ABSTRACT

Pea (*Pisum sativum* L.) is a principal commodity belonging to the leguminous family and is relatively inexpensive and highly nutritious agricultural crop. India is the second largest producer of pea next to China. The green pea production of India is about 2.3 million metric tonnes whereas the global production is 26.3 million metric tonnes. Pea peels turn out to be the major by-product of the pea processing industry. Annually in India, pea peel waste is discarded in large volume of almost 1 million ton.

Pea peels are main by-product of pea processing industries containing remarkable amount of dietary fiber, protein, phenolic compounds and also calcium in comparison with other vegetable by-products. The dietary fiber content in pea peels found to be around 58.6 g where the insoluble dietary fiber (54.4 g) was much higher than that of soluble dietary fiber (4.2 g) per 100 g. DF is the seventh important nutrients for organisms, which can be categorise into two parts based on solubility: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Based on the structural differences and degree of polymerisation, SDF and IDF possess different physiological functions. IDF contains cellulose, lignin, some hemicelluloses, while SDF contain sodium alginate, guar gum, pectin, dextran and so on. IDF exhibits water retention, swelling and oil retention capacity, as well as binding affinity. Also IDF has greater affinity towards increasing the faecal bulk, reducing the gastrointestinal transit time and preventing several disease like colonic cancer, obesity etc. SDF can be used to reduce plasma cholesterol and glycaemic response along with immune-modulatory activity. It also helps in preventing colorectal cancer and reduces the risk of angiocardiopathy. IDF is structurally more resistant to colonic fermentation than SDF. Based on health impacts, SDF plays a substantial character other than IDF, but the DF constituent in most of the plant-based products is IDF, about 60-80 %, which is mainly accountable for stimulating bowels function.

There are various techniques used in order to modify the DF constituents such as chemical (alkali and acid method), physical (such as ultrahigh pressure, extrusion technology and ultrafine pulverization) and using biotechnology (mostly enzymatic method) that break the glycosidic bonds of IDF by lowering the degree of polymerization. The modification leads to the breakdown of pectic polysaccharide or the degradation of partial cellulose and hemicellulose into simple saccharide structure. The modification removed the protein around the DF showing a spongy structure having more

porous structure exposed to hydroxyl groups. Biological and physical methods are highly recommended to modify DF where the enzymolysis modification can enhance the extraction yield and considered to an effective method for reducing the particle size because of their high efficiency, high specificity. In the colonic fermentation, the role of DF is significantly correlated with the digestive health. Research on prebiotic DF has evidenced that it modifies the specific gastrointestinal taxa by modulating the colonic bacteria (*Bifidobacterium*). Modification of IDF showed smaller particle size, specific large surface area as well as the roughness on fiber that contribute to structural changes of dietary fiber to simple matrix enhances the fermentability that convey prebiotic effects on appropriate general health.

This thesis explores the incorporation of DF in food model may change and improve the consistency, rheological behaviour, texture, and sensory properties of foods as well as the health directly. Yogurt is contemplated as symbiotic functional food because of its prebiotics and probiotics properties that provides therapeutic, nutritional implications to human health. The effect of different modifications in physicochemical, functional, and structural properties of pea peel dietary fiber has not been explored and also, limited studies have been carried out on the digestibility of pea peel insoluble dietary fiber and about its prebiotic potential. Moreover, there is scanty of research on lactose free yogurt with modified pea peel insoluble dietary fiber. So the use of DF as functional food with low cost, rich in bioactive compounds and promising therapeutic effects without compromising its quality parameters enrich with prebiotic compounds can be obtained.

Research Gap

1. Very limited studies have been done on the suitable extraction technique to enhance the yield and purity of the dietary fiber (DF) extracted from pea peels. There are no reports available on the modification of the DF from the pea peels using different techniques.
2. The potential of pea peel insoluble dietary fiber to exhibit prebiotic activity has not been explored. Further, pea peel insoluble dietary fiber digestibility in gastro-intestinal system has not been studied.
3. Further, incorporation of modified fiber into yogurt has not been reported so far.

The thesis is divided into six chapters which are briefly discussed below

1. Introduction
2. Impact of extraction methods on functional properties and extraction kinetic of insoluble dietary fiber from green pea peels: A comparative analysis
3. Effect of extrusion and enzyme modification on functional and structural properties of pea peel (*Pisum sativum* L.) insoluble dietary fiber and its effect on yogurt rheology
4. Prebiotic activity of enzymatically modified pea peel dietary fiber: an *in vitro* study
5. Lactose free yogurt with enzymatically modified pea peel dietary fiber: its effect on texture, microstructure and rheological properties
6. Conclusion including future prospects of the current study

Chapter-1 comprises of introduction into comprehensive profiling of chemical compositions and bioactives of fresh mature peas (specifically garden or field pea seeds) and by-products of pea processing (peels) that sets a foundation for assessment of their health benefits. It also includes different modification techniques using physical, chemical and biological methods. Gastrointestinal effects of dietary fiber also included.

Chapter 2 includes the utilization of green pea peel (GPP) by-product for its insoluble dietary fiber (IDF) which was estimated by employing different extraction techniques. A comparative evaluation using various extraction methods viz., alkaline extraction (ADF), ultrasound assisted extraction (UDF) and ultrasound assisted alkaline extraction method (ODF) was employed vis-à-vis its effect on yield, kinetics, thermal stability, morphological and functional properties of IDF. The ODF was found to be the best extraction method and standardized using rotatable central composite design with time 30 min, amplitude 30%, solid to liquid ratio (1:20), and NaOH concentration 1.2 mol/L. ODF evinced biologically rich and higher water and oil holding capacities with low peak and final transition temperature. The morphology of IDF also varied distinctly with extraction processes. ODF conclusively evidenced that it has immense potential to scale up in fulfilling the commercial demand of insoluble dietary fiber.

Chapter 3 includes the enzymatic and extrusion modifications of insoluble dietary fiber (IDF) of green pea peel to improve the functional and structural properties. The effect of the modified IDF on the yogurt rheology was also examined. The enzyme treatment was done with different concentrations of cellulase and xylanase while the extrusion modification was done with different cooking temperatures. The typical honeycomb morphology of control IDF got changed after both modifications. Brunauer-Emmett-Teller (BET) surface area ranged from 11.892 m^2/g to 15.358 m^2/g for enzyme modified sample. Enzyme modified IDF (M2) sample showed higher water retention capacity (3.25 g/g), swelling capacity (8.89 mL/g), oil absorption capacity (3.25 g/g), cation exchange capacity (0.86 mmol/g), glucose adsorption capacity (4.73 mmol/L) as compared to the extrusion modified samples. The glucose dialysis retardation index (GDRI) was significantly higher for enzyme modified samples (p<0.05). The addition of modified IDF to yogurt, changed oscillatory rheological and viscoelastic behaviour which was linear and enhanced supramolecular interaction during gel formation.

Chapter 4 comprises the prebiotic index of enzymatically modified and unmodified insoluble dietary fiber from pea peel by using three probiotic bacteria; *Lactobacillus rhamnosus* ATCC 7469, *Lactobacillus sakei* ATCC 15521, *Lactobacillus plantarum* ATCC 8014 and one pathogenic bacteria; *Escherichia coli* ATCC 4157. Interestingly, dietary fiber from pea peels that had been modified produced better results than its unmodified insoluble dietary fiber, as it promoted the growth of probiotic bacteria but not that of pathogenic *E. coli.* A positive prebiotic index value was determined which is greater than those from inulin, unmodified dietary fiber, and standard glucose. Lactic acid bacteria were grown on MRS medium with or without (control) dietary fiber and the effect of prebiotic substances on the kinetics of fermentation was examined. Throughout the 30 hours of cultivation, samples were taken for analysis in every two hours for glucose and dietary fiber concentration. Thus, dietary fiber obtained from pea peels as a prebiotic fiber that can boost cell biomass and relative growth rate of probiotic bacteria’s.

Moreover, *in vitro* simulated gastrointestinal model, the breakdown of DF may be attributed to the hydrolysis of compounds in the presence of probiotic *L. rhamnosus* ATCC 7469 (DFB) way more than fiber without bacteria (DF) with increased reaction time. These results suggest that the microbiome of the colon facilitates dietary fiber fermentation by bacteria.

Chapter 5 includes the addition of prebiotics which was modified pea peel insoluble dietary fiber to lactose-free set-type yogurt and its texture, rheology, microstructure and sensorial attributes along with its prebiotic potential, structure on dairy gel matrix were evaluated. Pea peel fiber concentration showed significant effect on *L. bulgaricus* growth count ($p<0.05$) whereas showed no significant effect on *S. thermophiles* counts ($p>0.05$). A significant change was observed in the gel firmness and cohesiveness when fiber was added from 1% to 2.5% during 28 days of storage, denoting reinforcement of structure caused by undistributed casein gel matrix.

Chapter 6 includes the overall conclusion and future prospects of the current study. The pea grown worldwide for its edible seeds is nutritionally very rich with high dietary fiber, antioxidants, numerous important biomolecules and having low glycaemic index. The ODF found to be the best extraction technique in terms of thermal stability, morphology, and the functional properties of IDF. The extrusion and enzymatic both treatment found to be effective method to modify the pea peel insoluble dietary fiber could improve the physicochemical (WRC, SC, OAC, BET) and functional (CEC, GAC, GDRI) properties effectively. Modified insoluble dietary fiber (MDF) might be used as prebiotic as it showed a significant positive prebiotic index and relative growth value. When dietary fiber was subjected to gastro-intestinal phases was more resistant to the enzymes present in the SIF and SGF. These results highlight the possibility of pea peel dietary fiber to reach the large intestine, target organ, to exert their potential prebiotic effects. Lactose free fiber enriched yogurt can be successfully utilized and gave huge scope for lactose intolerant people with improving gut problem by modulating the intestinal bacteria by incorporating prebiotic and probiotic supplements.

Extraction of phytochemicals, a detailed *in vitro* / *in vivo* gastrointestinal study and other health-beneficial properties of pea peel insoluble and soluble dietary fiber can be carried out in the future.