ABSTRACT

The aim of the current research was to analyze the physicochemical, functional, and nutritional properties of *Euryale ferox* kernel starch-polyphenols complex and its application. The research involves isolation of starch from the kernel of *Euryale ferox* seed, extracting phytochemicals from the shell of *Euryale ferox* seed, assessing its antioxidant, antidiabetic, and anti-inflammatory effects. *Euryale ferox* kernel starch-polyphenols complex was prepared by employing polyphenols rich *Euryale ferox seed* shell extract, and some of the predominant polyphenols present in the extract such as ferulic acid, quercetin, gallic acid, rutin, and catechin. The complexation was confirmed by various analytical techniques, the *in vitro* starch digestibility, predicted glycemic, and the antioxidant activity of the complexes was determined. *Euryale ferox* seed shell extract was added to wheat-based bread, and the EFKS-polyphenol complex was incorporated in gluten free bread. The nutritional and organoleptic properties of the formulated bread samples were evaluated.

The thesis is organized into 7 chapters, which are concisely discussed as follows:

Chapter 1 highlights the overall introduction of *Euryale ferox* plant and its utilization in the present work. It also includes information about its nutritional and pharmacological properties. The chapter also consists of information about starch-polyphenols complexation and the importance of modification of starch using polyphenols.

Chapter 2 includes the physicochemical, functional, and nutritional properties of *Euryale ferox* kernel starch of Assam and Manipur was evaluated. *Euryale ferox* kernels contained 68-71% of starch. *Euryale ferox* kernels starch exhibited polyhedral, angular and oval shape with remarkably small granule size of 1~3µm. *Euryale ferox* kernels starch displayed A-type XRD pattern. The chemical composition of *Euryale ferox* kernel starch reflects higher degree of purity. The amylose content of *Euryale ferox* kernels starch was in the range of 29-30%. The thermal decomposition pattern of *Euryale ferox* kernels starch shows two steps mass loss. The first thermal decomposition is due to the disappearance of bound water. Whereas, the second stage is due to pyrolysis and degradation of starch backbone. The resistant starch content of *Euryale ferox* kernel starch was enhanced in the retrograded starch.

Euryale ferox kernel starch displayed unique physicochemical and functional properties, possessing high pasting temperature, which is attributed by its high amylose content, small granules size which rendered better resistant against rupture, resulting low water absorption capacity and low swelling capacity. This characteristic of starch will be suitable for food products requiring high processing temperature and alteration in viscosity is unwanted in the course of heating and cooling. Due to its tiny granule size, it has also the potential to cater the ever-increasing demand of small granule starch in industries for food and non-food applications. The results have also shown that the characteristics of *Euryale ferox* kernel starch was influenced by the genotypes and environment where it is grown. The findings of our study credibly assist in recommending the utilization of *Euryale ferox* kernels starch as an alternative source of starch.

Chapter 3 focused to assess the phytochemical composition and bioactivities of *Euryale ferox* seed shell extract (EFSSE). The percentage of phytochemicals yield, TPC and TFC increased when the solid: solvent ratio was higher, regardless of the extraction techniques. The maximum TPC was observed in the MAE extract (659.45 mg GAE/g) in 1:50 w/v solid-liquid proportion, whereas, the lowest TPC (463.24 mg GAE/g extract) was observed in MC (1:25 w/v) extract. Similarly, the highest TFC in MAE (104.16 mg QE/g extract), and the lowest TFC in MC (45.42 mg GAE/g extract) was observed. From our findings, it was observed that *Euryale ferox* seed shell extract obtained by using microwave assisted extraction system had exhibited the strongest radical inhibition for DPPH radical (IC_{50}=17.76 µg/mL); ABTS radical (IC_{50}=26.36 µg/mL) and FRAP value of 1936.56 µmol/g. *Euryale ferox* seed shell extract could effectively hinder α-amylase and α-glucosidase in a concentration dependent fashion. Among all the extract, MAE extract had demonstrated the maximum level of α-amylase inhibition potential (73.35 % with IC_{50} of 65.64 µg/mL), while MC extract had the lowest level (56.44% with IC_{50} of 87.74 µg/mL). Similarly, MAE extract had the highest α-glucosidase inhibitory activity (80.45% with IC_{50} of 43.83 µg/mL). The results of the present study revealed that EFSSE inhibited α-glucosidase more efficiently than acarbose.

The MAE extract was chosen for further studies owing to its higher polyphenol concentration. From the HR-LCMS analysis, it was discovered that *Euryale ferox* seed shell extract contains various phenolics, flavonoids, terpenoids, alkaloids organic acids, nucleotides, amino acids, carbohydrates and derivatives, unsaturated fatty acids,

phenylpropanoid, and steroid etc. From the HPLC chromatogram, it was found that gallic acid was the major compound found in EFSSE which was followed by ferulic acid, catechin, quercetin, and rutin. *Euryale ferox* seed shell extract demonstrated a concentration dependent inhibition of DPP-IV *in vitro*. *Euryale ferox* seed shell extract did not show cytotoxic effects at any of the tested concentration in both L6 muscle cell line and THP-1 cell line. It was observed that EFSSE could successfully enhanced glucose uptake. The mRNA gene expression of the pro-inflammatory cytokine such as IL-1β, TNF-α, IL-23, Cox-2, IL-1β, and IL-6 was drastically reduced in LPS induced THP-1 cells co-tested with EFSSE.

Chapter 4 deals about the development of starch-polyphenols complexes in order to better understand the alterations brought to the *Euryale ferox* kernel starch by the polyphenols migrated from the shell. The whole *Euryale ferox* seed shell extract along with its major constituents such as ferulic acid, gallic acid, catechin, quercetin, rutin and quercetin were chosen to prepare the complexes. XRD, FTIR, ^{1}H NMR, DSC, and SEM analysis were performed to verify the complexation. Polyphenols complexation produces crystalline complexes and emergence of multiple additional peaks due to the aromatic hydroxyl groups migrated from polyphenols. The ^{1}H NMR spectra of starch-polyphenols complexes showed additional peaks between 6.00- 9.00 ppm due to the benzene ring and phenolic hydroxyl groups imparted from polyphenols. The shifting and widening of the characteristic peak observed in the DSC thermogram confirmed that polyphenols were successfully attached to starch. SEM micrographs have shown that polyphenols were attached on the exterior surface of the starch granules. The complexation of polyphenols onto starch could change the color, swelling capacity and solubility index. Complexation of starch with polyphenols could modulating the digestibility and thus lowered the glycemic index. The structural modification of the starch may be accountable for the observed effect. There was a substantial increase in the antioxidant activity of the complex. Therefore, the developed complex may be considered as a unique resistant starch with antioxidant potential.

Chapter 5 studies the consequences of *Euryale ferox* seed shell extract (0.25% to 2%) addition on the *in-vitro* starch digestibility and predicted glycemic index (pGI) of wheat-based bread. *Euryale ferox* seed shell extract addition could affect loaf volume, hardness, and color. EFSSE (2%) had a strong inhibitory impact, as evidenced by the drop in

glycemic index from 94.61 to 61.66. Swarm intelligence supervised neural network (SISNN) technique was applied for the predictive simulation of digestion kinetics and pGI, and was compared with the mathematical modelling approach. Principal component analysis (PCA) with proportional odds modeling (POM) was used to find the most sensitive component based on the sensory attributes of bread.

Chapter 6 discussed the impact of starch-polyphenol complex addition on the *in-vitro* starch digestibility and predicted glycemic index (pGI) of gluten free baked bread. Gluten-free bread flour mix was partially replaced with the complex to increase the amount of resistant starch and polyphenols in bread. The addition of starch-polyphenol complex could improve the moisture content of bread, thus reducing the firmness and chewiness of bread crumbs. The inclusion of complex could significantly lower the glycemic index of gluten-free bread, while enhancing the antioxidant activity. The findings, thus showed that, starch-polyphenol complex may be used to produced gluten free baked products with improved nutritional qualities without compromising sensory properties.

Chapter 7 consists of the conclusion of the study. It includes the specific objectives, salient findings, and future scopes of the present investigations. The variations in physicochemical characteristics of *Euryale ferox* kernel starch were influenced by the cultivars. *Euryale ferox* shell is an attractive raw material for polyphenols extraction and recovery. EFSSE had no negative impact on the viability of cells. *Euryale ferox* seed shell extract inhibited α-glucosidase more efficiently than acarbose. The current investigation revealed that *Euryale ferox* seed shell extract possessed anti-diabetic effect and enhanced glucose uptake. The findings of the present study suggested that *Euryale ferox* seed shell extract may be utilized as a novel therapeutic alternative for the management of inflammatory-based chronic illnesses. Starch-polyphenols complexation is a promising approach for modulating the digestibility of starch. The resulting complex may be regarded as an antioxidant rich resistant starch. However, the diverse attributes of starch were governed by variations in the types and levels of polyphenolic molecules. Further, *Euryale ferox* seed shell extract may be utilized as a curative component that can be used to produce lower glycemic index functional food. Additionally, the replacement of gluten free bread flour by starch-polyphenol complex could increase the resistant starch, phytochemicals content, and antioxidant potential of gluten free bread.