7. Significant findings

Investigation 1: Standardization of methods to estimate inhibitors of iron absorption in roasted coffee beans.

- Polyphenol estimation from roasted coffee beans by solvent extraction was standardized using methanol as solvent. Methanol% of 80%, solvent: solid ratio of 50 ml/g and time of extraction of 4 h were the standardized parameter levels. Average polyphenol content of roasted coffee beans was 78 mg GAE/ g dry sample.

- Phytate estimation from roasted coffee beans by anion exchange chromatography was standardized for the first time. Extraction time of 2 hours, sample dilution of 1:10 for column loading and column elution volume of 15 ml were the standardized parameter levels. Average phytate content of roasted coffee beans was 2.49 mg phytate/ g dry beans.

Investigation 2: Evaluation of the combined effect of roasting and irradiation on anti-nutrient levels and their iron binding ability in coffee beans.

- Polyphenol content in decreasing order with respect to roast degree is: CCL-M > CCL-L > CCL-D.

- Polyphenol content increased at lower dosages (4 and 5 kGy) and decreased at 6 kGy. At 4 kGy dosage, polyphenol content in CCL-L, CCL-M and CCL-D were found to increase by 36.32%, 6.70% and 44.35% respectively. At 5 kGy dosage polyphenol content increased in CCL-L and CCL-D by 27.73% and 47.70%, but was found to decrease in CCL-M by 2.34%. At higher irradiation dosage of 6 kGy 3.36% and 3.39% increase in CCL-L and CCL-D and a 23.73% decrease in CCL-M was seen.
Polyphenols in medium roast degree coffee beans (CCL-M) were highly sensitive to irradiation and showed high degradation compared to CCL-L and CCL-D.

Roast degree had insignificant effect on phytate content.

Irradiation of coffee beans lead to significant decrease in phytate levels with the effect more pronounced in CCL-M and CCL-D. Phytate levels in CCL-L decreased only at higher dosage of 6 kGy by 18% and remained relatively constant at 4 and 5 kGy. Phytate levels in CCL-M reduced by 15%, 35.80% and 75.58% in 4, 5 and 6 kGy respectively. On the other hand, in CCL-D, 33.73%, 60.40% and 78.18% reduction in phytate levels in 4, 5 and 6 kGy doses respectively were observed.

Iron binding ability measured in terms of percentage bound iron of coffee bean polyphenols was inversely proportional to polyphenol concentration. With respect to roast degree, CCL-D (238 %) had higher iron binding ability than CCL-L (209 %) and CCL-M (197 %).

With increasing irradiation dosage, % bound iron in CCL-L dropped from 209% in 0 kGy to 114% (45% reduction) in 6 kGy. Similarly in CCL-M, % bound iron reduced from 197% in 0 kGy to 127% in 6 kGy resulting in a 35% reduction. Whereas in CCL-D, the % bound iron decreased in 4 kGy samples, followed by increase in 5 and 6 kGy.

Uniaxial compression studies showed that roasting and irradiation made the coffee beans brittle with gradual decrease in initial fracture strength and hardness. The effect of roasting was highly significant in terms of hardness than fracturability.

With increasing roast degree, there was decrease in lightness (L) and yellowness (b*) of coffee beans. Whereas, redness (a*) of coffee beans increased with higher
roast degrees. L, a* and b* values indicating lightness, redness and yellowness decreased with increasing irradiation dosages.

- Peroxide value of coffee beans increased from 0.55 in CCL-L, 1.35 in CCL-M and 3.46 meq/kg coffee oil in CCL-D. There is a progressive increase in the PV value of coffee beans with increasing irradiation dosage. Highest peroxide values were observed in 6 kGy samples of 3.24 in CCL-L, 5.44 in CCL-M and 6.28 meq/kg coffee oil in CCL-D.

- The major fatty acids in coffee beans were palmitic and linoleic acids. Other fatty acids that were identified in the samples were myristic, stearic, arachidic, palmitoleic, oleic and linolenic acids.

- Fatty acid composition of coffee beans remained largely unaffected by irradiation treatments, except for levels of stearic acid and arachidic acid. At 4 kGy levels of stearic acid and arachidic acid increased by 14% and 60% respectively. At 5 kGy the increase was 8% and 63%. Whereas, in 6 kGy a lesser increase of 4% and 585 were observed.

- Sensory analysis using quantitative descriptive analysis shows that coffee prepared from 0 kGy CCL-L, 6 kGy CCL-L, 4 kGy CCL-D and 6 kGy CCL-M had high overall acceptability.

- In terms of level of anti-nutrients and their iron binding ability in coffee beans 5 kGy CCL-M, 6 kGy CCL-L and 6 kGy CCL-M were favorable.

- 6 kGy CCL-M was finalized product formulation and manufacture considering low anti- nutrient levels, low iron binding and high sensorial acceptability.
Investigation 3: Optimization of drying technology for the production of instant coffee powder mix.

- Spray drying technology was optimized using Taguchi design and optimized conditions for dryer operation are: usage of skim milk, 1kg/cm² compressed air pressure, 180 °C inlet temperature and 20 rpm feed rate. The product was aimed at having high dispersibility, density, yield and low free fat content, moisture% and overall acceptability.

- Freeze drying technology was also optimized using Taguchi design and optimized conditions for dryer operation are: temperature and pressure of 65 °C and 2 mTorr. The product was aimed at having high dispersibility, low time of drying, and high sensory quality.

Investigation 4: Evaluation and comparison of quality parameters of freeze dried and spray dried samples of instant coffee powder mix, skim milk powder and coffee extract.

- Comparison of physical properties of spray dried and freeze dried product showed that freeze dried instant coffee powder mix was easily rehydratable, more hygroscopic, had lower degree of caking, was lighter in terms of color, had lower bulk and tap densities, had lower moisture content, was highly compressible and was equally dispersible upon comparison with spray dried instant coffee powder mix.

- Thermograms of developed product comprised of two events: glass transition and lactose crystallization. $T_g$ of spray dried powder (29.76 °C) is lower than that of freeze dried powder (36.73 °C). $T_g$ value of freeze dried and spray dried skim milk powder is 32.16 °C and 26.53 °C showing comparable values with the respective products.
• Crystallization temperature (T_{cr}) of spray dried instant coffee powder (135.03°C) was lower than freeze dried sample (158.42 °C).

• Moisture sorption studies showed sigmoidal shape curves representing type II. Freeze dried sample absorbed more moisture than spray dried at all levels of relative humidity.

• GAB and Oswin models provided best fit for moisture sorption data in both spray dried and freeze dried instant coffee powder mix. Monolayer values for all samples were greater at 27±2 °C than in 37±2 °C. k constant in GAB equation were high of the order 0.90-0.98 indicating a highly structured state of water adsorbed on top of the monolayer (GAB layer).

• Energy required for mass transfer between the atmosphere and the product is lower at 37 ±2 °C compared to 27 ±2 °C. Free energy change of spray dried product at 25 °C and 37 °C were 30 and -6.12 cal/ g mol respectively. Similarly free energy change of freeze dried product at 25 °C and 37 °C were 36.24 and 23.22 cal/ g mol.

• Spray dried and freeze dried coffee powder remains stable having moisture content within acceptable limits up to 32.8% and 7.38% relative humidity respectively.

• The microstructure of spray dried instant coffee powder was rounded particles of uneven sizes adhering to neighbor particles. Freeze dried coffee powder had flattened, skeletal structured individual particles of uneven shape.

• Powder rheology of spray dried and freeze dried instant coffee powder mix showed that both the samples are stable under variable flow, compression, flow under aeration and shearing.
Investigation 5: Testing different iron compounds in instant coffee powder mix for fortification.

- Sensory analysis of iron fortified reconstituted spray dried and freeze dried instant coffee powder mix showed that samples fortified with encapsulated ferrous sulphate, ferric orthophosphate and ferrous pyrophosphate had the highest overall acceptability.

- Powders fortified with ferrous sulphate, ferrous fumarate and sodium iron EDTA were found to be least acceptable making reconstituted coffee rancid, metallic tasting, bitter and gritty.

- Ascorbic acid had higher effect as an enhancer of iron absorption from samples fortified with ferrous pyrophosphate than in ferric orthophosphate. Ascorbic acid had no significant effect in reducing iron binding by samples fortified with encapsulated ferrous sulphate.

- An iron: ascorbic acid ratio of 1:2 was found to cause 15% reduction in iron binding by samples fortified with ferrous pyrophosphate.

- When studying the iron binding from encapsulated ferrous sulphate and ferrous pyrophosphate by skim milk solids, coffee extract and their combination, it was found that iron binding in product was governed by coffee extract. Iron binding by skim milk solids was negligible.

- From iron binding ability, effect of ascorbic acid and sensory effects, encapsulated ferrous sulphate and ferrous pyrophosphate were chosen for shelf life studies.
Investigation 6: Physicochemical changes in iron fortified instant coffee powder during storage at ambient (25 ± 2 °C) and accelerated (37 ± 2 °C) storage conditions, in two packaging material (PFP and Metalized polyester).

- Spray dried instant coffee powder mix had higher TBARS values compared to freeze dried powder at ambient (25 ± 2 °C) and accelerated storage (37 ± 2 °C) conditions.
- Spray dried and freeze dried instant coffee powder mix stored in metalized polyester pouches had higher TBARS values than samples stored in Paper-Foil-Plastic pouches. Form of iron fortificant had no effect on rancidity. Although there was a gradual increase in mg MDA with storage time, the levels were under acceptable limits.
- Freeze dried instant coffee powder mix had lesser free fat content than spray dried product. Free fat contents of samples directly correlated with TBARS values. Free fat content of samples stored in metalized polyester and at 37 °C had higher free fat content than other samples. Fortificant added did not affect release of fat.
- With storage time, the L values gradually increased, making the product darker in instant coffee powder mix stored at 37 °C in metalized polyester laminates due to accelerated browning reactions at high temperatures. No significant differences were observed between samples fortified with encapsulated ferrous sulphate, ferrous pyrophosphate and control (non-fortified).
- Spray dried and freeze dried instant coffee powder mix remained sensorially acceptable up to 8 months when stored at 25 °C in Paper-Foil-Plastic laminates.
- Both products complied with FSSAI standards for microbial safety (Aerobic plate count: 30 x 10^3 CFU/g- 50 x 10^3 CFU/g; Coliform: 0.1 CFU/g- 50 CFU/g; Yeast and mold: 5/0.1 g) up to the 8 months tested in both the storage temperatures and packaging materials.
Both encapsulated ferrous sulphate and ferrous pyrophosphate had high retention throughout storage.

**Investigation 7: Establishing the bioavailability of fortified iron in instant coffee powder mix by in vitro study.**

- *In vitro* dialyzable iron measurements show that absorbability of encapsulated ferrous sulphate increased from 40% in non irradiated samples with no ascorbic acid to 85% in irradiated samples with added ascorbic acid.
- Similarly iron absorption in case of ferrous pyrophosphate increase from 30% in non irradiated samples with no ascorbic acid to 80% % in irradiated samples with added ascorbic acid.
- Individual effects of irradiation and addition of ascorbic acid to product fortified with encapsulated ferrous sulphate were 77% and 65% respectively.
- In samples fortified with ferrous pyrophosphate, irradiation and ascorbic acid individually resulted in 66% and 44% iron absorption respectively.
- Both encapsulated ferrous sulphate and ferrous pyrophosphate have similar absorption values. Absorption of a less soluble iron compound (ferrous pyrophosphate) was thus improved using irradiation and ascorbic acid to levels equivalent to that of highly absorbable encapsulated ferrous sulphate.
- Considering cost of fortificant, ferrous pyrophosphate is recommended for fortification of instant coffee powder mix.
- From *in vitro* bioavailability studies it is proven that by consuming the fortified product, considering two servings per day (30 g powder dissolved in 120 ml water containing 3.5 mg iron), 26% (approximately 1/4th) of RDA for women will be met.