Biopolymers are polymers obtained from botanical, animal, microbial or marine sources. They are used in a wide range of applications mainly owing to their biodegradable and non-toxic nature. Among the various biopolymers being explored for food applications, Aloe vera and chitosan have gained a lot of attention due to the wide spectrum of activity exhibited by these naturally occurring bio materials.

The mucilaginous gel obtained from Aloe vera is well recognized as a valuable ingredient in functional foods, cosmetics and medicines. This functionality is mainly contributed by its compositional heterogeneity, mainly consisting of carbohydrates (pectins, hemicellulose, glucomannan and acemannan) and others such as phenolics, vitamins, enzymes and low molecular substances. The other biopolymer, chitosan is widely recognized for its intrinsic property of biodegradability, biocompatibility with several substances, solubility in weak acids, antimicrobial activity and other biological activities.

The study was designed with an aim of exploring the use of these two biopolymers-Aloe vera gel and chitosan for applications in postharvest technology and functional foods.

The first application involved studying the efficacy of the selected biomaterials as edible coatings for postharvest quality maintenance of fruits and vegetables. In India and other developing countries, postharvest losses in fruits and vegetables are as high as 30-40%. Among the various methods employed to minimize the postharvest losses, edible coating using naturally occurring biopolymers is explored as an eco-friendly cost effective technology. The present study attempted to evaluate these two biopolymers for extending the shelf life of tomato and fig fruits. The local horticultural department and small scale farmers reported a huge postharvest loss in these fruits.
Another section of fresh produce gaining fast popularity is minimally processed fruits and vegetables. These are highly perishable in nature and have a very short shelf life. Several techniques have been employed to minimize these losses, one of them being use of edible coatings. However, most of the literature reports on edible coatings for MP produce use the dip or spray coating technique, which in most cases causes adverse changes in the coated produce. To avoid these problems, powder coating technique was explored in the present study to extend the shelf life and quality of minimally processed carrot and radish.

Functional foods and nutraceuticals have become a key focus in the health food industry due to the increased awareness among the consumers regarding the importance of diet in the management of health and chronic diseases (WHO, 2002). Other driving factors include the sedentary lifestyle, an aging population and increasing healthcare costs (Malla et al., 2013a and b). Increased functional food consumption supported with proper health claims such as “soluble fibre from barley/psyllium is good for managing CHD” has been reported to bring about an increased consumption of soluble fibre and potential savings in health cost up to an estimated $90.84 million per year (Malla et al., 2004). In this scenario, the other objectives were set to formulate suitable functional foods using the selected biopolymers and to evaluate their efficacy as natural food additives and as nutraceutical ingredients.

The salient findings of the studies in the three major areas of applications are summarized and discussed in the following sections

I. EDIBLE COATING APPLICATIONS
a) Whole fruits

The tomato (local seeded variety, color break stage) and unripe fig fruits (Poona cultivar) were procured from the marketplace of Anantapur town, India.

The effect of Aloe gel coating on whole tomato and fig fruits was investigated in comparison to chitosan, a well explored biopolymer coating substance. The selected
biopolymers were prepared in the laboratory from the *Aloe vera* leaves and crude chitosan. These were compared with those obtained commercially during low temperature storage followed by market simulated storage conditions. The different coating solutions prepared were as follows: PAG: Processed Aloe gel prepared without additives and extensive processing; CAG: Commercially sold *Aloe vera* gel (Excel Industries, India) and diluted 3 times with water; PCH: Purified chitosan prepared in the laboratory from crude chitosan flakes (Panvo Organics, Chennai, India) - 2% dissolved in 0.5% aqueous acetic acid solution, CCH: Commercial grade chitosan (SRL Chemicals) - 2% in 0.5% aqueous acetic acid solution.

The fruits were divided into five groups. They were coated with PAG, CAG, PCH and CCH coating solutions by dipping for one minute. The tomato fruits were designated as TPAG, TCAG, TPCH and TCCH, respectively, and fig fruits as FPAG, FCAG, FPCH and FCCH, respectively. One set was dipped in distilled water which served as control, designated as TC for tomato and FC for fig fruits. The fruits were dried and were stored at 10 ºC in macro-perforated LDPE packs (18 x 21 cm; 6 mm thickness) for shelf life study.

The control and coated fruits were analyzed for various quality parameters namely physiological loss in weight (PLW), titrable acidity (TA), total soluble solids (TSS), firmness, color, respiration rate, percent decay and sensory acceptability. The analysis was carried out on 0d, 7d, 14d, 21d and 28d followed by 1 week of market simulation study (MS) for tomato. For figs, the interval analysis was 0d, 5d, 10d, 15d, 20d and 25d followed by 1 week market simulation study.

The major findings of the study include:

✅ Shelf life extension of tomato by about 7 days and figs by 9 days under refrigerated conditions and by 1 week under market simulation conditions – using PAG, PCH and CCH coatings.
The biopolymer coated fruits had better gloss, reduced chilling injury; uniform ripening, lower degree of shriveling/browning of peel, minimal browning of the inner fruit pulp in figs.

This is one of the earliest studies to demonstrate the potential of Aloe gel and chitosan coating to preserve the quality of fresh fig fruits. Aloe gel was found to perform similar or better than chitosan, an established coating material. Minimally processed Aloe gel was found more suitable as coating in comparison to pharmaceutical grade gels commercially available in the market at higher costs. The cost of biopolymer coatings used in the present study was computed. The cost of coating 1 Kg of fruits (tomato/figs) was found to be Rs.2.50/- for PAG, Rs.50/- for CAG, Rs.2.50/- for PCH and Rs.189/- for CCH coating solutions [Appendix IIa(i)].

The present study demonstrated the efficacy of the biopolymers i.e. PAG and PCH as sources of cost effective and eco-friendly primary packing material for tropical and sub-tropical fruits. Transfer of this simple, low-cost and easily adaptable technology to the small scale fruit/vegetable growers could help in improving the marketable shelf life of the fresh produce. This could in turn reduce the postharvest losses and improve revenue for the farmers.

b) Minimally processed vegetables

Effect of Aloe gel and chitosan on the shelf life stability and nutraceutical quality of carrot and radish shreds was investigated using powder coating technique. Powder coating technique was selected to avoid the problems associated with the conventional dip and spray techniques. Purified chitosan powder and spray dried Aloe gel powder (200X) were used as the coating agents.

Carrot and radish roots were shredded and coated with Aloe gel and chitosan biomaterials at different concentrations (0, 0.1%, 0.2% and 0.3%). The resultant shreds were designated as KC and RC – uncoated carrot and radish shreds,
respectively; 0.1KAG, 0.2KAG, 0.3KAG (carrot samples) and 0.1RAG, 0.2RAG and 0.3RAG (radish samples) – coated with 0.1%, 0.2% and 0.3% of Aloe gel powder, respectively; 0.1KCH, 0.2KCH, 0.3KCH (carrot samples) and 0.1RCH, 0.2RCH and 0.3RCH (radish samples) – coated with 0.1%, 0.2% and 0.3% of chitosan powder, respectively. The samples were stored in LDPE packs at 10°C and periodically analyzed for various physicochemical, phytochemical and in vitro antioxidant activities on storage (0d, 5d, 10d and 15d). The major results obtained in the coated vegetables were as follows

- Reduction in PLW with better maintenance of moisture and freshness
- Minimal changes in pH, TSS and TA
- Reduced respiration rate
- Good microbial quality and higher sensory acceptability (reduced browning and better texture)
- ↓ exudates volume and higher juiciness in radish shreds
- Extension of shelf life by about 1 week could be observed for carrot and radish shreds with AG and CH biopolymer coatings.
- Aloe gel and chitosan treatment elicited antioxidant compounds such as phenolics and the antioxidant activity and also reduced the loss of the antioxidant potential on storage.

This is probably the first report on the effect of Aloe gel and chitosan powder coating on shelf life and nutraceutical quality of minimally processed carrot and radish. Procedures for juiciness index and exudates volume devised for characterizing these important marketability related parameters for radish shreds have not been previously reported. These could be adopted for characterizing other fresh cut produce.

The cost computation of the biopolymer powder coatings was carried out [Appendix IIa (ii)]. The cost of coating 1Kg of shreds was found to be Rs.12/- for spray dried Aloe gel powder and Rs.1.6/- for purified chitosan powder. Acceptability
of Aloe gel powder as a nutraceutical additive is expected to be higher compared to chitosan, as the former is obtained from a well-known herbal/botanical source.

The present study, therefore, demonstrated innovative use of powder coating technique along with macroperforation as a cost effective technology for extending shelf life of minimally processed vegetables stored at refrigerated temperatures. The selected biopolymers could act as natural preservatives and elicitors even when used at very low concentrations, proving to be a simple, practical and healthier alternative to preserve the quality and extend shelf life of fresh cut carrot and radish. Aloe gel and chitosan powders could be promoted and further explored as non-thermal, non-toxic, safe and eco-friendly alternatives in fresh-cut industry.

II. FUNCTIONAL FOOD AND NUTRACEUTICAL APPLICATIONS

Aloe gel and chitosan biopolymers were incorporated into suitable foods and the developed foods evaluated for various quality characteristics. Aloe gel was found to be suitable for incorporation into dairy and fruit based products. Hence dahi, papaya powder and papaya RTS beverage were chosen for incorporation of Aloe gel in either fresh or dried form. Chitosan was found to be more appropriate for incorporation into cereal based products. The study thereby investigated the effects of its incorporation into whole wheat flour and chapati. The results related to product development are briefly described below.

Since Aloe gel and chitosan are known to possess antioxidant and antidiabetic activity, the effect of addition of these biopolymers on the in vitro antioxidant and antidiabetic activity of the developed products were also evaluated. In vitro antioxidant activity was evaluated in terms of estimation of total polyphenols, DPPH radical scavenging activity (%), ferric reducing antioxidant potential FRAP (μmole Fe²⁺ equivalents/g) and reducing power. Fruit based Aloe gel enriched products namely papaya powder and papaya RTS beverage were also evaluated for total flavonoids and vitamin C content. In vitro antidiabetic activity of the developed products
products was evaluated in terms of α amylase and α glucosidase inhibitory activity, the key enzymes involved in carbohydrate metabolism.

**a) Aloe gel enriched products**

- **Aloe gel enriched dahi**

Preparation of dahi enriched with different concentrations of spray dried Aloe gel powder (0%, 0.1%, 0.15%, 0.2% and 0.25%) was carried out and the samples designated as CD, 0.1AGD, 0.15AGD, 0.2AGD and 0.25AGD, respectively. The developed products were analyzed for product quality characteristics (total yield, water holding capacity, whey syneresis, and viscosity), sensory acceptability and microbial quality. The sample found to be optimum (0.15AGD) was selected for 1 week storage analysis in comparison with CD. The samples (CD and 0.15AGD) were analyzed for the changes in product quality characteristics, total viable count of LAB and sensory acceptability on storage. The major results obtained for the Aloe gel enriched dahi samples are as follows:

- Better product quality in terms of lower whey syneresis, higher water holding capacity, better viscosity, higher sensory acceptability (0.15AGD) and better microbial quality
- Higher polyphenol content and enhanced *in vitro* antioxidant and antidiabetic activity in AGD in a dose dependent manner.
- Higher total viable counts of *S.thermophilus* and *L.bulgaricus* in fresh and one week stored 0.15AGD samples, indicating a prebiotic effect.

Apart from the above mentioned effects, Aloe gel added in powder form helped in masking the unpalatable taste of fresh Aloe gel. It is a known fact that dahi/yoghurt are very sensitive products and even small changes in composition could result in deleterious effects on the body and texture of the end product, as
reported in studies incorporating components such as probiotics, fruits, and honey which adversely affects the consumer acceptability.

Incorporation of Aloe gel into dahi conferred a two-fold benefit. Firstly, an improvement in the product quality characteristics could be achieved. Secondly, it could improve the nutraceutical quality of dahi, suggesting its utility as a functional food.

- **Aloe gel enriched papaya powder**

  Fully ripe papaya pulp was mixed with the commonly used drying adjunct maltodextrin (MD) in the ratio 25:75 of the dry papaya solids and skim milk powder (2.5%). The pulp was then spread on trays and oven dried overnight at 55-60°C. To the powdered flakes, TCP was added at 1.5% level and the flakes were powdered. The powder obtained was divided into 4 sets. One part served as control and was designated as formulated papaya powder (FPP). To the other 3 sets, spray dried Aloe gel (AG) powder was added at 1.5%, 3% and 4.5% level and the powders obtained were designated as 1.5AGFPP, 3AGFPP and 4.5AGFPP, respectively. The powders were packed in metalized polyester pouches, sealed and stored at room temperature (28±3°C) and refrigerated temperature (10±2°C) for 5 months.

  Periodic analysis of the samples was carried out at 0d, after 3 months and 5 months of storage at both the temperatures for various parameters such as functional quality characteristics, physico chemical characteristics (moisture content, degree of caking, solubility, rehydration ratio, bulk density, non enzymatic browning, total soluble solids and titrable acidity), instrumental color, sensory acceptability and microbial quality. The formulated papaya powders were also assessed for their phytochemical content, in vitro antioxidant and antidiabetic activity. Aloe gel enrichment conferred beneficial properties to papaya powders as given below.
✓ Improved functional characteristics such as reduction in degree of caking and improved flowability and rehydration
✓ Better color retention and sensory quality
✓ Minimal microbial count, thereby improving shelf life
✓ Enhanced nutraceutical quality

The present investigation suggests utilization of fruit/vegetable powders enriched with Aloe gel in foods like instant mixes, custards, soup powders to improve their nutraceutical potential.

- **Aloe gel enriched RTS beverage**

  The inner *Aloe vera* gel matrix was carefully separated from the leaf, washed thoroughly to remove the latex, processed into a liquid and filtered. Aloe gel juice (0%, 10%, 20%, and 30%), papaya pulp (15%), spice extract (5%) and citric acid (0.1%) were mixed with sugar syrup to obtain a TSS of 15\(^0\) Brix. The product was hot-filled into pre-sterilized glass bottles, screw capped, pasteurized and stored at room temperature.

  The developed beverages were evaluated for sensory acceptability and the 30% Aloe gel containing papaya beverage sample (30AGPB) was found to be most acceptable and hence, selected for further storage analysis, along with 0% Aloe gel added papaya beverage as control sample (PB). The selected beverages were stored at room temperature (28±2\(^\circ\)C) in dark up to five months. Analysis for various parameters was carried out on 0d and after 45d, 90d, 120d and 150d of storage. The beverage was evaluated for physico-chemical characteristics (Titrable acidity, total soluble solids, sugar profile and non-enzymatic browning), instrumental color, microbial quality and sensory acceptability.

✓ Fruit beverage enriched with Aloe gel resulted in better product quality characteristics (physico-chemical, sensory and microbial quality)
Enhanced nutraceutical profile with improved storage stability could be achieved with Aloe gel enrichment.

The present study suggests use of Aloe gel for delivering natural antioxidants, antimicrobials and other bioactive phytochemicals towards formulation of nutraceutical rich fruit beverages. Long term consumption of Aloe gel is reported to lower levels of potassium in the blood in some conditions. Blending Aloe gel with papaya, a rich source of potassium, could help in maintaining the desired levels of potassium. Aloe-fruit beverage blends also could offer an attractive means of increasing the consumption of unpalatable Aloe gel/juice.

The analysis of additional cost of Aloe gel enriched optimum products was computed (Appendix IIb). The additional cost for 1 serving of dahi (1 cup-150ml) was found to be Rs.1.35/- (0.15AGD); Rs.18/- for 100g of3AGFPpapaya powder; and Rs.1.6-2.0/- per serving (200ml) of30AGPBRTS beverage. This additional cost could be offset partially by the higher yield obtained in dahi (increase by 4%) and in papaya powder (increase by 10%). Further, the improved technological properties and enhanced health benefits in the enriched products could compensate for the additional cost by widening the scope of food applications, consumer acceptability, and marketability.

Overall, the study on Aloe gel enriched products revealed and characterized some of the scientifically less explored properties of Aloe gel such as its function as a natural stabilizer in products like dahi/yoghurt wherein whey syneresis is a major defect; as nutritive food additive and anti caking agent in fruit powders and as a flavour regulator (imparting a balanced taste) and antibrowning aid in RTS fruit beverages.
b) Chitosan enriched wheat flour and chapati

For the whole wheat flour study, wheat grains were milled in one lot and the resultant flour was divided into four portions. The four flour portions were mixed with purified chitosan at the level of 0%, 1%, 3% and 5%, respectively, and designated as 0%CHWF, 1%CHWF, 3%CHWF and 5%CHWF. The enriched flours were evaluated for functional parameters namely water and oil absorption indices, least gelation concentration (LGC), bulk density and whiteness index. Nutritional quality (total carbohydrates, total proteins, total fat, crude fibre, minerals- calcium, sodium, zinc and iron) was determined using standard methods. Sensory characterization of the dough was carried out for kneadability, elasticity, and rolling property. Rheological characteristics of the dough were determined in terms of farinograph and extensograph characteristics.

Chapati was prepared with thirty grams of unincorporated (0%CHWF) and chitosan incorporated (1%CHWF, 3%CHWF and 5%CHWF) whole wheat flours and designated as 0%CHCh, 1%CHCh, 3%CHCh and 5%CHCh, respectively. Chapatis prepared from fresh wheat flours with and without chitosan were stored in LDPE pouches for a period of 72h and evaluated for various parameters such as moisture content, water soluble starch and in vitro enzyme digestibility. Differential scanning calorimetry analysis of the stored chapati was also carried out to compare the extent of staling in control and chitosan incorporated chapati.

The major findings of the study are given below:

✓ Chitosan incorporation improved functional and nutritional profile of wheat flour.
✓ Better rheological characteristics, kneadability, and ease of rolling were exhibited by chitosan enriched wheat flour dough.
✓ Enhanced flavour (without salt addition) and appearance /doneness in chitosan chapati.
✓ Minimal changes in the parameters indicative of staling, corroborated by DSC analysis.
✓ Greater shelf life in chitosan enriched wheat flour chapati stored at room temperature.
✓ Improvement in nutraceutical quality of wheat flour and chapati with chitosan addition.

The analysis of additional cost of chitosan enriched optimum products was computed (Appendix IIc) and found to be Rs.24/ per Kg of 3%CHWF. This will amount to a total cost of about Rs.54/- per Kg of enriched whole wheat flour which is comparable to an average cost of Rs.50-55/- per Kg of multigrain atta. The additional cost of chapati was found to be Rs.1.6/- per serving of 3%CHCh (2 chapatis). The multiple benefits accrued from the single component chitosan are likely to counterbalance the additional cost.

Some of the beneficial effects of chitosan highlighted through this study on wheat flour include its role in improving dough functionality; and as an anti-staling agent, and as a biopreservative in chapati. This study also indicates the potential use of chitosan as a sodium substitute and flavour enhancer in cereal based products. This aspect has a good scope as chitosan is also reported to alleviate hypertension through its inhibitory action on ACE.

III. Post prandial glycemic response studies of selected formulated functional foods

Among the various products developed, Aloe gel enriched dahi and chapati prepared from 5% chitosan enriched wheat flour were evaluated for post prandial glycemic response (PPGR). This was carried out initially in normal subjects and subsequently in diabetic subjects. The products were incorporated as part of a typical Indian meal for eliciting the PPGR. The control meal consisted of whole wheat flour chapati (WFCh) with plain dahi (PD). Test meal 1 (Aloe test meal) comprised of whole wheat flour chapati (WFCh) with Aloe gel enriched dahi (AGD) and test meal 2 (chitosan test meal) consisted of chitosan enriched chapati (CHCh) with plain dahi (PD). Tomato chutney was
given along with both control and test meals as an accompaniment. The developed meals were also tested for their nutrient composition.

For the estimation of glycemic response, a ‘food-based GI’ approach is being recommended as an alternative to glycemic index (GI). This involves determining the glycemic response on a whole food basis including all its constituents. Relative glycemic effect (RGE) is one glycemic expression used to describe this approach, which is based on total carbohydrate instead of available carbohydrate. In this approach, the glycemic response elicited by a meal providing 50g total carbohydrate is measured and expressed relative to 50g glucose.

After consumption of the reference/test and control meals, capillary finger-prick samples were obtained from the normal subjects at 0 minutes and after 15, 30, 45, 60, 90 and 120 minutes of ingestion of meals. For diabetic subjects, blood glucose measurements were carried out at 0, 30, 60, 90, 120, 150 and 180 minutes. The incremental area under the blood glucose response curve (IAUC) to reference, control and test meals were calculated geometrically using the trapezoid rule (FAO/WHO, 1998). The glycemic response for each of the experimental meals was calculated for each subject as a percent ratio between IAUC of test and control meals and the same subject’s IAUC for reference food (Wolever et al., 1991). The mean glycemic response was expressed as RGE.

Significant reduction in the blood glucose responses of both normal and diabetic subjects could be achieved with biopolymer enriched meals. These results correlated well with the in vitro testing wherein the AGD and CHCh recorded significantly higher inhibition of the carbohydrate hydrolyzing enzyme compared to the control products, suggesting their potent antidiabetic effect.

This is one of the initial studies to evaluate relative glycemic response of the biopolymers Aloe gel and chitosan as a part of a food matrix and not as pure Aloe gel/juice or chitosan tablet/capsules. In vivo results suggest that in the food matrices
evaluated, biopolymers retained their bioactivity and were assimilated to produce the desired effect as revealed earlier under *in vitro* conditions.

The nutraceutical activity of studied biopolymers suggests feasibility of developing antioxidant rich, low glycemic functional foods with promising commercial potential as value added health foods. These functionally enhanced products could be utilized by populations to ameliorate oxidative stress related disorders.

The present investigation thereby helped in evaluating Aloe gel and chitosan biopolymers and in identifying their multi functionality in foods. These biopolymers could cater to the current focus of the modern food industry, for safe natural ingredients with a wide array of useful properties. Addition to suitable products can create opportunities for blending technological aspects these biopolymers with their health enhancing properties.