a significant effect on the responses. The protein content increased with increase in WMP and pulse flour. The energy content was mainly influenced by the fruit pulp i.e. Bhim kol followed by WMP. On the other hand, WAI was observed to decrease with increase in WMP and pulse flour due to the high protein and fat content. WSI was observed to increase with an increase in malted rice as malting increased the soluble sugars. The optimization of the weaning formula was performed based on the responses and the optimized formula obtained was WF 10 %, RF 10 %, pulse 10 %, WMP 35 %, fruit 30 % and MR 5 % each keeping a constant level of 10 % refined vegetable oil. The validation was performed and the experimental values were found to be reasonably close to the predicted ones which confirmed the validity and adequacy of the predicted models. The proximate analysis of the optimized formula was evaluated and a high protein and energy content was observed. The WHC, WBC and SP were 3.37±0.062 g/g, 2.33± 0.27 g/g and 9.32±2.04 g/g respectively and was comparatively lower than another complementary formula which was due to the incorporation of malted rice in the weaning formula. The flowability and cohesiveness of the optimized weaning formula were very poor and highly cohesive which may be due to the presence of free surface fat in the formula. The mineral composition analysis showed the presence of calcium, phosphorous, chlorine, magnesium, iron and zinc. The amino acid composition was also analyzed and the essential amino acid detected were histidine, lysine, methionine, phenylalanine, threonine and valine while the

non-essential amino acid observed were alanine, arginine, glutamic acid and cysteine. The results also showed that the weaning formulation met 33.087 % of the total essential amino acids and 49.04 % of the total available amino acid (detected in the weaning formula) required for infants. Among the amino acids, the highest was detected for histidine which might be due to the ingredients such as WMP, wheat and pulse flour. The solid water ratio was also estimated to determine the consistency of the developed weaning formula and 50:50 ratio was observed to have the optimum viscosity range (2 Pa.s) since the viscosity range from 1-3 Pa.s was considered spoonable for the feeding of infants.

In vitro starch digestibility of the reconstituted weaning formula (50:50) was investigated in two different particle sizes i.e. 75 and 105 μm in a simulated human digestive system. The results showed that the starch digestibility in the mouth was lowest due to the short residence time in the mouth and it increased significantly from the stomach to the intestine which was due to the action of enzymes such as pepsin, pancreatin and glucoamylase. Moreover, the smaller particle size of the weaning formula showed higher starch digestibility than a larger particle in a simulated digestive system which might be due to the larger surface area of smaller particles. Similarly, the rheological property of the weaning formulation was also studied under the same conditions with the help of a rheometer. The results revealed that the viscosity of the weaning formulation decreased significantly as it passed from the mouth to the stomach and then to the intestine due to the action of the enzymes causing hydrolysis of starch. The modeling of the rheological behaviour of the weaning formula was also performed which showed that the shear stress decreased with increase in shear rate representing the shear thinning behaviour of the reconstituted weaning formulation at 50% solid concentration. The viscosity of the formula also showed a similar trend with an increase in shear rate and the smaller particle size (75μm) showed a higher decrease in viscosity as compared to larger particle size (105μm). Two rheological model such as power law model and Herschel-Bulkley model were employed for fitting the shear stress-shear rate data. The Power law model was taken as the best-fitted model and the weaning formula showed a pseudoplastic shear-thinning since flow behaviour index (n) was observed to be <1.

The cytotoxicity of the developed weaning formula was analyzed by two methods i.e. XTT and trypan blue assay. In both, the assay acute monocytic leukemia cell line (THP-1) were employed and both the assay showed more than 90 % viability indicating the absence of any cytotoxicity of the weaning formula.

The moisture sorption isotherm of the developed weaning formula was generated at the three different temperatures 20, 30 and 40°C in the water activity (a_w) range of 0.11-0.90. The results presented a sigmoid shape isotherm and resembled type II BET classification which might be due to the high protein content of the formula. It was observed that the equilibrium moisture content decreased with increase in temperature at constant water activity which was due to the loss of the hydrogen bonds that takes place at a higher temperature or breakaway of some water molecules occurs from their sorption sites due to activation to higher energy levels at elevated temperatures. In this study, the GAB and Peleg model was found to be the best-fitted model with R^2 values greater than 0.99. The estimated monolayer moisture varies between 0.0516 – 0.0462 and both the BET and GAB model showed decreasing trend in M_0 with an increase in storage temperature. Clausius-Clapeyron equation was found to adequately describe the dependence of isosteric heat of sorption on the equilibrium moisture content and values of net isosteric heat of sorption and sorption entropy were found to be slightly higher (less than 10%) based on Peleg model parameters as compare to GAB model parameters. Moreover, a strong dependence of differential entropy on moisture content with an exponential trend similar to that exhibited by differential enthalpy was observed. On the other hand, the Gibb's free energy (ΔG) decreased exponentially for both GAB and Peleg model with increasing EMC indicating that water adsorption was a spontaneous phenomenon.

The sensory evaluation of the reconstituted weaning formula and a commercial formula was performed on a 9-point hedonic scale and observed that overall acceptability of the developed weaning formula was higher than the commercial formula indicating that the properties of the food ingredients used in the blend were very much compatible. The quality evaluation of the developed weaning formula was performed by analyzing the peroxide value (PV) and free fatty acid (FFA). The peroxide value was observed to be low and well below 10 meq/kg fat and similarly, FFA was also observed to be low during the storage period indicating good quality of the developed formula during storage.

5.2 Conclusions

The overall objective of the present work is to understand the process of malting of rice in relation to the development of weaning food formulation. Two paddy varieties like low-amylose rice (LAR) and intermediate amylose rice (IAR) were considered for this study and the physico-chemical changes during the germination were evaluated. The enzyme reaction rate and germination process were modelled. The starch hydrolysis mechanism during germination based on structural changes were also predicted. The changes in pasting properties and thermal properties during germination were evaluated. The developed

weaning formulation was optimized and analysed to study the nutritional and rheological behaviour. Further, the moisture sorption isotherm and thermodynamic properties of the formulation were evaluated. Based on the above experimentations and findings of data analysis, following conclusions were drawn:

- Physico-chemical changes of the two rice varieties during the germination were significantly affected by the germination conditions i.e. temperature and time
- IAR paddy was found more suitable for malting as it showed higher malting potential as compared to LAR leading to higher starch degradation and production of amylolytic enzymes.
- The enzyme reaction kinetics followed the modified Michalis-Mention equation.
- An artificaial neural network (ANN) model was proposed to relate germination conditions and products obtained where the 2-6-4 ANN model showed good agreement with the experimental data.
- The vibrational spectroscopy, which was used to predict the starch hydrolysis mechanism, may be applied as a potential tool to study structural changes.
- PCA and PLS may also be used to corelate quality parameters or physical properties to analytical instrument data.
- An inter-relationship existed between the pasting properties and thermal behaviour of malted rice which was affected by germination conditions and amylose-amylopectin content.
- The developed weaning formula was high in energy and protein content along with essential minerals and amino acids.
- The invitro starch digestibility of the reconstituted product in a simulated human digestive system, showed high digestibility.
- The rheological changes in a simulated human digestive system showed shear-thinning behaviour and the power law model provided the best fit to correlate shear stress and shear rate.
- The developed weaning formula did not exhibit any form of cytotoxicity.
- The empirical and semi-empirical isotherm i.e. Peleg and GAB fitted well to the experimental data and the thermodynamic properties viz. net isosteric heat of sorption and sorption entropy, enthalpy-entropy compensation parameters and change in Gibb's free energy.
- The overall acceptability of reconstituted weaning formula was high and the quality of the product could be maintained during storage as the peroxide value (PV) and free fatty acid content (FFA) were low.

Thus, the present study concluded that the germination conditions offered the significant effect on paddy having different amylose contents and the obtained malted rice flour could be potentially used for the preparation of energy rich weaning food to combat calorie-protein malnutrition.

Future scope of work

The result obtained from the current thesis can form the basis for further studies on malting of grains and its application on various food products. The following scope for future study can be considered from the findings of the present work:

- The germination of locally available paddy with different amylose amylopectin ratio (waxy, normal and high amylose content) may be carried out at wider variation of temperature and time to evaluate their malting potential and changes in physico-chemical characteristics.
- The modelling of enzyme kinetics and the process of germination of paddy with different amylose content needs to be developed which shall provide a better insight in to the reaction process that occurs during the complex process of germination.
- The *in situ* structural mechanism of starch hydrolysis during the process of germination may be further studied in detail by using other sophisticated instruments which shall provide a clear idea to predict the actual changes inside the grain.
- The nutrient bioavailability of the developed weaning needs to be studied to get an idea about the proper absorption and utilization of nutrients by the body of the infants. Moreover, in vitro studies of mineral and protein in the simulated human system may be conducted.
- The prepared malted rice can also be utilized for the preparation of other food products such as energy drink for sportsperson, energy bars for children, gluten free confectionaries, preparation of beer and other bakery products.