Summary

The Performance Report of CFTRI highlights progress of the work done with respect to R&D projects during 2007-08. During this period, research and development activities under in-house, network, grant-in-aid and industrial projects were carried out. Some of the measurable outcome are given below:

Achievements at a glance 2007-08	
Patents filed in India	24
Patents filed Abroad	5
Research Papers Published	194
Number of Reviews	20
Number of Book Chapters	7
Number of Papers in Proceedings	1
Number of Grant-in-aid Projects	98
Industrial Achievements	
Number of Consultancy Projects	47
Number of Sponsored Research Projects	10
New Processes Developed	6
Technologies Transferred to Industries	42
Product Samples Analyzed	2468
Number of Technical Counselling	481
Number of Technical Enquiries	4322
Human Resource Development	
M.Sc. Students Passed Out	24
ISMT Students Passed Out	16
Number of Short Term Courses Conducted	44
Number of Ph.Ds Awarded	24

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Summary of the R&D Projects

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Pigmented variety of paddy was found to be highly susceptible to breakage on milling and its bran contained considerably lower levels of riboflavin than the non-pigmented varieties. High swelling power and solubility values of pigmented varieties of rice make it suitable for use in specialty foods. Health bar containing fenugreek and garden cress seeds, in addition to the other cereal/legume ingredients, particularly targeted for lactating mothers, was formulated. A laboratory scale machine for the production of health bars was designed and a prototype has been fabricated. Carbohydrates, fats, apparent amylose fatty acid profile and triglycerides of *Njavara* rice were comparable to *Jyothi* and IR 64 varieties. However, *Njavara* rice had 16.5% higher protein and higher dietary fiber content compared to the other two rice varieties.

Licorice, Pippali and *Talisapatra* extracts were used in the candy formulation. Chocolates prepared adding *triphala* powder at known levels are acceptable, in spite of lesser colour. Nutritive attributes of porridge could be enhanced by adding *dalia*, rich in protein and carbohydrates. Ready-mix formulations for pizza base of improved quality characteristics and whole wheat bread were developed. Multigrain *pasta* and high fibre pasta were formulated. Decorticated finger millet (*Ragi-rice*) possessing soluble fiber and straight chain fraction of starch in a larger proportion than the native millet, has good shelf life. Processes were developed for preparation of ready-to-prepare stiff porridge (*mudde*) and flakes from finger millet. Total polymeric protein content in the wheat flour protein increased dough hardness and *chapati* texture while high thiol content in the flour decreased the same.

A pilot scale process for health oil blends and a method for the preparation of pure phytosterols from soybean oil deodorized distillate, were developed. The nutraceutical concentrate isolated oil from treated sesame seeds was used to stabilize sunflower oil. Chemical refining and bleaching of coconut oil were standardized in the laboratory scale. Coconut stearin and palm stearin were suitable for the preparation of trans fatty acid free plastic fats acids. Nutraceuticals-rich interesterified fat, was prepared using a blend of coconut oil solid fraction and palm oil liquid fraction. Blending solid fraction of coconut oil with sunflower oil and rice bran oil were useful as frying oils. Lipase catalysed

Prof. M.S. Swaminathan, Chairman, CFTRI Research Council, releasing the CFTRI Performance Report 2007

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interesterification extended the plasticity of sal-mango blends with palm stearin was a suitable substitute for commercial hydrogenated fats. The interesterified fats were trans fatty acid free, and had better hypolipidemic effects compared to blended oils of similar fatty acid composition.

Ajowan ethanolic extract was found effective to prevent ochratoxin (a potent mycotoxin) contamination in foods. Oregano-based beverage, a mouth freshener, herbal candy, instant dip-mix from Indian borage and beverage from nutraceutical-rich concentrate of *ajowan* have been also developed in the laboratory. Sesamol could inhibit lipoxygenase and tyrosinase. Sesame lignans were supplemented in *chutney* powder for deriving health benefits. Karanjin inhibited H^+ - K^+ ATPase from parietal cells with an IC_{50} value of 39.5μg. Protein isolate prepared using the combination of micro filtration and ultrafiltration was found to be superior to the traditional isoelectric protein in terms of functional properties, yield and purity. The presence of food acidulants - lime juice / amchur and antioxidant spices, turmeric/ onion- proved to be beneficial in deriving maximum β-carotene from the vegetable sources. Addition of milk enhanced the bioaccessibility of provitamin A from the fruit pulps of mango and papaya. Oils of safflower and flax seed could be encapsulated upto 60-65% compared to 70 - 78% of bakery fat. Fat powder with bakery fat was more stable against oxidative rancidity than that from other oils. Fat rich powders were useful in the preparation of instant mixes as they facilitate uniform mixing.

Intense sweeteners could only impart sweet taste but not the characteristics of the traditional sweet products. Crackers and cakes, prepared replacing bakery shortenings with structured lipids, possessed highly acceptable sensory attributes. Chocolate prepared by replacing cocoa butter with a known structured lipid, though soft, exhibited acceptable overall quality and sensory attributes. This structured lipid, free of trans-fatty acids, was found to be an ideal replacement for hydrogenated shortening.

Coconut oil was blended at 20% level with vegetable oils, to provide medium chain fatty acids to non - coconut oil consumers. The blends did not have any coconut oil smell and retained all the natural antioxidants, A process for the production of coconut spread based on mature coconut-water concentrate and coconut dietary fibre was developed. Coconut protein powder was obtained by spray drying coconut whey concentrate.

Roasted fenugreek seeds showed significant anti-lithogenic influence similar to raw seeds. Enzymatic treatment enhanced the yield of essential oil from garlic bulbs without affecting the antioxidant activity. Garlic bio-oleoresin, free from toxic solvent residues, could find application as a flavourant and nutraceutical for incorporation into food. A water soluble turmeric colourant, for food applications, was prepared from spent turmeric oleoresin, a disposable turmeric industry waste. Curcuminoids-enriched odour free turmeric powder was prepared for use as natural colourant, without the turmeric odour. Demethoxycurcumin and

bisdemethoxycurcumin and curcumin could be used as nutraceuticals as well as preservatives in food formulations.

Growing *Pleurotus* mushroom using caffeine containing coffee processing industry wastes such as coffee pulp, coffee hull and spent caffeine, as substrates, is found to decaffeinate the substrates. Theophyline, at 150-450 ppm concentration, can suppress the growth of Hep3B. Pre-treatment with calcium chloride and calcium lactate improves the texture of *amla* and effectively maintains the colour, texture and ascorbic acid content. Process conditions were optimized for the preparation of vegetable cereal flakes. Conditions for dehydration of Ivy gourd slices and Ivy gourd beverage formulations with lime and ginger have been standardized. Process parameters for the preparation of blended RTS beverage from a 1:1 mixture of grape and phalsa juices with or without carbonation, is also standardized.

The influence of the incorporation of *makhana* in *kheer* and *laddoo*, was assessed. Amylographic studies were carried out with a blend of makhana flour along with wheat flour, *maida*, rice flour and water chestnut flour. Banana pseudostem juice was found to solubilise calcium oxalate and this could provide a good counter to kidney stones. Freeze concentration of banana juice, had characteristic banana flavor.

A new process integrating liquid-liquid extraction with either aqueous two-phase or reverse micellar and a membrane process for downstream processing, extraction or purification of biomolecules, was explored.

Recovery of bioactive components from fruit and vegetable by supercritical fluid extraction was also examined. Aqueous two phase extraction (ATPE) was used to remove free sugars from betalains to enhance their stability and shelf life. Higher purity of C-phycociyanin (4.32) with an yield of 80%, was achieved by ATPE. The interference of food matrices in the detection levels of *Bacillus cereus* in liquid foods by PCR, pointed to the need to develop protocols using ATPE, for removal of PCR inhibitors. Polyvinyl pyrilidones, at appropriate concentrations, have found to help in countering the inhibitors of PCR in saline and fruit juices.

An integrated process, involving ultrafiltration (UF) and direct osmosis (DO)/osmotic membrane distillation (OMD), was developed for the purification and concentration of bromelain from pineapple waste. Concentrated bromelain extract was found to be more stable (in terms of proteolytic activity) than the dilute enzyme extract at refrigerated as well as ambient conditions. Reverse micellar system of sodium bis(2-ethylhexyl)sulfosuccinate (AOT)/isooctane was used for the extraction and primary purification of beta-galactosidase from the aqueous extract of barley (*Hordeum vulgare*).

The phenolic components of *Garcinia mangostana* had considerable radical scavenging activity. Supercritical fluid extraction (SCFE) was used to extract key bioactive components from *chiba* seed (*Psoralea corylifolia L*) oil. The formation of the glycosylated compounds of bakuchiol was more pronounced in the SCFE method. Solanesol oleate was synthesized from oleyl chloride and

solanesol by condensation using sub-critical carbon dioxide.

Capsaicinoids and colour components from chilli pepper (*Capsicum chinense*) were extracted by SCFE. The temperature, 40^0C, was optimal to obtain the best yield. Moringa leaf protein exhibited maximum antioxidant activity in erythrocyle ghost model system. Drying spices (mint and *tulsi*), using a combination of hot air and RF dying not only improved the product quality but also reduced the processing time. Root extracts of *D. hamiltonii* exhibited protective action against hepatotoxic effect of ethanol and carbon tetrachloride in laboratory rat models.

Of the four wheat varieties studied, GW322 and NI5439 had better *chapati* making properties compared to MACS2496 and HD2781. Incorporating of enzymes did not alter the total amylose and soluble protein contents in *chapatis. Chapatis* made from partially baked dough sheet samples had appearance and overall quality almost similar to control. Fully pre-baked *chapatis* had slightly lower aroma and textural characteristics.

DEAE cellulose chromatography of pancreatic alpha-amylase (PPA) revealed two activity peaks, PPA-I and PPA-II, both of which are found to be isoforms due to their molecular weights being almost the same. Citric acid and oxalic acid inhibited PPA and its isoforms in a concentration dependent manner. Maize WSNSP inhibited PPA and its isoforms to a larger extent compared to the WSNSP of *ragi,* wheat and rice. The spectral characteristics of glycosylated and deglycosylated PPA and its isoforms, in the far-UV region, did not reveal any significant differences. Addition of Ca^{2+} was found to enhance the activity of wheat bran peroxidase, purified in the absence of Ca^{2+}.

Raw garlic extract was subjected to gel filtration and cation exchange chromatography for obtaining purified mannose specific garlic lectins, ASA I and ASA II. The anti-lectin IgG response for ASA I and ASA II was fairly significant compared to that with the reference protein ovalbumin. Only ASA I had a marked mucosal adjuvant activity. Dietary garlic could normalize the marginally reduced diet intake of diabetic rats, in addition to decreasing degree of polyurea. Garlic powder in the diet could mitigate embryo lethality in diabetic pregnant rats. While dietary garlic did not affect the basal levels in the fetal organs of non-diabetic controls, it restored, partially, the augmented levels in the fetal organs of diabetic dams. Garlic extract (GE), was found to prevent cognitive decline by protecting neurons from A β neurotoxicity and apoptosis. Garlic compounds were shown to reduce A β induced neuronal apoptosis, possibly by enhancing the endogenous antioxidant defenses.

Dietary antioxidants - quercetin and curcumin could ameliorate the complications of diabetic nephropathy induced by the productions of advanced glycation end products. Feeding dietary fibre and butyric acid diet could regulate alteration in the protein structure function and its turnover rate in glomerular basement membrane of the diabetic kidney, in diabetic rat models. *Withania somnifera* supplementation was found to marginally lower the enhanced glucose levels and elevated urine output in diabetic rats, apart

from providing protection against embryolethality in diabetic models in addition to combating fetal weight loss. Pectic polysaccharide (PPS) and antioxidant fractions prepared from ginger (GR) and swallow root (SR) were found to effectively alleviate acetic acid induced ulcers. Galectin-inhibitors from dietary sources appeared to block and modulate galectin-3 levels, effectively.

Evaluation of the *Nanjanagudu rasabale* plantlets for acclimatization to green-house conditions revealed that the partial immersion system (PIS) derived plants performed better than complete immersion system derived ones. The possibility of delaying ripening of *Nanjanagudu rasabale* through chemical inhibition was investigated. The transcript abundance calculations of the various genes in comparison with those of control, established the possibility of regulating the ripening genes by both genetic as well as chemical methods. Acetone extract of elephant foot yam exhibited highest antioxidant activity in superoxide radical scavenging, lipid peroxidation inhibition and beta-carotene linoleic acid model system assays. Acetone extract from karonda fruit showed higher antioxidant activity than methanol extract. The fruit extracts of *D. indica* showed higher minimum inhibitory concentration against *Bacillus cereus, Yersinia enterocolitica* and *Escherichia coli,* than the bark extracts.

Sardine and cod liver oils could be hydrolysed to an optimal degree using commercial lipase at 1% (w/v). The enzymatic method of PUFA enrichment of fish oil hydrolysed fraction, did not appear to be as good as the classical urea adduction method. Solid state fermentation conditions were optimized for the production of chitinases from shrimp bio-waste as the solid substrate. Indian brown seaweed incorporated up to 2.5%, in cooked *pasta* from Indian durum wheat, resulted in *pasta* of better quality and biofunctionality. Marine xanthophylls carotenoid-fucoxanthin, the major pigment in brown seaweeds, exerted protective influence on vitamin A deficiency condition by maintaining membrane integrity and decreasing oxidative stress. Antioxidant activities exhibited by cleavage products of hydrolysis of protein were independent of the protease used, indicating the importance of degree of hydrolysis and size of peptide for antioxidant activity. Biscuits, traditional pickle and *vadiams* (rice fryums) prepared with the incorporation of protein concentrate from defatted *rohu* fish egg (protein content 80%) at 5 and 10% level were found to be acceptable.

Preliminary studies have confirmed the beta carotene richness of *Tinospora* fruit extracts, betalines (Rivinarin) dominance in *Rivina humilis*, and anthocyanins in *Santalum album* fruits, respectively.

Different biotic and abiotic elicitors applied as a spray to the fully opened flowers of *Bixa orellana,* could enhance the annatto colour content 2-4 fold, depending on the nature of the elicitor. Neurotransmitters MEL and SER influenced the production of somatic embryos in *Coffea canephora* and shoot organogenesis in *M. pudica*. Genomic walking has been initiated to determine site of integration towards characterization of transgenic coffee plants for caffeine content. Soybean genotypes from Bulgaria had higher levels of isoflavones

compared to Indian genotypes. Astaxanthin, a keto carotenoid having high antioxidant activity, produced from *Haematococcus*, a green alga, is biosynthesized from beta-carotene using beta carotene ketolase and beta carotene hydroxylase. Its high antioxidant activity has contributed to its nutraceutical and pharmaceutical importance. Basta (Glufosinate ammonium), at a concentration of 12 mg/l, exhibited efficiency in killing *Dunaliella bardawil* in AS100 medium.

Water soluble alpha-tocopheryl glycosides were enzymatically synthesized in di-isopropyl ether organic medium using amyloglycosidase from *Rhizopus* mold and beta-glucosidase isolated from sweet almond. Conditions for the reaction with D-glucose were optimized for amyloglucosidase. Alpha-Tocopheryl glycosides showed antioxidant activity as well as angiotensin converting enzyme (ACE) inhibitory activity.

Nitrocellulose strips, with immobilised antiatrazine antibodies, were treated with atrazine/atrazine-HRP mixture with horse radish peroxidase (HRP) as a tracer molecule. Antibodies raised against DDT metabolites in rabbit (IgG) and chicken (IgY) were found suitable for biosensor applications. DDT could be detected at 1ppb level, by chemiluminescence. Investigations into the possible production of vitamin B_{12} by two lactic acid bacteria (LAB) isolates, such as *Lactobacillus plantarum* and *Pediococcus acidilactici* gave positive indications. Different methods were evaluated for extraction of Vitamin B_{12} from *Spirulina* without interference of pigments.

In vivo studies on the oxidative damage in testis caused by iron dextran (ID) revealed that while no significant damage was observed at low doses, MDA levels increased at higher doses. Elevation in ROS levels was more significant in mitochondria than in cytosol. A mathematical approach to monitor the kinetics of synuclein aggregation with nucleation and elongation constants based on fluorescence studies has been evolved. Attempts to identify the major allergens in eggplant resulted in the detection of 6 allergens in the green variety of eggplant.

Treatment of endoglucanase with diethylpyrocarbonate modified the histidine residues in the enzyme with a concomitant loss enzyme activity. Endoglucanase could be useful in the development of targeted functional foods. Enzymatically as well as physically modified soy flour was used in the preparation of products like saltine crackers and noodles. Supplementation with 10% defatted soy flour improved the nutritional value without altering the quality parameters. Influence of the acid protease from *Aspergillus oryzae* MTCC 5341 and neutral protease from *Bacillus licheniformis*, on the quality of bread and crackers, was explored. The natural and acidic proteases could be used as a replacer of chemical additives in the baking industry. Pepstatin inhibited acid protease competitively, confirming that the enzyme belongs to the aspartate class of proteases. Obestain, shown to reduce feed intake and gain in body weight in rodents.

A Kunitz type trypsin inhibitor (ESTI) from the tree legume, sword bean (*Entada scandens L.*), is found to exhibit a mixed type competitive inhibition at lower concentration and pure competitive inhibition at higher concentrations. Smaller cyclic peptides, based on the inhibitory loop structures of HGIII, have been designed. A truncated version of HGI III (189 bp) was cloned between the *Sap-1* restriction sites of pTWIN - 1 vector. The protein was expressed in *E.coli. B ER* 2566, purified by affinity chromatography on chitin beads. A continuous decrease in chlorophylls and increase in carotene content has been observed during ripening of tomato fruits. Beta-carotene accumulation which showed a steady increase initially decreases at later stages. A differential expression of carotenoid biosynthetic pathway genes is found to coincide with the color change in the ripening fruit. Detoxified meals of tree borne oil seeds have been analyzed for the presence of antinutritional factors and toxic constituents. Animal feeding experiments in rat models, suggest the need for further improvement in the methods of detoxification.

A new method, for determining concentrations of nigerloxin in crude samples was developed. Reaction parameters were optimized for the production of xylooligosaccharide (XOS) from pretreated corncob. Evaluation of antioxidant activity XOS obtained from corncob showed that maximum free radical scavenging activity of 80% was found at a concentration of 0.765 mg/ml of crude XOS.

Reverse phase HPLC methods for the detection of the neurotoxin in *Khesari dhal* and for the separation, identification and quantification of Sudan dyes (I-IV) in chilli powder and chilli powder based pickles and sauces were developed. In carbonated cola and orange beverages, sucralose was the most stable artificial sweetener, whereas aspartame degraded upto a maximum of 85%. A simple and easy sample clean-up procedure using acidic alumina was standardised. A laboratory isolate *Leuconostoc mesenteroides* was used to prepare fermented milk. Pathogenic microorganisms were isolated by plating on selective media, following first occurrence of spoilage. Bromofuranone was less effective than 2(5H)-furanone in inhibiting *pseudomonas* growth. Quorum sensing molecule involved in enterocin biosynthesis, in probiotic cereal blend prepared from malted wheat and *ragi*, was characterized.

Aromagram of cardamom essential oil revealed the association of 1,8 cineole and a terpinyl acetate with several aroma notes. Pepper odour perception was more in semi-solid matrix than in dry matrix. Blending of mango, orange and lemon juices exhibited overlapping of aroma profiles of the juices. The commercially available lateral flow strips (LFS) for EPSPS protein did not show any positive reactions with plant species other than RUR soybean. LFS could provide visual detection of the presence / absence of the recombinant protein with in a short time, without the need for any instrumentation, making it suitable for screening of raw ingredients. Two commercial kits and the conventional cetyltrimethylammonium bromide (CTAB) method have been evaluated for their efficiency in extracting genomic DNA from the

fruits and leaves of Bt-Brinjal harboring the Cry1Ac gene. A compound with insecticidal ability, isolated from the edible roots of *D. hamiltonii*, was characterized using LCMS and NMR spectroscopy. P-anisaldehyde could completely inhibit seed-borne fungi at 10-20% concentration. A chemical staining method was developed for the detection of insect's eggs in the stored refined wheat flour that would enable the flour mill industry to supply insect-free products to the consumers. *C. elegans,* as a model for enzyme inhibition kinetics, was validated by comparison of AChE activity *in vitro* with that of rat brain AChE and on exposure to DDVP. The sorption of sulfuryl fluoride by various food commodities following exposure to the fumigant at known doses for 24 hours revealed that sulfuryl fluoride, unlike methyl bromide, has the advantage of low-level sorption with most of the food commodities.

Personnel from Central Food Laboratories, Public Health Departments, customs and ports were imparted training in the detection of living modified organisms through various workshop conducted by the institute's faculty.