CHAPTER 6

Formulation of milling by-product based Nutrient Dense Health Drink and Detox Tea-Substitute

"The food you eat can be either the safest and most powerful form of medicine or the slowest form of poison"- *Ann Wigmore*

6.1. Introduction:

Tea-coffee is considered to be the popular beverages, consumed by most of the population without noticing the amount of caffeine intake. Presence of caffeine in these drinks, exerts psychoactive actions (Al Reef and Ghanem, 2018). However, due to rising awareness of health, nowadays caffeine free legumes-cereals based non-dairy drinks are getting popularized (Chavan *et al.,* 2018, Elsaid *et al.,* 2018) due to beneficial properties along with different flavour (Wang *et al.,* 2019). Moreover, milling by-products, fruit peels based beverages are also formulated due to higher amount of fibre in these by-products (Zeng & Lazarova, 2014; Elkhatim *et al.,* 2018). Our present interest is to study the role of chickpea husk as innovative food ingredient to develop caffeine-free drink and their characterization.

Besides food formulation, it is also essential to retain the quality of the product to avoid the adverse effect of contaminants or present adulterants upon health. Instead of using expensive analytical techniques to find out the adulterants, gamma irradiation technology can play a key role in the detection of added elements in liquid products like health drinks, milk etc. in a cost-effective way (Hendrichs *et al.,* 2009). Based upon the obtained attenuation coefficient of product, presence of adulteration can be detected. For the understanding of the storage stability of synthetic liquid products and the role of gamma radiation to detect the adulterants, milk has been chosen as 'Test sample' for the experimental design.

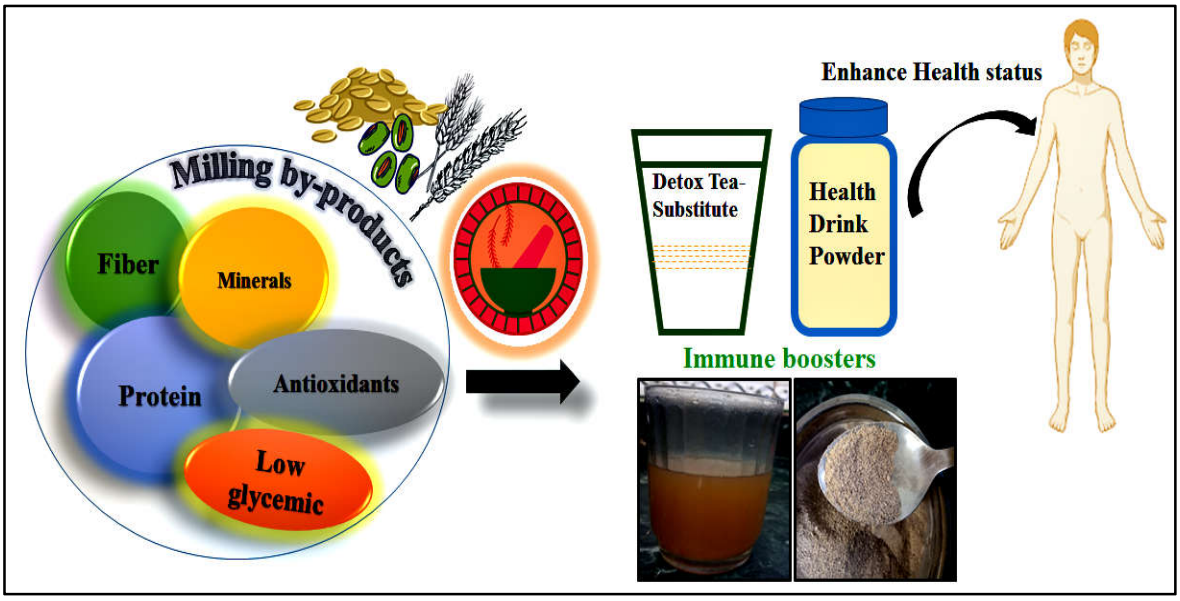

Figure 6. 1: Incorporation of milling by-product based products to enhance health status

In the following section, we will discuss about the role of processed milling by-products in Health Drinks Powder (HDP) and orange peel incorporated Detox Tea-substitute (DTS) production followed by estimation of proximate composition, in vitro digestibility, anti-oxidant activity, sensory parameters, and storage stability of the formulated products. Moreover, use of gamma radiation as a detector of the adulterant through estimation of attenuation coefficient also has been portrayed.

6.2.Experimental Design to characterize the formulated products

Physico-chemical composition of the raw ingredients had been discussed in Chapter 3. Processed orange peel powder and Milling by-product flours were taken for the product formulation process. Further development of value added functional products and their characterization had been discussed.

For the study of gamma radiation as adulterant detector, Milk samples were collected from the local village of Mahendergarh, Haryana. Double toned milk samples (Milk brand 1,2,3) had been procured from the local market of the same place. All the milk samples used for the study was not boiled (raw). For the collected milk from village, the breed was Murrah buffalo and time of milking was 5.00 am.

6.2.1. Value added product (Health Drink Powder and Detox Tea-substitute) formulation

6.2.1.1.Formulation of Health Drinks Powder

According to the percentages disclosed in the Table 6.1, the dried by-products were utilized to formulate mixtures for the development of variants of HDP. The mixture was first roasted for 1-2 min followed by addition of jaggery with continuous stirring on low flame to blend properly. The mixture was cooled down and fine powder was obtained by using laboratory grinder. In the mixture, cardamom powder, fennel seed powder, and clove powder were also added to obtain desired flavour (Figure 6.2). Finally, the mixture was sieved and stored for further use.

Ingredients (g)	HDP0 (g) (Control)	HDP1 (g)	HDP2 (g)	HDP3 (g)	HDP4 (g)
Rice bran	20.98 (48.92%)	20.81 (45.35%)	20.71 (43.24%)	20.59 (40.34%)	20.41 (38.77%)
Wheat bran	10.85 (25.30%)	10.74 (23.40%)	10.62 (22.18%)	10.5 (20.57%)	10.41 (19.77%)
Broken rice	10.01 (23.34%)	10 (21.79%)	9.98 (20.84%)	10.05 (19.69%)	10.01 (19.01%)
Chickpea husk	0 (0%)	3.09 (6.73%)	5.12 (10.69%)	8.22 (16.11%)	10.01 (19.01%)
Orange peel	1.05 (2.45%)	1.25 (2.72%)	1.46 (3.05%)	1.68 (3.29%)	1.81 (3.44%)
Total composition of composite flour	42.89	45.89	47.89	51.04	52.65

Table 6. 1: Ingredient composition to formulate Health Drinks Powder (HDP) (Percentage composition of formulated composite flour)

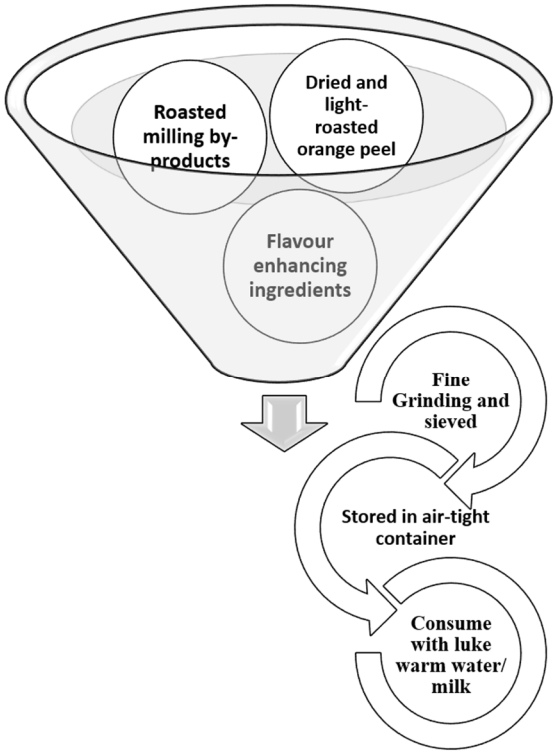

Figure 6. 2: Formulation Procedure of milling by-product based Health Drinks Powder (HDP)

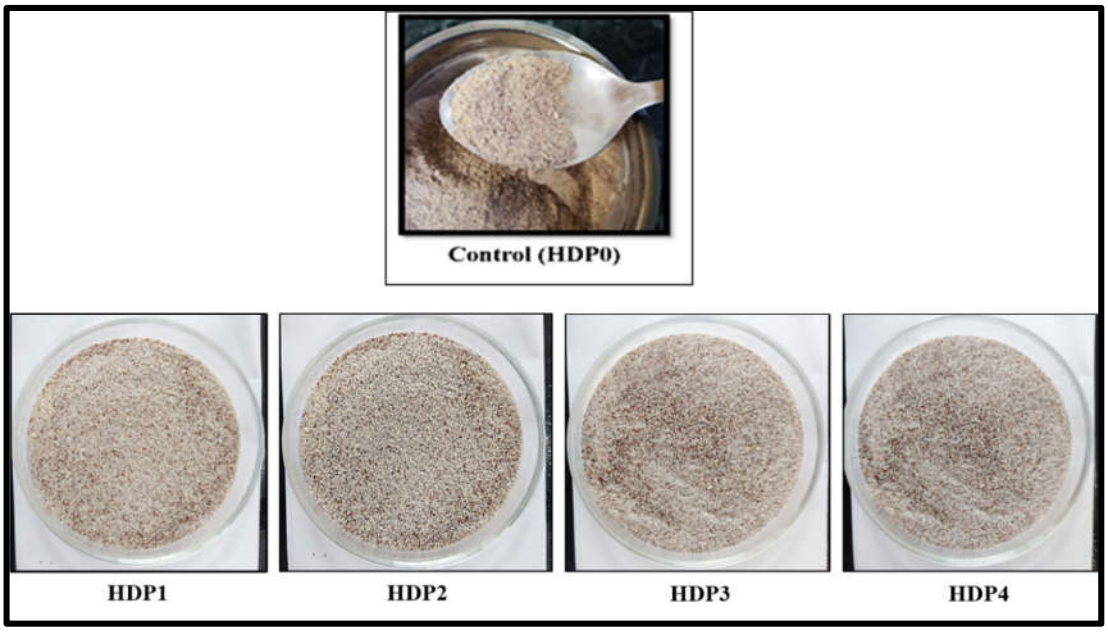

Figure 6. 3: Health Drink Powder variants (HDP0, HDP1, HDP2, HDP3, HDP4) developed form different percentage compositions of milling by-products

6.2.1.2. Formulation of Detox Tea-substitute

Based upon the combination percentages given in Table 6.2 by-product mixture was first roasted for few minutes and partially grinded the mixture to have a similar texture of dried tea leaf. Dried orange peel (taken amount given in Table 6.2) and jaggery were mixed followed by roasting on low flame and grinding at low speed. These jaggery-blended orange peel fractions were added to the partially blended by-product mixture and mixed properly. The mixture was again roasted for 1-2 min, cooled down and stored in small dip-pouches to use as dip using hot water to consume further (Figure 6.4).

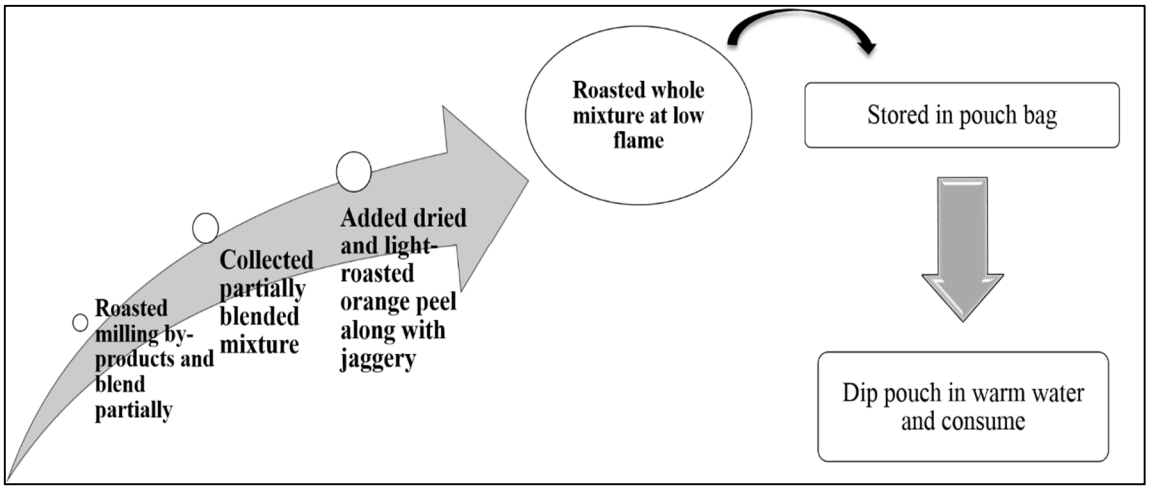

Figure 6. 4: **Formulation Procedure of Nutrient rich milling by-product based Detox Tea-substitute (DTS)**

Ingredients (g)	DTS0 (g) (Control)	DTS1 (g)	DTS2 (g)	DTS3 (g)	DTS4 (g)
Rice bran	20.29 (41.26%)	20.11 (38.94%)	19.79 (36.15%)	19.51 (33.27%)	19.34 (31.52%)
Wheat bran	20.85 (42.40%)	19.97 (38.67%)	18.85 (34.44%)	17.9 (30.53%)	16.89 (27.53%)
Broken rice	5.03 (10.23%)	5.55 (10.75%)	5.34 (9.76%)	5.21 (8.88%)	4.98 (8.12%)
Chickpea husk	0 (0%)	5.02 (9.72%)	8.86 (16.19%)	12.1 (20.63%)	15.01 (24.46%)
Orange Peel	3.01 (6.12%)	6.01 (11.64%)	10.76 (19.66%)	16.02 (27.32%)	20.15 (32.84%)
Total composition of composite flour	49.18	51.64	54.74	58.64	61.36

Table 6. 2: Ingredient composition to formulate value added milling by-product based Detox Tea-substitute

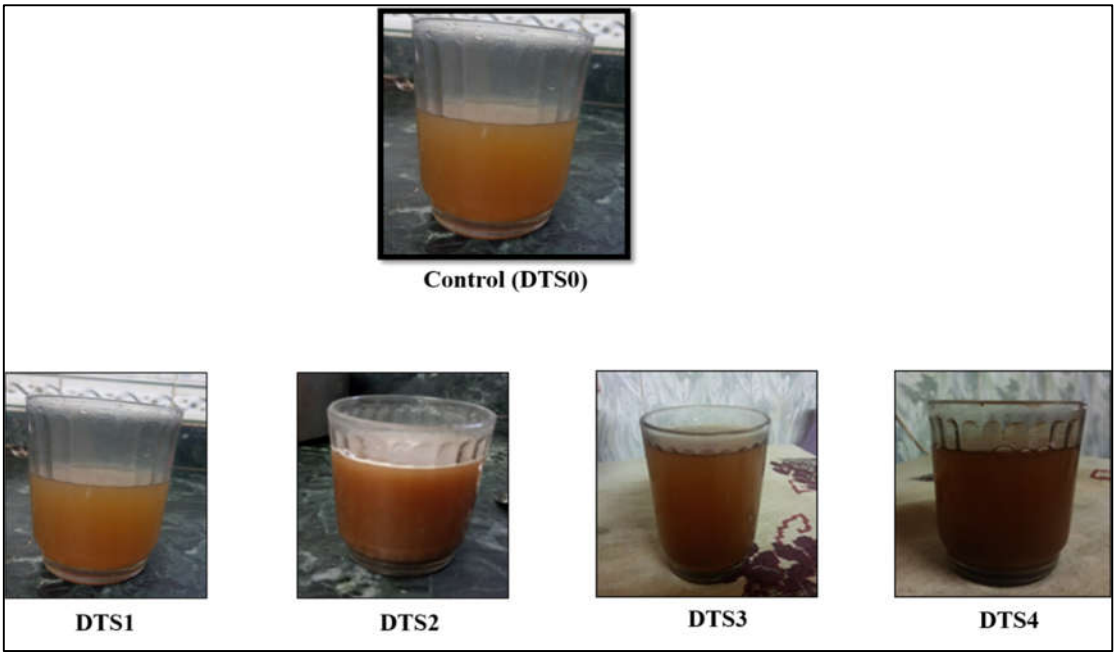

Figure 6. 5: **Detox Tea Substitute variants (DTS0, DTS1, DTS2, DTS3, DTS4) formulated using different percentage composition of milling by-products**

After the formulation of the value added health drink and tea substitute, the products were characterized based upon their nutrient composition, storage stability and sensory parameters. Later on, we performed adulteration detection using gamma radiation.

6.2.1.3. Detection of adulterants in liquid products

^{137}Cs gamma source was used to carry out the study of the adulteration detection in liquid product. The mass attenuation coefficients of different milk samples were determined using GM counter.

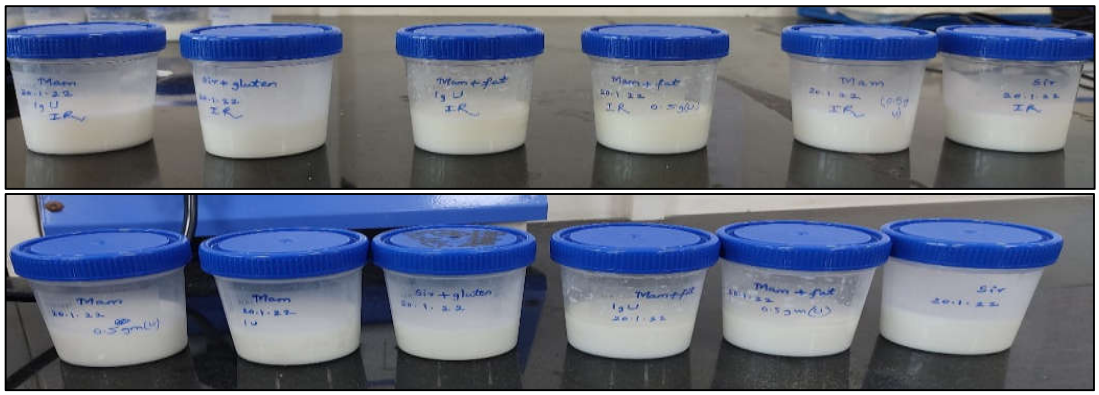

Figure 6. 6: Imitated milk sample prepared using known amount of adulterants: Urea, Gluten, vegetable oil and water

The experiment was carried out in two phases. In first phase, pure buffalo milk sample was taken and known amount of adulterants were added to imitate the synthetic milk composition. Pure milk sample was distributed into six groups in below mentioned manner:

Samples	Type of adulterants added	Preparation of mixture	Total volume of the final mixture sample taken
sample+ Vegetable oil+ 1gm urea	Vegetable oil, water and urea	20 ml of luke warm water was added to 20 ml pure milk. 1ml vegetable oil and 1g urea were added to the mixture	40 ml
sample+ Vegetable oil + 0.5 gm urea	Vegetable oil, water and urea	20 ml of luke warm water was added to 20 ml pure milk. 1ml vegetable oil and 0.5g urea were added to the mixture	
sample+ 1 gm urea	Urea and water	20 ml of luke warm water was added to 20 ml pure milk. 1g urea was added to the mixture	
sample+0.5 g urea	Urea and water	20 ml of luke warm water was added to 20 ml pure milk. 0.5g urea was added to the mixture	
sample+gluten	Gluten and water	20 ml of luke warm water was added to 20 ml pure milk. 1g gluten was added to the mixture	
sample	-----	-----	

Table 6. 3: Preparation of imitated sample compositions using adulterants (urea, gluten, and vegetable oil)

These prepared imitated milk samples were exposed to gamma source (^{137}Cs). Attenuation co-efficient of the same was determined and the effect of gamma radiation upon the samples upon spoilage was also studied.

In the second phase of the experiment, collected market milk samples were exposed to ^{137}Cs followed by determination of attenuation co-efficient and effect of gamma radiation on the milk samples. Results obtained from both the phases were compared to understand the role of gamma radiation in the detection of adulterants in milk sample. All the milk samples were also boiled upon spoilage to observe the structure of curdled milk protein (mass particles). Effect of adulterants as well as radiation on the curdled particles were also studied.

6.3. Result and discussions

The sensory profiling and characterization of the products which are Health Drink Powder and Detox Tea-substitute has been described along with their shelf life study. The adulteration detection in the liquid samples using gamma radiation also has been discussed in this section.

6.3.1. Sensory evaluation of prepared products

Based upon the obtained sensory scores (Figure 6.6), addition of higher percentage of chickpea husk flour and orange peel powder in composite flour formulation affected the acceptance ($p \leq 0.05$). Overall acceptability of HDP1 was 7.79 ± 0.01 whereas HDP4 showed acceptability score 7.26 ± 0.18. With increasing flour incorporation colour of DTS was slightly effected ($p \leq 0.05$). Although other sensory parameters were enhanced until DTS2 (8.18±0.11), further increased substitution of by-product flour lowers the sensory quality (8.03±0.05 for DTS4). It might happen due to bitter taste of orange peel, as higher percentage of the peel may abolish the taste and flavour. Mishra and Chandra (2012) also disclosed same observation in the sensory parameters of developed rice bran and soy flour based biscuits. Addition of wheat bran in product development also indicated the same pattern (Sozer *et al.,* 2014). Bose and Shams-Ud-Din (2010) disclosed that pre-treated chickpea husk can be used as innovative food ingredient for food formulation.

6.3.2. Detailed study of the nutritional attribute of the formulated products

Based upon the obtained sensory score, the most preferred products that is, HDP1 and DTS2 were analysed to determine nutrient composition, antioxidant activities, digestibility and shelf life.

6.3.2.1. Proximate compositions

According to the obtained data, crude protein was 18-19% ($p \leq 0.05$) and moisture present in HDP1 and DTS2 was 4-4.5%. among these products, moisture in HDP1 was lesser [(4.19±0.02) %] (Table 6.4). Less carbohydrate was found in these by-product based products. Crude fat (%) was 2% to 3%. Lower fat content due to use of milling by-product in frankfurters was perceived by Choi *et al.,* 2015 where the product supplemented with 2% rice bran showed lower fat percentage. Total carbohydrate found in HDP1 and DTS2 was (63.59±0.11) % and (64.18±0.09) %. Generally, 100g of malt-based market health drinks possess carbohydrate content around 85g, of which almost 32g is sugar (Sandhya Ramesh, 2018). Low carbohydrate was also found in the study by Choi *et al.,* 2015 in rice bran utilization in food production.

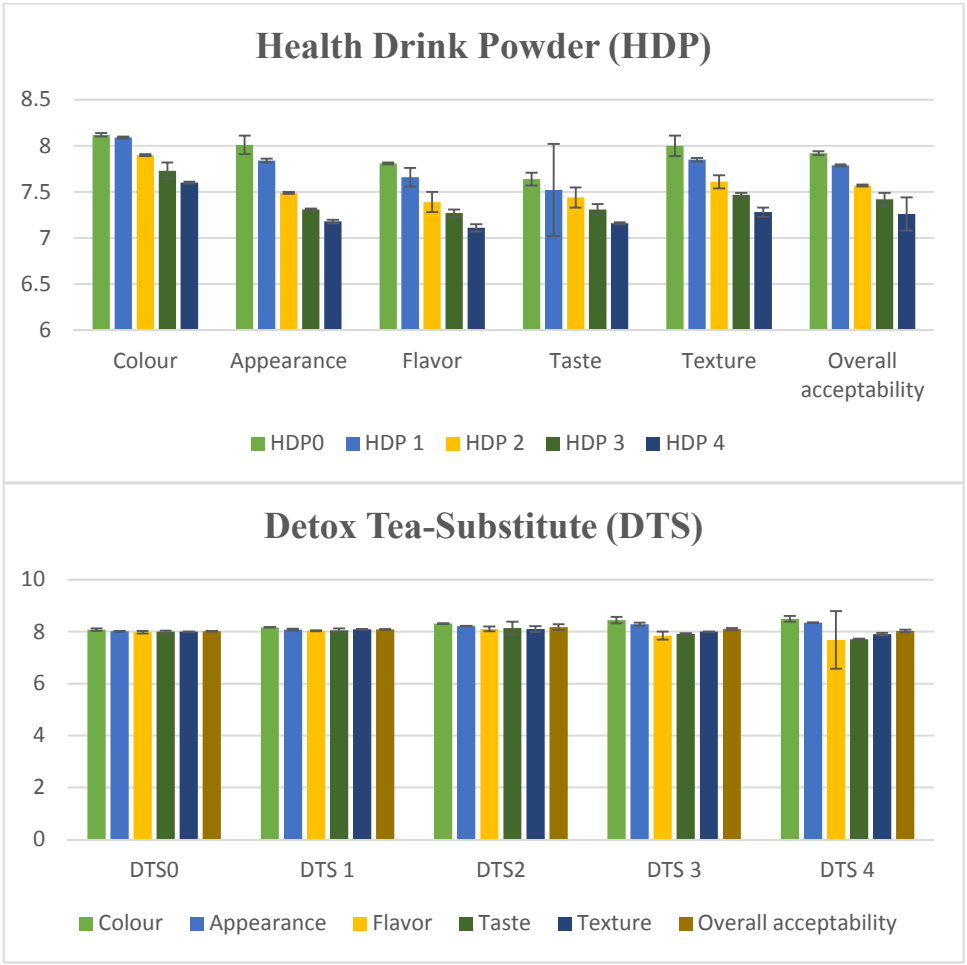

Figure 6. 7: Sensory evaluation of prepared value added products- Health drink powder and Detox tea substitute

Proximate composition	Health Drinks powder (HDP1)	Detox tea-substitute (DTS2)
Moisture	4.19±0.02[b]	4.47±0.06[a]
Crude protein	19.27±0.01[c]	18.21±0.19[a]
Crude fat	2.98±0.01[b]	3.09±0.04[a]
Crude fibre	4.95±0.06[b]	5.07±0.11[a]
Ash	5.02±0.12[a]	4.98±0.12[a]
Total carbohydrate	63.59±0.11[a]	64.18±0.09[b]

Table 6. 4: Proximate composition of formulated Products per 100g (%, dry matter basis). [Data presented are proximate compositions of developed Products. significantly different, $p < 0.05$ (Statistical analysis has been done row wise)]

6.3.2.2. Sugar and starch level

Non- reducing sugar obtained in the products were around 1.5 g/100g. Recorded reducing sugar level in HDP1 and DTS2 was 3 g/100g (Table 6.5**)**. Presence of broken rice in the formulation indicated the obtained data of available starch in the products (9-11 g/100g). although broken rice contain higher level of starch, during the processing of the by-products for the formulations the level of starch got decreased (Torres *et al.,* 2018).

6.3.2.3. Minerals

Ash (%) was significantly moderate in all the formulations (5%). Commercial Health show lower ash (1-2%), and so as the mineral contents. However, these by-products based formulations showed rich mineral levels. Iron in HDP1 and DTS2 was 5.29±0.08 mg/100g and 6.87±0.05 mg/100g respectively. Calcium was higher in HDP1 (81.21±4.03 mg/100g) than DTS2 (78.96±4.36 mg/100g). Phosphorus was more in DTS2 (211.52±0.22 mg/100g) than HDP1 (139.58±0.20 mg/100g) ($p \leq 0.05$). Enhanced protein, fibre, and ash content due to legume fortification already was depicted by several studies (Cheng & Bhat, 2016). Rich mineral profile (Table 6.4) of the milling by-product based formulations also indicate to the possible use of these agricultural by-products for the product development with higher essential micronutrients to prevent the risk of micronutrient deficiency (Frossard *et al.,* 2000). Rice bran in the formulation contributed a potent role in available iron level (Sohail *et al.,* 2017) resulting in development of nutrient dense products.

6.3.2.4. Antinutrient factors

Antinutrient levels were comparatively higher in these novel formulations due to presence of legumes. Trypsin inhibitor activity was found 1.95±2.01 TIU/mg along with

197.01±1.32 mg/100g of phytic acid content in HDP1 and 2.97±2.06 TIU/mg Trypsin inhibitor activity along with 201.25±1.38 mg/100g of phytic acid content (Table 6.5) in DTS2 which belongs to the healthy range of antinutrient in body. Though antinutrient is popular because of their hindrance effect on mineral or other nutrient absorption in body, a minimal level of present antinutrient can also act as an antioxidant contributing in enhanced health status (Kumar *et al.,* 2021). Due to the processing methods during the product formulations such as roasting and heating (Patterson *et al.,* 2017; Shi *et al.,* 2018), HDP1 and DTS2 showed negligible antinutrient in comparison to source.

Nutrient composition	Health Drinks Powder (HDP1)	Detox tea-substitute (DTS2)
Non-Reducing sugar (g/100 g)	1.42 ± 0.21^{a}	1.57 ± 0.19^{b}
Reducing sugar (g/100 g)	2.79 ± 0.08^{a}	3.11 ± 0.01^{b}
Total soluble sugar (g/100 g)	4.21 ± 0.05^{a}	4.68 ± 0.01^{b}
Starch (g/100 g)	9.90 ± 0.01^{b}	11.01 ± 0.05^{a}
Iron (mg/100g)	5.29 ± 0.08^{b}	6.87 ± 0.05^{a}
Calcium (mg/100g)	81.21 ± 4.03^{b}	78.96 ± 4.36^{a}
Phosphorus (mg/100g)	139.58 ± 0.20^{b}	211.52 ± 0.22^{a}
Trypsin inhibitor activity (TIU/mg)	1.95 ± 2.01^{b}	2.97 ± 2.06^{a}
Phytic acid (mg/100 g)	197.01 ± 1.32^{b}	201.25 ± 1.38^{a}
Total phenolic content (mg GAE/ g)	4.01 ± 0.05^{b}	4.04 ± 0.05^{a}
DPPH Radical Scavenging Activity (%)	56.09 ± 0.05^{b}	57.12 ± 0.06^{a}
In vitro digestibility of Starch (mg maltose released/ g of product starch)	28.01 ± 0.07^{b}	28.19 ± 0.07^{a}
In vitro digestibility of Protein (%)	70.09 ± 0.15^{b}	70.11 ± 0.16^{a}
Soluble dietary fibre(g/ 100g)	2.96 ± 0.12^{b}	3.29 ± 0.12^{a}
Insoluble dietary fibre (g/ 100g)	7.05 ± 0.11^{b}	7.21 ± 0.11^{a}
Total dietary fibre (g/ 100g)	10.01 ± 0.01^{b}	10.5 ± 0.06^{a}
Vitamin C (mg/100g)	60.23 ± 0.11^{a}	57.36 ± 0.09^{b}

Table 6. 5: Analysed nutrient composition of formulated products per 100g. [Data presented are nutrient compositions of developed Products. significantly different, $p < 0.05$ (Statistical analysis has been done row wise)]

6.3.2.5. Phenolic compounds and antioxidant properties

According to research data, in vitro evaluation of DPPH scavenging activities of the Chickpea husk indicated potential antioxidant properties (Niño-Medina *et al.,* 2019) along with rice bran (Ghasemzadeh *et al.,* 2018) and wheat bran (Ramos-Enríquez *et al.,* 2018). Thus determination of antioxidant activity of these husk based products was necessary to analyse the effects of cooking and other processing method (Myint *et al.,* 2017). Total Phenolic content as well as DPPH Radical Scavenging Activity was enhanced with the substitution of developed composite flour. Total phenolic activity of HDP1 and DTS2 were 4.01±0.05 mg GAE/g and 4.04±0.05 mg GAE/g. there was no significant difference ($p \leq 0.05$) between the phenol content of the products. DPPH Radical Scavenging activity of HDP1 was lower [(56.09±0.05) %] than that of DTS2 [(57.12±0.06) %] (Table 6.5). Enhanced Total phenolic content along with improved antioxidant activity due to substitution of chickpea husk in the formulation of bread was also reported in a study (Niño-Medina *et al.,* 2019).

6.3.2.6. In vitro protein and starch digestibility

In vitro digestibility of protein and starch was notable. In vitro starch digestibility was around 28 mg maltose released/g (Table 6.5). Obtained data showed improved in vitro protein digestibility (70%) of these products. Improved in vitro digestibility of starch and protein was also observed in a study (Singh Sibian and Singh Riar, 2020) due to incorporation of germinated legume grains lacking gluten protein.

6.3.2.7. Dietary fibre

Substitution of composite flour in product formulation showed significant quantity of soluble dietary fibre (around 3 g/ 100g). whereas, it has been found that market food generally shows fibre somewhat like 1 g/ 100g or less, especially the 100% wheat products. Insoluble dietary fibre (g/ 100g) level was also elevated (around 7 g/100g) in these recipes. Total dietary fibre (g/ 100g) of the formulated products was almost 10 g/100g (Table 6.5). Similar enhanced level of dietary fibre was also observed in a study after substitution of chickpea husk fibre (Niño-Medina *et al.,* 2019). Due to a much higher level of fibre (83.45g/100g) in Chickpea husk studies are going on to formulate high fibre food products

using chickpea husk flour (Niño-Medina *et al.,* 2019). Formulated value-added products in this study could contribute to fibre requirement to much extent.

6.3.2.8. Vitamin-C level in formulations

It has been found that the formulated recipes contain remarkable amount of Vitamin- C. HDP1 showed 60.23±0.11 mg/100 g and DTS2 contains 57.36±0.09 mg/100 g of Vitamin C (Table 6.5). Due to fortification of the recipes with orange peel, both the formulations showed significant Vitamin-C level. Though Orange peel contains Vitamin-c around 110 mg/100 g (Sir Elkhatim *et al.,* 2018), blanching and roasting during the formulation of the recipes may be responsible of Vitamin-C loss and comparatively lower level of presence in products than the orange peel (Lee *et al.,* 2018)

6.3.2.9. Shelf life of the prepared food products

Preferred products (HDP1 and DTS2) based upon sensory evaluation were further stored to check their shelf life and storage stability. Figure 6.8 showed that the sensory attributes of the stored products was intact until 90th day that is 3 months from the manufactured date. Peroxide value and free fatty acid value of both the products was observed to increase moderately until 90th day. They showed almost 2 meq peroxide/1000g and 12 meq peroxide/1000g peroxide value on initial day and 90th day regarding the assessment of storage stability (Table 6.6). Free fatty acid value also increased in the range 0.3 to 0.8 mg KOH/100g in these novel formulations. The range of increased peroxide value was quite moderate and the increased levels, recorded on 90th day were quite acceptable as the peroxide value should not be above 10–20 meq/kg fat (Connell, 1975) to avoid rancidity flavour. Interpretation of the data indicated higher storage stability of the products as ready-to-eat food.

6.3.2.10. Microbiological safety of food

The assessment of the microbial count of the products is presented in Table 6.7. It is fundamental to determine the microbial activity as this is an important aspect to any food product. The *Total Plate Counts* (Log CFU/g) were 2.12±0.01 and 2.08±0.12 found to be in freshly prepared HDP1 and DTS2 respectively under evaluation**.** Then successively the Total Plate Count (Log CFU/g) increased up to the mark of 2.5. Yeast and mold counts in freshly prepared formulations were negative. The yeast counts **(**Log CFU/g**)** increased to

2.09±0.05 after 75th day and 2.01±0.11 after 60th day in HDP1 and DTS2 respectively (stored room temperature). All formulations of prepared foods from 0th day to 90th day were within the parameters, and can be said microbiologically safe. Based upon another studies formulating food recipes using milling by-products viz., bran of cereals and pulses were found microbiologically safe and healthy enough to incorporate in diet (Ahmad *et al.,* 2021; Aktaş and Akın, 2020; Hasani *et al.,* 2017).

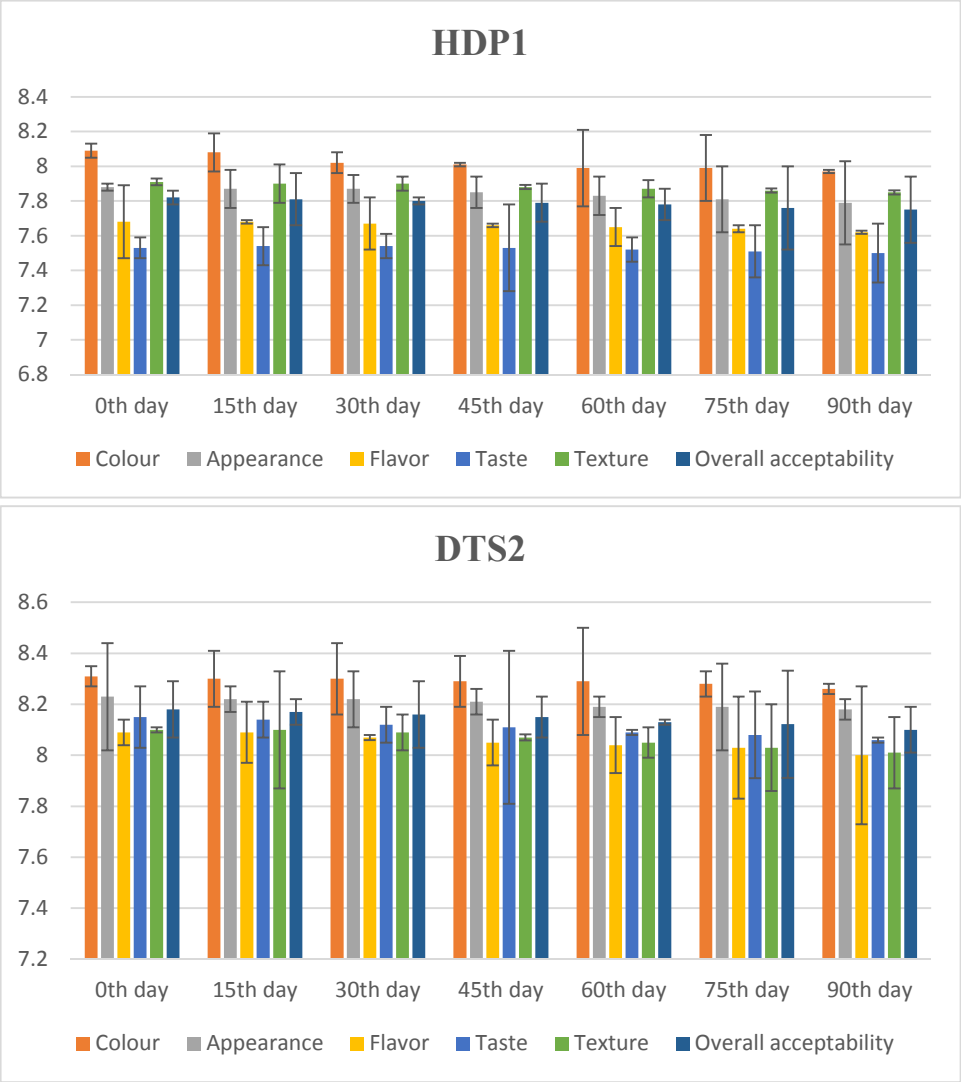

Figure 6. 8: The effect of storage on hedonic quality characteristics analysis of Products incorporated with composite flour

6.3.2.11. Antioxidant activity of the stored products

Based upon the estimated data (Table 6.5), the antioxidant activity of the stored products (after 90th day) was lower than that of freshly prepared products. Total phenol contents were 3.85±0.12 mg GAE/ g and 4.09±0.01 mg GAE/ g for HDP1 and DTS2 respectively. Whereas, DPPH Radical Scavenging Activity was (55.81±1.02) % and (56.95±0.14) % for HDP1 and DTS2 respectively.

Day intervals	0th day	15th day	30th day	45th day	60th day	75th day	90th day
Health Drink powder (HDP1)							
Peroxide value (meq peroxide/ 1000g	2.04±0.01^{e}	2.75±0.08^{b}	4.92±0.04^{c}	6.88±0.11^{a}	8.89±0.01^{e}	10.98±0.04^{c}	12.20±0.03^{d}
Free fatty acid (mg KOH/10 0g)	0.32±0.08^{b}	0.41±0.10^{a}	0.52±0.12^{a}	0.59±0.11^{a}	0.67±0.16^{c}	0.72±0.08^{b}	0.79±0.02^{d}
Detox tea-substitute (DTS2)							
Peroxide value (meq peroxide/ 1000g)	2.11±0.01^{e}	3.95±0.08^{b}	4.99±0.04^{c}	6.84±0.11^{a}	8.79±0.01^{e}	10.78±0.04^{c}	12.02±0.03^{d}
Free fatty acid (mg KOH/10 0g)	0.34±0.08^{b}	0.47±0.10^{a}	0.59±0.12^{a}	0.63±0.11^{a}	0.75±0.16^{c}	0.80±0.08^{b}	0.84±0.02^{d}

Table 6. 6: **Shelf life determination of novel products. [Data presented are significantly different, p < 0.05 (Statistical analysis has been done row wise)].**

Based upon the evaluated scores and the overall analysis, it can be said that the novel formulated food products showed an ample amount of nutrients as well as satisfactory sensory acceptance. A positive effect was observed in proximate composition and in vitro digestibility of the formulated food due to the addition of milling by-products. Available antinutrient present in the foods was less (within the tolerable level) due to cooking and processing of composite flour. Incorporation of these herbs and spices,

underutilized greens and their by-products is essential and recommended to consume through formulation of novel food products or by renovating the local recipes to improve health status, to prepare body to survive in pandemic situations. Popularization of these products (discussed in Chapter 3) also had been done in collaboration with KrishiVigyan Kendra, Mahendergarh.

Health Drink Powder (HDP1)		
Total Plate Count	0^{th} day	2.12±0.01
	15^{th} day	2.19±0.05
	30^{th} day	2.26±0.12
	45^{th} day	2.31±0.01
	60^{th} day	2.35±0.09
	75^{th} day	2.41±0.21
	90^{th} day	2.47±0.08
Yeast and Mold Count	0^{th} day	-ve
	15^{th} day	-ve
	30^{th} day	-ve
	45^{th} day	-ve
	60^{th} day	-ve
	75^{th} day	2.09±0.05
	90^{th} day	2.11±0.14
Detox tea-substitute (DTS2)		
Total Plate Count	0^{th} day	2.08±0.12
	15^{th} day	2.15±0.05
	30^{th} day	2.23±0.02
	45^{th} day	2.29±0.22
	60^{th} day	2.31±0.19
	75^{th} day	2.39±0.20
	90^{th} day	2.45±0.04
Yeast and Mold Count	0^{th} day	-ve
	15^{th} day	-ve
	30^{th} day	-ve
	45^{th} day	-ve
	60^{th} day	2.01±0.11
	75^{th} day	2.14±0.04
	90^{th} day	2.20±0.07
[–ve: not detected]		

Table 6. 7: **Microbial load (Log CFU/g) analysis of freshly formulated products**

After 90th day of the storage		
Antioxidants	HDP1	DTS2
Total phenolic content (mg GAE/ g)	3.85±0.12[b]	4.09±0.01[a]
DPPH Radical Scavenging Activity (%)	55.81±1.02[b]	56.95±0.14[a]

Table 6. 8: Estimation of antioxidant activity in the products after 90th day of the storage

After the products (HDP and DTS) formulation and characterization, according to the next phase of the experimental design that is to detect the adulterants in liquid products using gamma radiation, we here recorded attenuation coefficient of the 'Test samples' (Milk samples).

Samples	Nc	Density	Linear attenuation	Mass attenuation
Sample+ Vegetable oil + 1gm urea	122	1	0.188	0.188
Sample+ Vegetable oil + 0.5 gm urea	119	1	0.201	0.201
sample+ 1 gm urea	105	1.025641	0.279	0.272
sample+0.5 g urea	101	1.025641	0.302	0.294
Sample + gluten	102	1.025641	0.296	0.288
Sample (pure)	90	1.0526316	0.379	0.360
nb- 31; Height of milk samples (cm)				

Table 6. 9: Attenuation co-efficient of prepared milk samples containing different adulterants

6.3.3. Detection of adulterants in liquid products using gamma radiation

Obtained data depicted the relation between attenuation coefficient and composition of different milk samples. Milk with greater density showed larger value of attenuation coefficient and vice versa. Decrease in the value of mass attenuation coefficient showed higher photon interaction probability at lower energy and photon interaction probability decreases as the energy of photon increases (Limkitjaroenporn *et al.,* 2012).

Based upon the obtained data of the imitated milk samples (prepared in lab), attenuation coefficient of pure milk sample was the highest (Table 6.9). The linear attenuation coefficient of pure milk sample was 0.379 and mass attenuation of the same was 0.360. In case of imitated milk samples, sample containing gluten showed lesser attenuation coefficient than the pure milk sample. The attenuation coefficient value decreased with the increasing concentration of added urea in the milk sample. Upon adding vegetable oil along with urea in the milk sample, attenuation coefficient decreased even more. Sample containing fat and 1g urea showed least attenuation co-efficient value (Figure 6.9) which was 0.188.

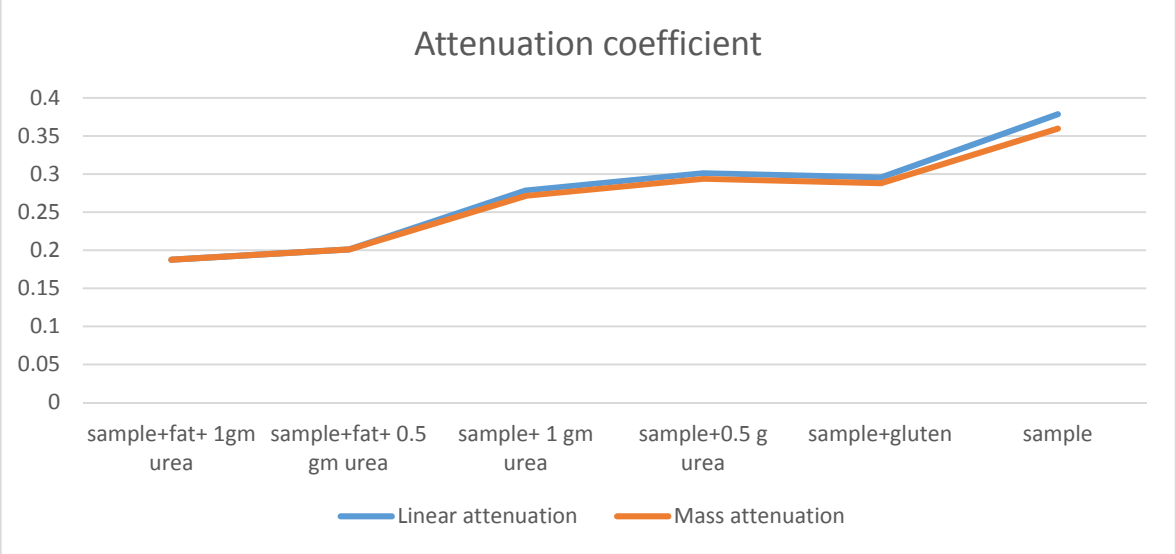

Figure 6. 9: Graphical representation of obtained attenuation co-efficient of imitated milk samples

	24 hours	48 hours	72 hours
Sample + Vegetable oil + 1gm urea	5	3	0
Sample + Vegetable oil + 0.5 gm urea	7	2	0
Sample + 1 gm urea	2	1	0
Sample +0.5 g urea	7	0	0
Sample + gluten	9	2	0
Sample	9	2	0

Table 6. 10: Observed counts in gamma irradiated different milk samples (Synthetic and natural milks)

After 24 hours of exposure of these samples to gamma source, all the samples were stored at room temperature (20°C) in plastic container and left the lid of container open. Present counts in the samples were estimated by the interval of 24 hours. According to the estimated data, all the sample showed zero count on third day. Spoilage of milk occurs due to excess growth of microorganism resulting in production of lactic acid. Increasing acidity of milk leads to coagulation of milk protein, forming soft lumps. Upon heating milk protein become over saturated and gain visible curdling. Upon obtaining zero count of the exposed samples, they were boiled to check whether the samples got spoiled or still retaining required consumable quality. Exposed 'pure sample' and 'sample with gluten' exhibited bit sour smell with visible protein coagulation on the third day. Rest exposed samples containing urea and vegetable oil showed sign of spoilage on seventh day. Besides determination of storage stability, effect of radiation on milk protein coagulation were also noted. Upon boiling of the samples, irradiated pure sample showed larger sized solid particle/curdled mass in comparison with that of gluten containing irradiated sample. Irradiated urea and fat containing samples showed smaller size of solid particles/curdled mass. Increase in the concentration of urea in milk sample resulted in visible effect on coagulated particles in sample. Irradiated milk sample containing 0.5 g urea showed smaller grains of coagulated milk protein (curdled mass). Moreover, irradiated milk sample containing 1g urea showed even more smaller grains of coagulated milk protein. The observed analysis depicted inversely proportional relation of size of grains of coagulated milk protein with the concentration of added urea. Non-irradiated pure sample and sample with gluten showed spoilage on third day. Upon heating larger sized lumps of coagulated milk protein was observed in case of spoiled pure milk. Curdled lump obtained from non-irradiated gluten containing sample was smaller than the previous one. Non-irradiated urea and fat containing samples showed spoilage on sixth day. From the obtained observation, it can be depicted that implication of gamma radiation to the milk sample might cause obstacle to the excessive growth of lactic acid producing bacteria, resulting in balance of pH of milk samples and their storage stability. Spoiled urea containing samples showed smaller grains of coagulated milk protein in comparison with that of pure milk sample. Presence of fat in the milk also led to smaller grains of curdling. In comparison between

irradiated and non-irradiated samples, size of curdled mass of coagulated milk protein obtained from radiated samples was smaller than that of non-irradiated samples. Smaller the size of curdled mass, more binding property was observed.

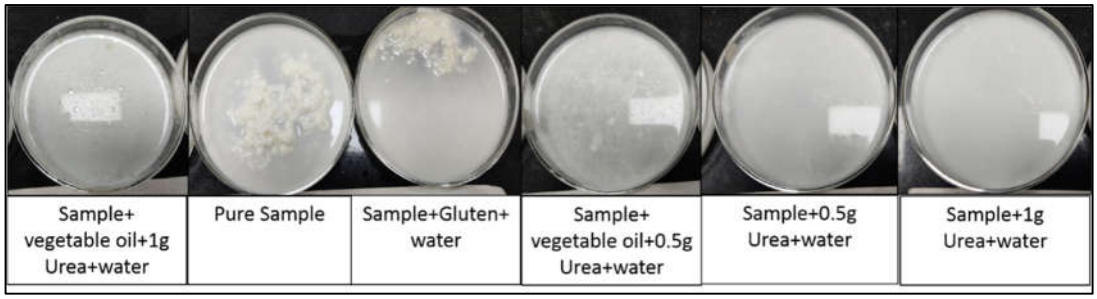

Figure 6. 10: **Curdled mass/particle obtained from different composition of milk sample (not radiated) on boiling**

SAMPLES	Fat (%)	Protein (%)
sample+ Vegetable oil + 0.5 gm urea	4.38	3.88
sample+ Vegetable oil +1 gm urea	4.45	3.9
sample+ 0.5gm urea	4.19	3.88
sample+1g urea	4.25	3.91
sample + gluten	3.83	3.23
sample	3.81	3.19

Table 6. 11: Fat and protein percentages of adulterated milk samples

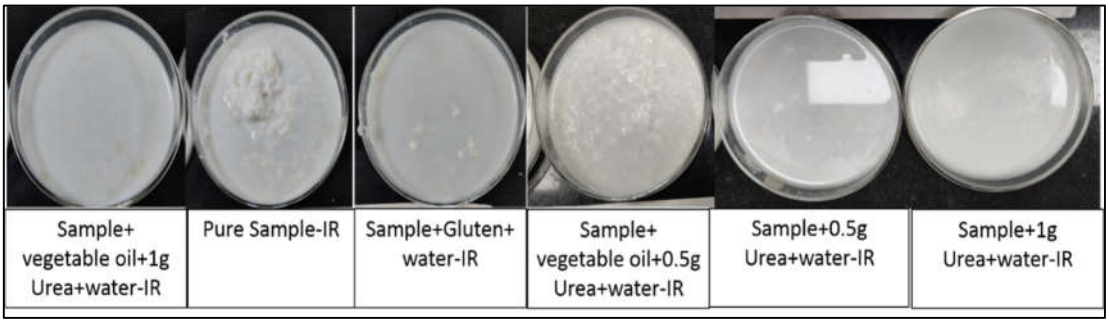

Figure 6. 11: **Curdled mass/particle obtained from different composition of radiated milk sample on boiling**

Based upon the estimated fat and protein percentages of the fresh pure milk and imitated milk samples, fat percentage (4.45%) was found highest in the sample containing fat and 1g urea. Although among fat containing samples amount of added fat was same, due to presence of more urea, sample containing fat and 1g urea might show more fat percentage. According to previous studies, it had been established that presence of urea in milk increase amount of fat. In case protein estimation, sample containing urea showed

higher amount of protein percentages. Sample with only 1g urea showed highest protein (3.91%). The samples with Vegetable oil + 0.5g urea and sample with 0.5g urea showed 3.88% protein.

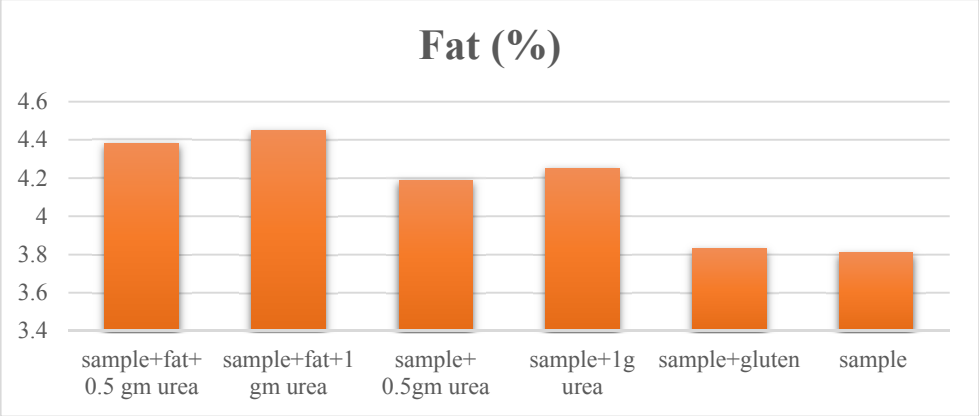

Figure 6. 12: **Graphical representation of fat present in synthetic and natural milk samples**

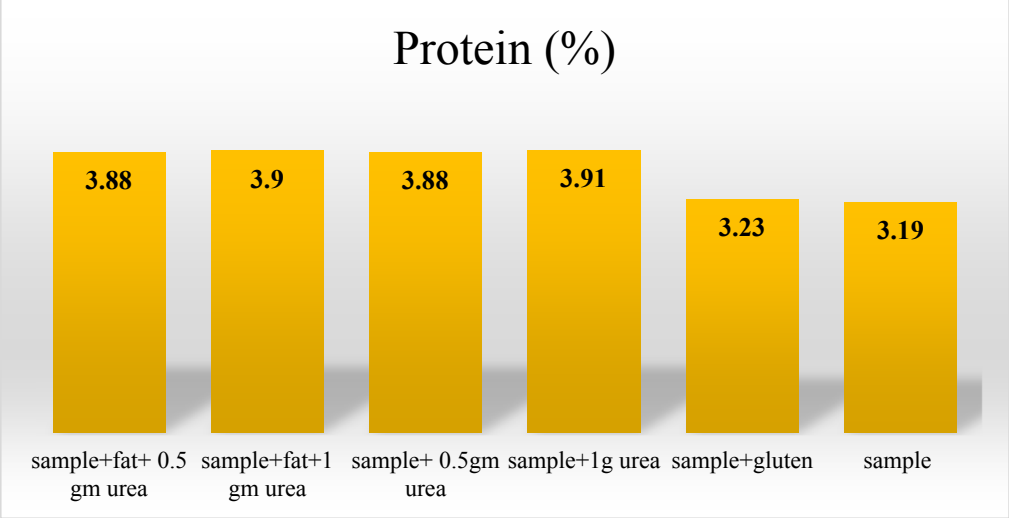

Figure 6. 13: Graphical presentation of protein present in synthetic and natural milk samples

Obtained data of attenuation coefficient of the imitated milk samples disclosed a range of reference values which indicates certain concentration of urea, vegetable oil, gluten and water present in milk. Further use of gamma radiation to check adulteration in commercial milk samples using these obtained reference values can be helpful to understand the added concentration of urea, vegetable oil, gluten in the tested sample in a cost effective way.

	nb	nc	Height of milk samples (cm)	Density	Linear attenuation	Mass attenuation
Milk brand 1	33	109	2.5	0.8	0.312810994	0.391013743
Milk brand 2	33	121	2.5	0.833	0.276976961	0.332372353
Milk brand 3	33	105	2.5	0.851064	0.351183516	0.412640631

Table 6. 12: Determined Attenuation coefficient of market milk samples using gamma source (^{137}Cs)

In the next phase of experimental design, each of 40 ml of procured commercial milk samples were taken for the analysis. Milk brand 3 showed highest attenuation coefficient and milk brand 2 showed least attenuation coefficient. The obtained attenuation co-efficient of market samples were similar to that of imitated milk samples containing urea, gluten, vegetable oil and water. Attenuation coefficient of milk brand 2 (0.2769) was approximately similar to that of imitated milk sample containing 1g urea (0.2787). Attenuation coefficient of milk brand 1 was approximately similar to that of imitated milk sample containing 0.5g urea. As attenuation coefficient depicts transparency of a material (Ghosh & Das, 2014), from the obtained data it can be disclosed that the transparency of milk brand 2 and the imitated milk containing 1g urea is approximately similar. Same disclosure can be made in case of milk brand 1 and milk sample containing 0.5g urea. Similarity between the imitated and market samples can indicate the potent use gamma radiation to detect adulterants.

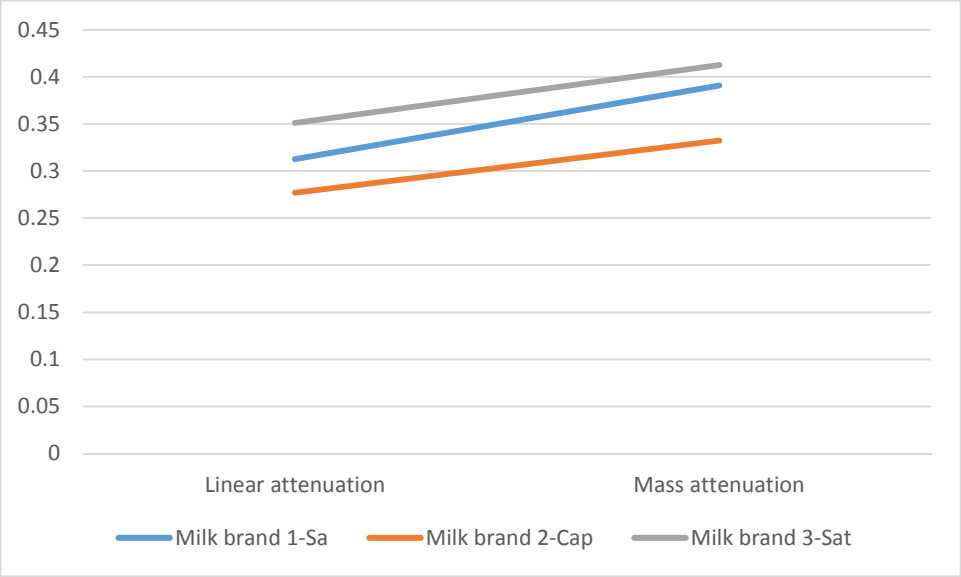

Figure 6. 14: Graphical representation of attenuation coefficient of market milk samples

Place	Samples	Total Fat	Protein
Churu dairy	Milk brand 1	1.5 g	3.4 g
Gurugram	Milk brand 2	0.5 g	3.3 g
Kanumangarh, Rajasthan	Milk brand 3	1.5 g	3.2 g

Table 6. 13: Determination of Fat and protein present in different market milk samples

According to the obtained analytical data, Milk brand 1 and 3 showed similar fat content that is 1.5g. Milk brand 2 possessed 0.5 g fat. Protein content was found higher in milk brand-1 (3.4g). The lowest protein was obtained in milk brand-3 (3.2g).

	24 hours	48 hours	72 hours
Milk brand 1	3	1	0
Milk brand 2	1	0	0
Milk brand 3	5	2	0

Table 6. 14: Obtained counts in gamma irradiated different market milk samples

Upon 24 hours of exposure to gamma source, the commercial milk samples were stored maintaining the same condition that is at room temperature (22°C) and the lids of

the container were left open. Milk brand 2 showed zero count after 48 hours. Rest samples showed zero count after 72 hours. Irradiated commercial milk samples did not show any sour smell or spoilage until fourth day, although the samples showed zero count after 72 hours. Samples with radiation exhibited mild foul smell and showed lesser precipitation after boiling on fifth day of storage. Milk brand 2 showed smallest grainy particles of coagulated proteins among other two samples. Milk brand 3 showed the largest coagulated lumps. Non-irradiated all the commercial milk samples exhibit sour smell on fourth day indicating initiation of protein coagulation due to low pH. For more proper visualization of protein coagulation, samples were heated and visible grainy particles of coagulated milk protein were observed. After exhibition of sour smell upon boiling of the commercial milks, irradiated samples and non-irradiated samples showed the same pattern of visible coagulated proteins. Irradiated samples showed smaller grainy texture of coagulated proteins than the coagulated lumps obtained from non-irradiated milk samples.

Besides the study of nutrients available and effect of gamma radiation on milk samples, fat separation pattern of the imitated sample during storage was also observed. Fat separation of milk samples showed inversely proportional relation with concentration of added adulterants in the sample. Pure milk showed more fat separation in comparison with imitated milk and commercial milk samples. Milk sample containing urea, vegetable oil and water showed lesser or no fat separation. Market milk samples showed negligible fat separation. According to previous studies in case of market milk, natural fat present in milk is extracted and replace them with vegetable oil to compensate profit (Mudgil & Barak, 2013).

Estimated data obtained from the comparative study of the interaction between gamma radiation and different milk samples viz., pure milk, imitated adulterated milk, commercial milk, disclosed that gamma radiation can be utilized as potent detector of adulterants present in milk sample in cost-effective way. By studying the pattern of attenuation coefficient of different milk samples, added elements in sample can be predicted.

Figure 6. 15: **Curdled mass (Coagulated milk protein) obtained from market milk samples**

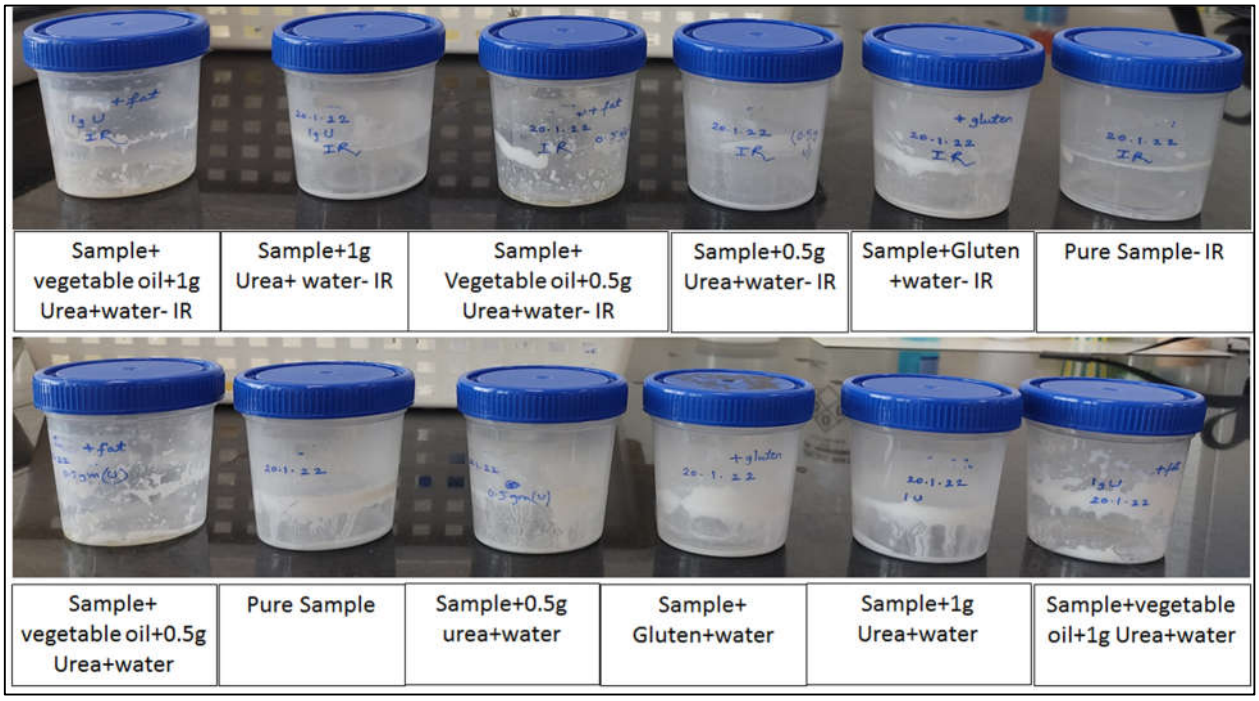

Figure 6. 16: Fat separation of imitated milk samples (irradiated and non-radiated)

Size of obtained curdled lumps of coagulated milk proteins also depicts the degree of concentration of added adulterants in milk samples in inversely proportional manner. According to the obtained data, grainier the texture of coagulated proteins, higher concentration of adulterants might be present in the milk. Moreover, the nature of the

texture of the curdled coagulated proteins obtained from the milk samples can depict the binding property the obtained curdled mass which are used to formulate dairy products like paneer. The curdled mass is obtained due to excess growth of lactobacillus in milk and generation of lactic acid in milk resulting in low pH (acidic media). Due to low pH, milk protein starts to coagulate. Upon boiling, in the presence of heat and acidic environment, the protein gets oversaturate and results in visual grainy or lumpy texture of coagulated milk proteins. Imitation of the same environment is carried out using citric acid or lemon juice in paneer preparation. Generation of low pH and heating results in curdled mass from which paneer is made. In case of smaller size of curdled mass/crystals (coagulated milk proteins) observed in market milk sample, blending of the obtained mass gave smooth and consistent structure leading to proper shaped paneer. This paneer did not break easily upon cooking and gave rubber like texture. On the other hand, large sized lumps of curdled mass obtained from fresh milk did not give smooth blend. Paneer prepared from fresh milk, easily de-structured on cooking which is an appropriate characteristic to differentiate composition of milk used to prepare the paneer.

Addition of adulterants like cheaper and harmful materials in food products is a crucial problem faced by the society. A study mentioned mixing of cheaper solvent, diethylene glycol in products viz., alcohol based beverages to enhance sweetness which indirectly lower the cost (Risk Assessment studies, 2003; Spink 2014). Depending on the consumption of toxic adulterants over time, effect of such adulterants on health can be graded (Schep *et al.,* 2009; Ferrari 2005; Megarbane *et al.,* 2005). In previous study, gamma ray spectroscopic method was used to detect such adulterants based upon the alteration in linear and mass attenuation coefficients. Although gamma ray spectroscopy method is more popular in nuclear science research, the study of interaction of gamma radiation with solid food products or liquid products like juice, milk etc. can depict the composition of the provided sample. The linear attenuation coefficients of potassium chloride, ammonium chloride, ferrous sulphate, carbohydrates, phenol, urea and lactose in milk at different concentrations had been studied using gamma ray spectroscopic technique (Gerward 1996; Teli and Chaudhari 1996; Teli 1998; Chaudhari and Nathuram 2010;

Chaudhari and Rathod 2013; Mitkar and Dongarge 2012; Mudahar *et al.,* 1991). The detection of adulterants like melamine in milk powder using gamma ray spectroscopy (Udagani and Ramesh 2014) also had been reported. According to the obtained data, linear attenuation coefficient gets higher with the increasing added amount of the melamine in milk powder.

6.4. Summary

In this chapter, we have discussed formulation of Health drink powder (HDP) and Detox tea substitute (DTS) using milling by-products and fruit peel followed by estimation of proximate composition, nutrient availability, minerals, antioxidant and storage stability of the products. Most acceptable variants among the formulated products were estimated through sensory profiling where, HDP1 (7.79±0.01) and DTS2 (8.18±0.11) showed higher acceptability scores among others. Crude protein present in HDP and DTS were (19.27±0.01) % and (18.21±0.19) % respectively. Calcium was higher in HDP (81.21±4.03 mg/100g), whereas phosphorus was higher in DTS (211.52±0.22 mg/100g). Total phenolic contents of both the products were around 4 mg GAE/g. Vitamin C level was higher in HDP (60.23±0.11 mg/100g). Shelf life study and microbial load assessment indicated longer storage life of the formulated products. The *Total Plate Counts* (Log CFU/g) were 2.12±0.01 and 2.08±0.12 found to be in freshly prepared HDP1 and DTS2 respectively under evaluation**.** The yeast and mold counts (Log CFU/g) was observed after 75^{th} day and 60^{th} day in HDP1 (2.09±0.05) and DTS2 (2.01±0.11) respectively (stored room temperature). The overall acceptability of these novel formulations as determined by sensory evaluation throughout the storage duration was satisfactory.

In the succeeding part of the chapter, we have also described use of gamma radiation to check the presence of adulterants in liquid products in a cost-effective way. Milk was chosen as 'Test sample' for the experiment. The present study gives a way out to use gamma radiation in adulteration detection along with identification of natural milk and synthetic milk. Structural changes in the curdled mass obtained from different milk samples based upon heating and irradiation also had been observed which might depict the reason behind the perfect shape of paneer in market as compare to that prepared in home. Milk

samples are adulterated for commercial purpose, profit as these milk samples possess longer shelf life. But the after effect of daily consumption of such milk and milk based products are hazardous. Due to expensive and time consuming methods, conventional adulteration detection sometimes become problematic. Hence, use of cost effective gamma radiation based rapid detection of presence of added elements i.e., adulterants in liquid products can be helpful to maintain good health and beneficial to the society.