

## Summary

The report comprises of a brief on achievements, outcomes from R&D projects including the societal, supra-institutional and network projects. Some of the measurable outcome from the R&D activities during 2008-09 are listed below:

<b>Achievements at a glance 2008-09</b>	
Patents filed in India	18
Patents filed Abroad	10
Research Papers Published	140
Number of Reviews	12
Number of Book Chapters	3
Number of Papers in Proceedings	4
Number of Grant-in-aid Projects	76
<b>Industrial Achievements</b>	
Number of Consultancy Projects	44
Number of Sponsored Research Projects	9
New Processes Developed	14
Technologies Transferred to Industries	49
Product Samples Analyzed	2454
Number of Technical Counselling	477
Number of Technical Enquiries	5233
<b>Human Resource Development</b>	
M.Sc. Students Passed Out	25
ISMT Students Passed Out	16
Number of Short Term Courses Conducted	41
Number of Ph.Ds Awarded	25

## Summary of the R&D Projects

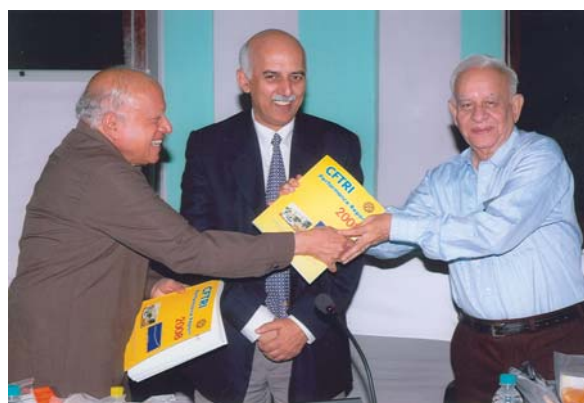
Achievements under R&D projects have been highlighted under nutrition, nutraceuticals, functional food, processes, products, automation, biotechnology, biomolecules, ameliorative studies, analytical strategic research, societal programs. This also comprises work carried out under the supra-institutional project and network project in which CFTRI is the nodal laboratory.

### Nutrition

Enhancement of bioaccessability, nutraceutical oil blends, role of enzymes on *Chapati* making quality, fresh water fishes as the source of lipids and the use of fish eggs for the preparation of health-beneficial bakery products were the major topics covered.

Influence of therapeutic levels of exogenous iron and calcium on the bioaccessability of zinc from selected staple grains were examined. Soy protein had negative effect on iron bioaccessability, whereas the raw and cooked grains enhanced the zinc bioaccessability by 50% and 90% respectively. Beta-carotene rich sources such as carrot and amaranth and curcumin were found to have positive effects on zinc and iron bioaccessability from food grains. Antioxidant rich oil blend was prepared using rice bran oil, sesame oil, lignan and tocopherol concentrates. The sensory attributes were found to be comparable to that of any commercial samples. Garden cress (*Lepidium sativum*), an edible underutilized herb was assessed for fatty acid composition, Sn-2 composition of fatty acid in triglycerides, quality, storage and stability. The

oil was stable up to four months when stored at 4°C. In order to enhance the nutritional status of coconut oil and also to make it pourable at low temperature, coconut oil was blended with linseed, safflower and sunflower oils. The blend of 70% coconut oil to 30% sunflower seed oil improved the pourability and nutritional status. Trans-fatty acid free plastic fats as an alternate to hydrogenated *fat* and cocoa butter substitutes were prepared. *Sal*, mango, palm, palm oil fractions and coconut oil fractions were used for interesterifications. The coconut oil using membrane filters to improve its nutritional quality was carried out. Coconut oil blends with sunflower oil, rice bran oil and palmolein ensured high stability and sensory acceptability. Blends of coconut oil were prepared with sunflower oil, rice bran oil, safflower oil, groundnut oil, sesame oil and palm oil. The incorporation of coconut oil to sunflower and rice bran oils provided oxidative



Prof. M.S. Swaminathan, Chairman, CFTRI Research Council, releasing the CFTRI Performance Report 2008



stability, where as the addition of sunflower oil and rice bran oil to coconut oil contributed to radical scavenging effects of the blends. Two structured lipids containing 29% behenic acid were prepared by enzymatic interesterification. These lipids have the physical characteristics similar to that of bakery products, making it suitable for the preparation of low calorie biscuits and cakes. Palm oil was fractionated and its suitability was assessed for its use as a vanaspati substitute. Transfatty acid free bakery shortening was prepared from palm oil fractions blended with rice bran oil.

Role of enzymes such as superoxide dismutase and phenol oxidases in the cross-linking of wheat protein on quality of different wheat varieties were studied. Proteins and pentosans were isolated from different wheat varieties to study their effect on the *chapati* making quality. High pentosan content was observed on wheat cultivars with good *chapati* making properties, along with high arabinose - xylose ratio. Effect of peroxidase on textural and physico-chemical parameters of wheat dough was carried out. Significant increase in dough hardness and decrease in adhesiveness was observed, upon incorporating peroxidase. The extent of cross-linking of gluten subunits by peroxidase was found to be dependent on enzyme concentrations.

Fresh water fishes such as *Catla*, *Rohu*, *Mrigal*, Common carp and *Tilapia* were analysed using their meat, head and viscera parts for total fat content, lipid classes and quality. The study indicated the potential of fresh water fish wastes as a source of lipids. Fish egg lipids of most of the fishes contained significant amount of phospholipids. The use of fish egg protein

concentrates into bakery and extruded products resulted in improved protein content.

Detoxification of oilseeds, *Jatropha*, *Karanja* and *Simrouba* were carried out using different methodologies. Rats were fed with casein, 50% detoxified meal and 100% detoxified meal and the efficacy and the level of detoxification of the meal was assessed. Removal of the toxic and anti-nutrients in all the three oilseeds were found to be partial.

Sugar was fully replaced with alternate sweeteners and its effect of these sweeteners on viscosity, texture and sensory quality of chocolate was studied. It was evidenced that polyols like maltitol, xylitol and isomalt could be used to prepare sugar free chocolates without affecting the rheology and sensory quality significantly. Butter spread enriched with polyunsaturated fatty acids (PUFA), blend consisting of *Sal* and RBO along with hardened oil showed a melting profile comparable to that of commercial butter. A process for cereal bar preparation with dry fruits and nuts was standardized.

### Nutraceuticals

Antioxidant activity of hydrolysates prepared from Japan seaweed, *Akamoku*; antiinflammatory, antioxidant and antiulcer properties of the nutraceutical molecules derived from sesame, marigold and *karanja*; influence of xylooligosaccharides (XOS), a non-nutritive sweetener; and the radical scavenging and antioxidant properties of the *Mangosteen* (*Garcinia mangostana* L.) pericarp extracts were assessed.

*Njavara* rice, cooked along with herbs is used for ayurvedic treatments. The rice is easily digestible with good high fiber content and vitamins, it could be a good source for infant and geriatric foods. Brown rice obtained by bioprocessing (controlled germination, hydrothermal treatment, drying, devegetation and dehusking) had oryzanol retention up to 100%, tocotrienols (80-96%), vitamin E (70-76%) and antioxidant activity (45-77%). *Njavara* and *Jyothi* were found superior compared to IR 64 due to the presence of higher content of lipid soluble antioxidants. Thermal stability of antioxidants *Chilli* and pepper extracts incorporated in to soyabean oil, sunflower oil and groundnut oil were evaluated.

### Functional Foods

Lactic acid bacteria cultures isolated from selected food materials were studied for inhibition zone of *V. cholerae*. *Pediococcus pentosaceus* MTCC 5151 culture showed maximum inhibition to *Shigella* from feces. *Lactobacillus plantarum* has high antimicrobial activity against toxic food pathogens such as *Klebsiella*, *Acetobacter*, *S.typhi*, *L.monocytogens*, *Shigella*, *E. coli* and *Pseudomonas*. An oat based beverage supplemented by this culture was prepared. Bacteriocinogenic cultures were isolated from vegetables, cereals, intestinal sources and fermented foods. Screening of the pediocin, PA-1 processing lactic acid bacteria from vegetables, dairy and fermented food was done.

Biological activities of enzyme hydrolysis of proteins and synthesis of peptides generated from food protein hydrolysates were studied for their

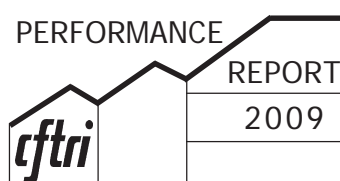
use as ingredients in functional foods. ACE inhibitory activity of proteins such as whey, casein from bovine milk, watermelon seeds and ground nut proteins were evaluated. The whey protein hydrolysates showed ACE inhibition and antioxidant activity with  $IC_{50}$  values of 0.23 mg/ml and 6.9 mg/ml respectively.

Onion agglutinin (*Allium agglutinin*, ACA), a 50 kD mannose-specific lectin having subunit was obtained from onion. ACA agglutinates rabbit erythrocytes. Onion agglutinin has moderate immunomodulatory activity with respect to lymphocytes proliferation.

Oligosaccharides are popular as a probiotic and there is a large demand for it as functional foods. Water soluble polysaccharides (WSP) and water insoluble residues (WIR) from bengal gram husks subjected to xylanase treatment yielded 8.8 and 5.2% of xylooligosaccharides (XOS) respectively. XOS from WSP consisted of xylose and arabinose in the ratio 1:3.5 where as from WIR consisted xylose, arabinose and galactose in the ratio 1:1:2.

### Processes

Recovery of value-added products from the wastes of poultry industry wastes and sea foods processing were taken up. Protein was extracted from poultry intestine. Conditions were optimized for the maximum proteases activity in the extractants. The effect of ensilaging on the proteases activity was assessed. Studies were also conducted for the stabilization of the enzymes using various organic solvents.



To evolve an integrated biocompatible methods for downstream processing for natural colorants and enzymes, separations with membrane processing, aqueous two phase extraction (ATPE) and reverse micelle extraction (RME) were carried out. *Kokum* extract, was made using direct osmosis and osmotic membrane distillation at ambient temperature and atmospheric pressure. Integrated membrane processing (IMP) using suitable MF and UF membrane enhanced the specific activity of the submerged fermentation (SmF) PG nearly fivefold. The IMP approach revealed its potential for purification as well as concentration for the SmF-PG. The experimental results showed UF has the potential to be a single step process for desalting and concentration of eluted PG. *Okara*, a byproduct obtained during processing of soymilk is an underutilized source of protein. To find out the effect of particle size on the recovery of protein, solubility of soybean and *Okara* protein was studied at different pH levels. The outcome in terms of protein recovery, quality and productivity indicated the use of MF 450 nm membrane for the optimum results.

Extension of storage life and quality parameters of pink flesh guavas (CV.L-49), brinjals and cauliflowers were evaluated for different packaging films and temperatures. Coffee industry substrates such as coffee pulp, coffee cherry husk, silver skin, spent coffee and a mixed combinations were evaluated for its efficacy to be used as a sole carbon source for the synthesis of alpha-amylase in solid state fermentation (SSF). Maximum alpha-amylase activity was observed in the case of coffee pulp and the mixed combination with steaming. Conserves and value added products were prepared using pepper and

ginger oils extracted from pepper powder and dry ginger powder dried with spent residues. Sensory analysis and consumer acceptance studied showed that the bengal gram flour based *Sohan papdi* was more acceptable than the one made using wheat flour.

Bacterial PHA production using starch as a carbon source was investigated. Around 200 cultures from different soil samples were isolated, purified and maintained on nutrient agar slants. Twenty of these isolates were found to produce a maximum of 63% PHA in the biomass with sucrose or glucose as carbon source.

## Products

The *Chapatís* prepared using whole wheat flour adding selected preservatives, stored for 21 days were compared against the fresh *Chapatís* and sensory studies showed acceptable quality. In order to make fabricated wheat based breakfast cereals, moisture and powdery mixture of the whole wheat flour, corn grit along with other ingredients were extrusion cooked and flaked. Flakes were dried and coated to prepare the breakfast cereals. Bengal gram dhal and green gram dhal flours were selected to prepare legume based pasta and their chemical characteristics were analyzed. Multigrain mix (MGM) prepared using oat flakes, soyabean, fenugreek, flax seeds and sesame seeds by milling them into grits of equal quantity. Replacement of whole wheat flour with 15% MGM was used as the control with the combination of different additives. MGM (15%) showed significant improvements in the volume and decrease in crumb firmness with enhanced sensory attributes. A formulation for the

preparation of multi-grain bread was standardized. Selected raw materials viz. wheat flour, *Aestivum* and durum semolina, white oats, coarse wheat bran, defatted soy flour (DSF) and whey protein concentrate (WPC) were analyzed for the protein content and dietary fibre for the preparation of instant vermicelli,. Vermicelli with *Aestivum* semolina was found to be more suitable compared to durum semolina. Non-wheat protein supplementation with DSF and WPC blended at different levels was evaluated for rheological characteristics of the wheat flour dough. Wheat flour was substituted by finger millet flour showed that 60 % finger millet flour was optimum in muffins. Thermally processed *Chapaties* kept in refrigerated storage for a period of 6 weeks were evaluated in comparison with the freshly prepared ones. Standardization of the recipes for the preparation of *Wadian* with various blends of cereals, pulses and vegetables were carried out. Blends of ready-to-serve pulpy fruit juices (smoothies) was prepared from selected regional varieties of fruits; papaya, mango, pineapple, watermelon, banana and grapes.

Under the supra-institutional projects, nutrition intensive products were formulated using quality proteins fortified with micronutrients. These include: Supplementary foods for children, Fruit and vegetable based snacks, Egg albumin *paneer*, Vegetable oil, Nutra-rich *chikki* and fortified beverages.

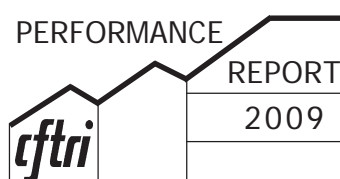
Shelf-life extension of *Capsicum* using chitosan and chitosan blended poly-psi-capralactone was tried. *Capsicum* samples packed in chitosan blended could delay-the ripening and extended shelf life for 2 weeks.

## Automation

Conceptual design of an automatic weighing machine for wheat products, extrusion and encrusting of traditional snack, kargikai and machineries for integrated coconut processing were at different stages for the automation. In the network project, design and development of machineries were made for *Roti* making units, Forming and frying units for *Murukku/Sev*, *boondi* and *poori*; Lemon cutting machine, *Jilebi* forming unit, Rotary drum roaster, Mini *chakki* mill, low-moisture foods, Garlic peeling machine, Pressure frying units for meats and Laboratory freeze dryer. Also the isolation of bioactive and nutraceutical compounds from curry leaves and fenugreek seeds were carried out.

## Biotechnology

Two fold increase in the production of nigerloxin was achieved supplementing wheat bran with appropriate content of sweet lemon peel, methanol by controlling various process parameters such as moisture content, initial inoculum size and incubation period. The product was found stable between the pH 4-5. Efforts were made to detect and quantify the intracellular vitamin B<sub>12</sub> produced by lactic cultures. Competitive ELISA was used for the detection of cobalamin concentration in the culture extracts of lactic acid bacteria. A simple, quick and sensitive chemiluminescence (CL) based method in which parameters such as luminol concentration, hydrogen peroxide concentration and pH were optimized for obtaining the maximum CL. *Spirulina* biomass extracted was evaluated for B<sub>12</sub> using HPLC, microbiological assay, AAS and chemiluminescence methods.





Coffee *in vitro* cultures were taken to find out the role of *Coffea canephora* and caffeine production in the embryos. Transformation studies to develop plants with antisense and RNAi constructs of N-methyl transferase is in progress.

Characterization of one of the transgenic coffee plant for correct integration and functioning of the inserted fragment i.e. RNAi or antisense construct of N-methyl transferase was completed. *Bixa orellana* roots obtained by *in vitro* normal root cultures was able to produce ~0.36% of the total annatto pigment. Different abiotic and biotic elicitors were used to find out effect on annatto pigment production in the normal roots. The total annatto pigment content during the ontogeny of *Bixa orellana* L. fruit was analyzed. Extraction of RNA from the seeds and seedlings of *Bixa* and cDNA from extracted RNA were standardized. Primers were designed for three important enzymes involved in the bixin biosynthesis pathway. Genetic transformation of *Dunaliella bardawil* with *astaxanthin biosynthetic gene* from *H. pluvialis* was tried. Amplification of approximately 1.9 kb was obtained from the genomic DNA of *H. pluvialis* for beta-carotene ketolase gene. Cloning of beta-carotene ketolase gene into binary vector is under progress. Suitable shoot multiplication media using Thidiazuron (TDZ) was standardized for explants such as cotyledonary leaf segments, hypocotyls and leaf segments.

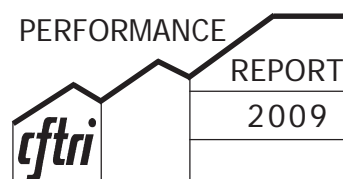
*Monascus purpureus* MTCC 410 fermented rice (red mould rice) is one of the food supplements used in order to lower the blood-lipid levels. Safety of the red mould rice (RMR) was studied in albino rats. Rats fed with the RMR showed significant

reduction in the cholesterol and triglycerides levels in both serum and liver without causing any toxic effects in the rats. UV mutant of *Aspergillus carbonarius* grown in shake-flask cultures over produced polygalacturonase and accumulated carotenoids like partially saturated astaxanthin and canthaxanthin in their biomass. Differential display RT-PCR techniques are being standardized for the pigment synthesis.

The pattern of gene expression for several genes involved in carotenoid biosynthesis in tomatoes was studied. Arka Ahuti showed highest carotenoid accumulation at BR7 stage, where typically a higher expression of upstream genes coincided with sudden drop in the expression of downstream genes. Also tissue culture protocols for the *in vitro* growth of tomato (Cv. *Arka Ahuti*) were also standardized. mRNA-differential display technique coupled with silver staining was developed for the identification and isolation of cDNAs representing transcripts, differentially expressed during banana ripening process.

## Biomolecules

The allergens from egg plant pulp, (43, 45, 64 and 71 kD) were characterized as glycoproteins by periodic acid-Schiff staining. Zymograms exhibited beta-fructofuranosidase activity in 52 and 54 kD proteins. Coffee diterpenes, cafestol and kahweol are known to be cholesterol elevating factors present in the saponifiable fraction of coffee brew. Documentation of the levels of cafestol and kahweol in coffee and the effect of various brewing and roasting methods on diterpene profile are in progress.



N-terminal peptide (residues 1-13) mimicked obestatin, a 23 amino acid C-terminus amidated peptides produced in stomach. Middle fragment (residues 6-18) reduced epididymal fat without altering the feed intake or gain in body weight. Phe5 and Gly8 were substituted and synthesised to obtain Nt5cha and Nt8U peptides and by replacing both Gly3 and Gly8 in the middle fragment, Mf38dU was obtained. The substitution of glycine with alpha-aminoisobutyric acid at position 8 in the N-terminal fragment rendered the peptide as effective as obestatin. Also the double substitution of glycine at position 3 and 8 by alpha aminoisobutyric acid in the middle fragment has led the peptide, Mf38Du to be effective in reducing the gain in body weight, total cholesterol without affecting its ability to reduce the epididymal and perirenal fat.

A potent trypsin inhibitory activity detected in sword bean (*Entada scandens*), was evaluated against a trypsin like proteinase from the fifth instar larvae of rice moth, *Corycyra cephalonica*. The Kunitz inhibitor ( $K_i$ ) value suggests that it exhibits a greater affinity to the mid gut proteinase.

Glycosidases were employed to prepare selective phenolic and vitamin glycosides like retinol, ergocalciferol, cholecalciferol, capsaicin, DL-dopa, L-dopa and dopamine glycosides which are more water soluble than their aglycons and are used as food additives and nutraceuticals.

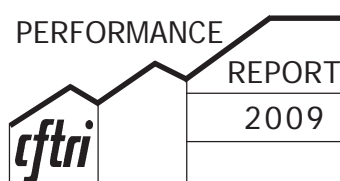
Amyloglucosidase from *Rhizopus* mold and beta-glucosidase isolated from sweet almond were used for catalysis. Synthesized glycosides were tested for antioxidants and ACE inhibitory activities.

## Ameliorative studies

*Bacopa monnieri* ethanolic extract (BME) and brahmi capsules were supplemented in diets to adult males of *Drosophila melanogaster* for a period of 7 days. Preliminary studies have indicated that BME has a potential to alleviate rotenone induced neurotoxicity in *Drosophila* model. *Bacopa monnieri* standard extract offered significant protection against paraquat induced mortality.

*Banana*, a rich source of bioactive compounds and dietary fiber is considered effective as an anti-diabetic agent. Methanol and water extracts of flower showed 3.9% and 3.4% antioxidant effects respectively. DPPH assay of methanol and water extracts for flower and stem were found 17 and 19% respectively.

*Alpha-Synuclein* is found in the form of aggregates in Parkinson disease affected brains and it was found glycosylated in the aggregation. Effect of glycation on DNA binding was studied using conformation specific oligonucleotides, 5' CGCATATATGCG 3'. Circular dichroism spectroscopy showed that *alpha-Synuclein* bound to 5' CGCATATATGCG 3', but not induced any conformational change in the oligonucleotide. *H.pylori* was isolated from endoscopic samples of gastric ulcer patients. The Swallow Root Pectic Polysaccharide (SRPP) showed effective healing of acetic acid induced gastric ulcer (~88%) at 200 mg/ kg body weight. It was inferred that healing was due to the ability of SRPP in enhancing the mucin synthesis, inhibition of H<sup>+</sup>K<sup>+</sup>-ATPase enzyme which controls acidity and also inhibition





of *H. pylori*. SRPP treatment showed clear evidence of restoration of epithelium and reduced inflammatory exudates after 5 days of treatment. The treatment also increased mucine synthesizing cells and regenerated completely similar to that of controls after 10 days by upregulating the production of PGE2 and by down-regulation of matrix metallo proteinases.

Amelioration of hyperglycemia and other associated complications were studied with finger millet, pearl millet and foxtail millet. Lipid soluble antioxidants such as tocopherols and carotenoids are rich in finger millet and pearl millet, and the polyphenols was lower in foxtail millet. In terms of total antioxidants and free radical scavenging activity, finger millet was more potent. Galectin-3 is a galactoside-binding protein, which plays a key role in cell-cell matrix interactions leading to invasions and metastasis. Stages of the diseases with galectine-3 level and Swallow Root Polysaccharide (SRPP) inhibition of metastasis were correlated. Diabetic induced *prepubertal* (PP) rats of 4 and 6 weeks old were investigated for oxidative dysfunctions in *mitochondria/microsomes*. Evidence suggested during acute phase, testis mitochondria/ microsomes were less susceptible to oxidative stress compared to stress during progressive phase. Amyloid beta-peptide is a key etiological factor involved in the pathogenesis of *Alzheimer's* disease. Investigations were carried out to understand the mechanism of amyloid beta-enantiomers binding to DNA and to characterize nicking property of amyloid. The effect of dietary supplementation of *Withania somnifera* (WS) during pregnancy for protection against preeclampsia induced oxidative stress in rat models was studied.

## Analytical strategic research

A simple analytical device was developed for noninstrumental immunoassay. The device was used to estimate low concentrations of DDT in food samples by using an improved catalysed reporter deposition. Spiked and contaminated samples of milk, soft drinks and vegetable samples were analyzed. The dipstick chemiluminescence method was able to detect the DDT at 50 ppt level where as the gold nanoparticles based dipstick assay could show the sensitivity in the range 1000-30 ppb.

Bioactive molecules having insecticidal activity against *Rhizopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum* and *Callosobruchus chinensis* were characterized. The compounds were also found effective against wheat and green gram infestation in grain treated at 0.1 g/kg. Usefulness of *C. elegans* as a model for toxicity of anticholinesterase compounds was demonstrated. *C. elegans* could be suitably exploited for studying the oxidative stress due to toxicity by pesticides. Suitable detection methods of GMOs in insect resistant Bt-brinjal and Bt-cauliflower, DNA based methods targeting 126 bp fragment of the junction region between CaMV 35S promoter (not duplicated) and cry1AC gene of the transgenic construct inserted in Bt-brinjal were developed and validated.

Bacterial cultures belonging to DDT degrading microbial consortium were screened for the DDT-dehydrohalogenase activity. The cell free extract of *Pseudomonas putida* T5 showed higher DDT-dehydrohalogenase activity and enzyme was purified to apparent homogeneity with 73% overall

recovery. The primary sequence of HPLC purified enzyme protein was deduced by LC-ESI-MS.

### **Societal Programs**

Food Processing Training Centres (FPTC) were established at Nizamabad and Anantapur districts (Andhra Pradesh) in coordination with the District Rural Development Agency (DRDA), Government of Andhra Pradesh. Various equipments and

machineries were installed for the dehydration of fruits and vegetables. Awareness and hands on training programmes were arranged for the entrepreneurs and DRDA personnel in which the Ready to serve beverages (pineapple), Squash (orange), Mixed fruit jam, Tomato ketchup, Chilly sauce, Ginger and onion brine, Ginger and garlic pastes as well as dehydrated onion and potatoes were demonstrated.

