CHAPTER-3

Development of fibre and micronutrient enriched fermented products

"Even in this high-tech age, the low-tech plant continues to be the key to nutrition and health."- Jack Weatherford

3.1. Introduction

Fermented foods are considered as a potential sources of micro and macronutrients and cereal-legume based fermented foods are already popular enough among consumers due to their health beneficial aspects (Yao *et al.,* 2020). Cereals and pulses provide health beneficial nutrients either through direct consumption or in the form of composite flour based food products. According to a study reported, lactic acid fermentation has come out as an extraordinary choice to valorise these by-products (Spaggiari *et al.,* 2020). Thus to analyse the impact of the milling by-products (rice bran, broken rice, wheat bran, chickpea husk) as an alternative source to prepare fermented products, among most consumed fermented recipes, *idli* (Rice cake) and paneer (Indian cottage cheese) were chosen as target foods due to their popularity, especially among vegetarians (Mandhania *et al.,* 2019).

Our present interest is to process the legume by-product (chickpea husk) along with the cereal by-products (rice bran, broken rice, wheat bran) through fermentation along with various domestic culinary treatments and to study the food properties of those processed by-products-based fermented products. These products were developed using domestic cooking methods (soaking, blanching, roasting) and thermal processing (hot air oven drying).

In the following section, author has performed the detailed study of the raw materials which are the milling by-products of legumes and cereals. Processing of the selected milling by-products through fermentation and domestic culinary methods to develop fermented products i.e., Instant mix for the preparation of *idli* (Rice cake) and Bran Paneer (Indian cottage cheese analogue) followed by evaluation of the nutrient composition, sensory attributes and shelf life of the products also have been disclosed.

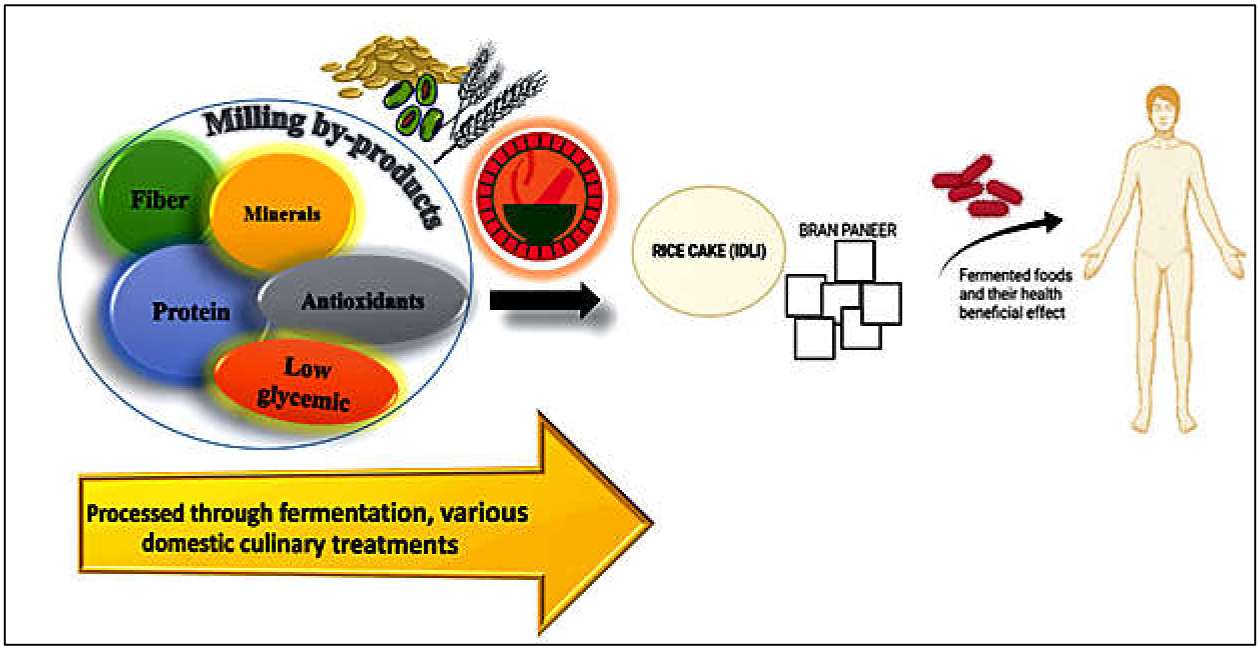

Figure 3. 1: **Formulation of nutrient dense fermented products using processed milling by-products**

3.2. Processing and valorisation of the milling by-products

Prior to the value added product formulation, proximate composition and nutrients present in the milling by-products were estimated according to the methodology of AOAC (Discussed in Chapter 2). Further, the milling by-products were processed through fermentation and various domestic culinary treatments for the product development. The detailed formulation composition of Instant mix (IM) (for *idli* preparation) combining different percentages of by-product flours has been provided in Table 3.1. The *idli* batters were prepared by mixing Instant mix (IM0, IM1, IM2, IM3, IM4) with required amount of fruit salt and yoghurt/curd. Cardamom powder, cumin seed powder, black pepper, and black salt were added further to the batter according to the taste followed by a proper mixing and were kept for 12 hrs. at room temperature (24±2°C). After the proper fermentation of the batter, it was poured into greased *idli* moulds and steamed using an *idli* cooker (Pigeon stainless steel 4 plates *idli* maker) for 10-12 min (Figure 3.2) (Rani *et al.*, 2019). The cooked *idlis* were kept a while to cool down and then stored in HDPE bags for further analysis.

Detailed composition of composite flour formulation for the preparation of Bran paneer variants is given in Table 3.2. Five variants of Bran paneer (BP0, BP1, BP2, BP3, BP4) were formulated separately, using Calcium Chloride ($CaCl_2$), an acidic agent as a coagulant (Kumar *et al.,* 2011). Composite flour was soaked in water and blended to get smooth mixture. Then muslin cloth was used to strain the blended mixture by squeezing properly. Collected milling by-product isolate (Composite flour milk) was heated up in a heavy bottom pan to 100°C for 20 minutes. Then it was cooled down and stored.

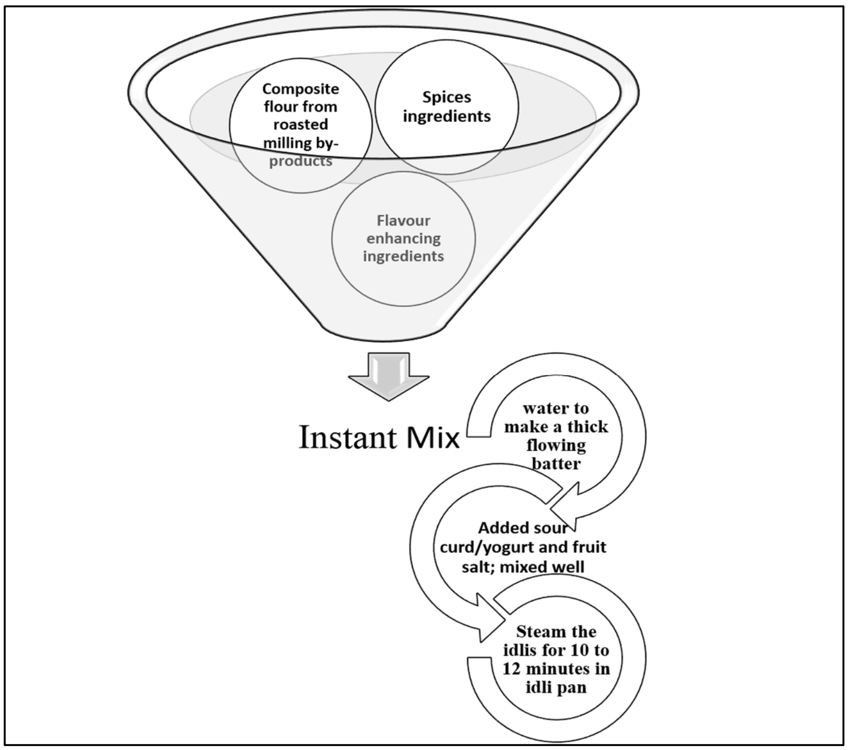

Figure 3. 2: Processing of milling by-products to formulate Instant mix (IM) and *Idli* (IM-I)- The figure represents schematic diagram of the preparation of *idli* using Instant mix formulated using composite flour of milling by-products

Due to presence of certain protein (casein), it gives appropriate curdle when an acid (coagulant) is added to the hot milk. The selected milling by-products do not contain sufficient amount of protein to give appropriate curdle upon addition of the coagulant. Thus, a mixture of the milling by-product isolate (Composite flour milk) and full cream milk was made where added composite flour milk was 30% of the volume and rest was the full cream milk that is 70% of the volume. While heating up the final mixture, the coagulant was added to it when the temperature reached 60° C (Figure 3.3). The mixture was heated up till 80°C to get the complete coagulation. Later, the whey (remaining liquid after the curdle) was separated and the collected curdled mass was pressed in paneer mould. It was kept under a heavy static load for at least 15 min. Then formulated paneer (BP) was drained for few minutes under cold water and packed into a HDPE bags for further analysis.

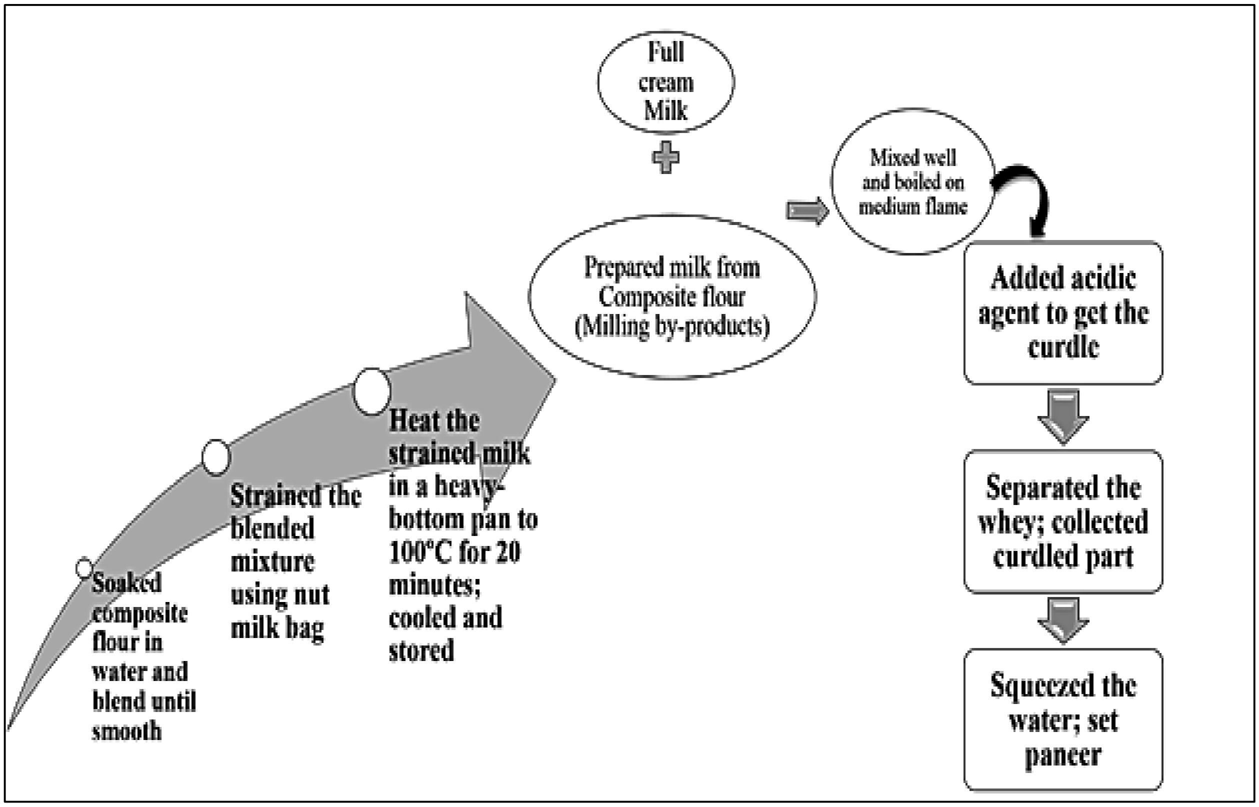

Figure 3. 3: Processing of milling by-products to formulate value added product-Bran *Paneer*

As this study mainly focuses upon possible food value of chickpea by-product along with other cereal by-products (rice bran, broken rice, wheat bran), the composite flour composition which did not contain chickpea husk is considered as 'control' regarding the formulation of the fermented products in this current study. Instant mix 'control' that is IM0 (Table 3.1) does not contain chickpea husk. IM0 formulation composition only contains rice bran, wheat bran, and broken rice. Same consideration has been applied for the formulation of another fermented product, Bran paneer and 'control' is labelled as BP0 (Table 3.2).

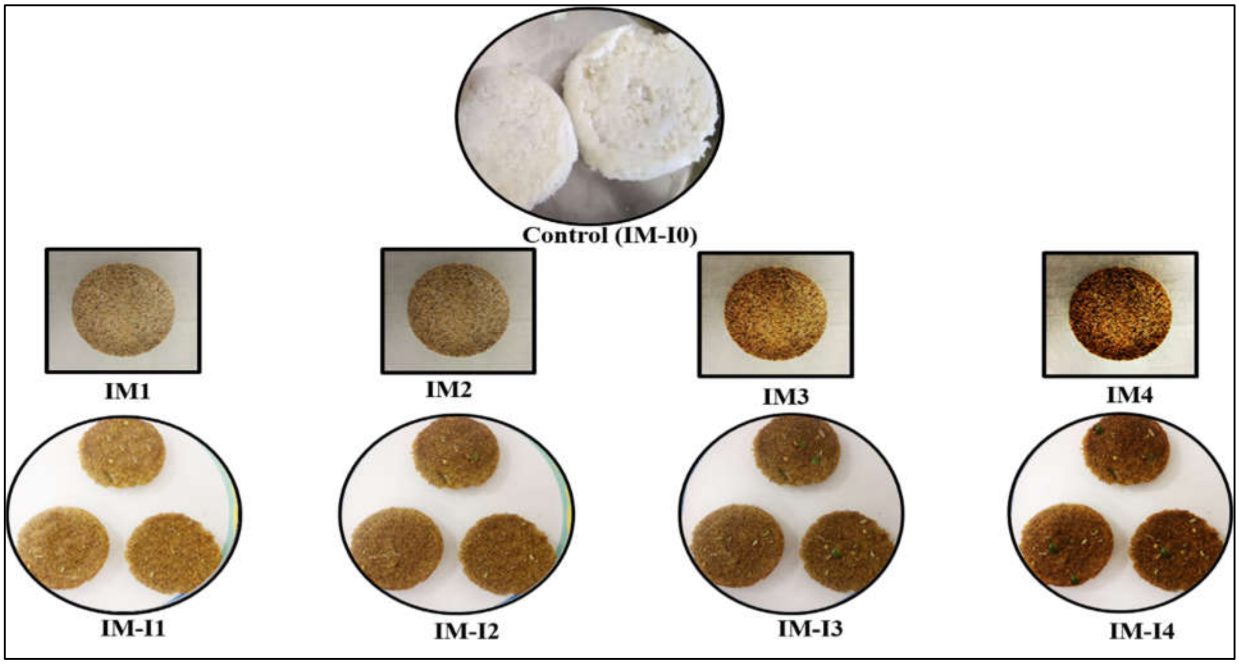

Figure 3. 4: **Formulated variants of Instant Mix for *idli* (IM-I0, IM-I1, IM-I2, IM-I3, IM-I4) using different percentage compositions of milling by-products**

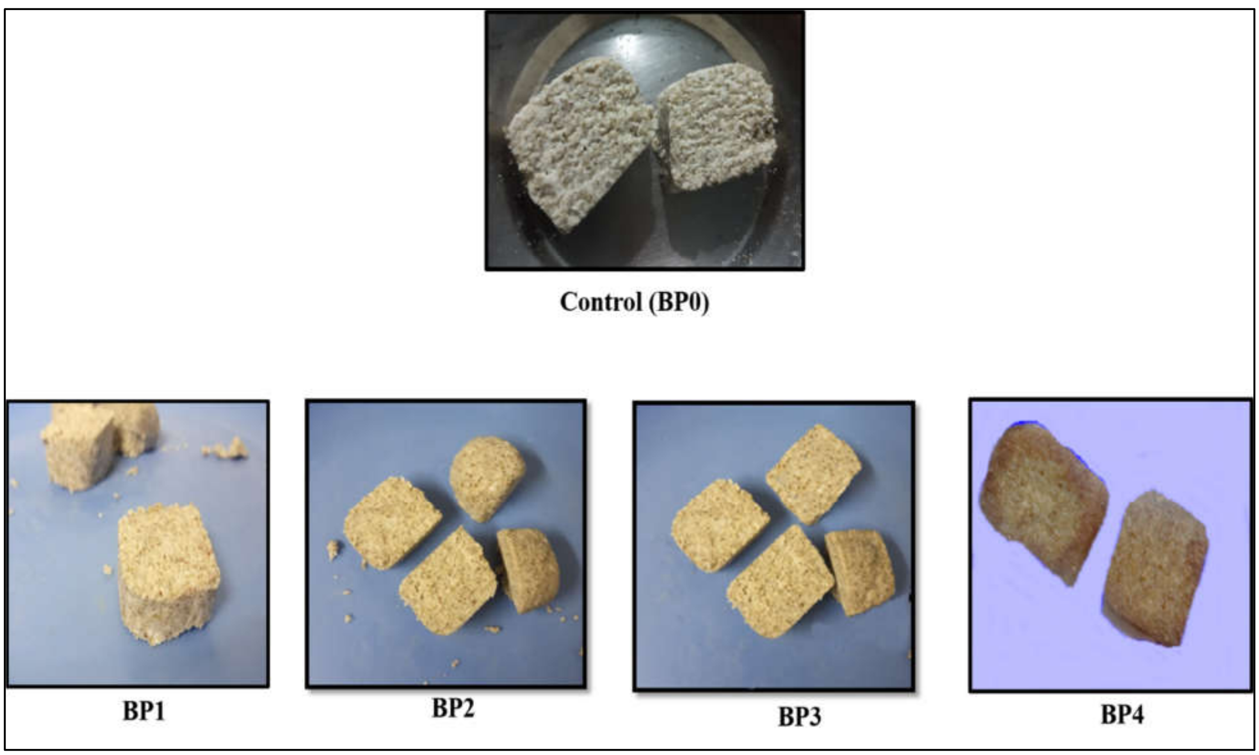

Figure 3. 5: **Prepared variants of Bran *Paneer* (BP0, BP1, BP2, BP3, BP4) using different percentage compositions of milling by-products**

Ingredients (g)	IM0 (g) (Control)	IM1 (g)	IM2 (g)	IM3 (g)	IM4 (g)
Rice bran flour	10.92 (20.73%)	10.55 (20.82%)	10.02 (19.95%)	9.53 (18.97%)	9 (18.14%)
Wheat bran flour	10.75 (20.41%)	10.07 (19.87%)	9.35 (18.61%)	9.04 (17.99%)	8.75 (17.63%)
Broken rice flour	31 (58.86%)	27.05 (53.37%)	25.1 (49.97%)	23.65 (47.07%)	21.72 (43.77%)
Chickpea husk flour	0 (0%)	3.01 (5.94%)	5.76 (11.47%)	8.02 (15.96%)	10.15 (20.46%)

Table 3. 1: Ingredient composition to formulate value added milling by-product based Instant Mix (IM) for *Idli*/ Rice Cake

Table 3.1 is comprised of the amount of husk/bran flour taken (g) to formulate the composite flour. Percentage of the used quantity of husk/bran flour in the formulated flour is also mentioned.

Ingredients (g)	BP0 (g) (Control)	BP1 (g)	BP2 (g)	BP3 (g)	BP4 (g)
Rice bran flour	20.62 (20.46%)	20.15 (19.88%)	18.98 (18.92%)	18.56 (17.97%)	17.66 (17.08%)
Wheat bran flour	20.15 (19.99%)	18.67 (18.42%)	18.25 (18.19%)	18.01 (17.43%)	16.65 (16.11%)
Broken rice flour	60.03 (59.55%)	56.55 (55.78%)	52.34 (52.17%)	50.72 (49.095%)	48.92 (47.32%)
Chickpea husk flour	0 (0%)	6.01 (5.93%)	10.76 (10.72%)	16.02 (15.51%)	20.15 (19.49%)

Table 3. 2: Ingredient composition to formulate nutrient rich milling by-product based Bran *Paneer*

Table 3.2 is comprised of the amount of husk/bran flour taken (g) to formulate the composite flour. Percentage of the used quantity of husk/bran flour in the formulated flour is also mentioned.

3.3. Results and Discussions

In this section, we have discussed the estimation of the physico-chemical properties and nutrient composition of the milling by-products followed by the formulation of the value added products along with sensory profiling, characterization and shelf life study of product.

3.3.1. Physico-chemical properties

3.3.1.1. Bulk density

Estimated bulk density of the raw materials varied significantly ($P<0.05$). Wheat bran showed highest bulk density (0.40±0.26 g/ml) and Bengal gram husk had least bulk density (0.22±0.01 g/ml). Rice bran and broken rice showed bulk density in the range of 0.56-0.51 g/ml (Table 3.3).

3.3.1.2.Water absorption capacity

Bengal gram husk had highest water absorption capacity i.e., 5.59±0.05 g/g and that of broken rice was the least (1.99±0.16 g/g). Wheat bran and rice bran showed water absorption capacity at the range of 2.44-2.15 g/g (Table 3.3).

3.3.1.3.Oil absorption capacity

According to the analysed data, oil absorption capacity of the rice bran was the highest i.e., 1.66±0.12 g/g and that of the Bengal gram husk was the lowest (0.85±0.11 g/g). Wheat bran and broken rice showed the oil absorption capacity in the range of 1.15-0.89 g/g (Table 3.3).

Physicochemical properties	Bengal gram husk	Wheat bran	Rice bran	Broken rice
Bulk density (g/ml)	0.22±0.01	0.40±0.26	0.56±0.14	0.51±0.23
Water absorption capacity (g/g)	5.59±0.05	2.44±0.09	2.15±0.23	1.99±0.16
Oil absorption capacity (g/g)	0.85±0.11	1.15±0.01	1.66±0.12	0.89±0.03

Table 3. 3: Physicochemical properties of the milling by-products of cereals and legumes

3.3.2. Proximate composition of the milling by-products

Proximate composition of the selected milling by-products was estimated and the obtained data varied significantly ($P<0.05$) (Table 3.4).

Moisture content of the broken rice was the highest among all other by-products i.e., (12.02±0.08) % and that of the rice bran was the lowest [(4.66±0.06) %]. Legume by-products i.e., Bengal gram husk and moong bean husk showed moisture (5.60±0.11) % and (7.09±0.03) % respectively. Wheat bran had (6.77±0.10) % moisture content.

Ash content of the broken rice was the lowest i.e., (0.49±0.05) %. Bengal gram husk, moong bean husk, wheat bran, and rice bran showed ash content in the range of 4.79-3.79 %. Rice bran showed the highest ash content [(4.79±0.03) %].

Crude fat of rice bran was the highest that is (17.88±0.31) %. Crude fat of the other by-products ranged in between 2.32-0.85 %. Bengal gram husk and moong bean husk showed (1.36±0.21) % and (2.21±0.12) % crude fat content respectively. Other two cereal by-products i.e., wheat bran and broken rice showed (2.32±0.15) % and (0.85±0.24) % crude fat respectively. Crude fat present in the broken rice was the least.

Crude protein of Bengal gram husk was (4.12±0.21) % and that of moong bean husk was (7.75±0.16) %. Milling by-products of rice i.e., rice bran and broken rice showed (12.96±0.01) % and (6.01±0.16) % crude protein respectively. According to the obtained data, wheat bran possessed (11.63±0.06) % crude protein.

Crude fibre of broken rice was the lowest among all other by-products which was (0.22±1.03) %. Rice bran and wheat bran showed (10.01±0.32) % and (8.43±0.03) % crude fibre respectively. Moong bean husk showed (18.70±0.35) % crude fibre. However, Bengal gram husk seemed to possess the highest amount of crude fibre among all which was (40.01±0.22) %.

Total carbohydrate of broken rice was (80.41±0.61) % which was the highest carbohydrate level. Wheat bran and moong bean husk possessed total carbohydrate in the range of 60.46-66.65%. Rice bran and Bengal gram husk showed (49.7±0.58) % and (44.86±0.26) % total carbohydrate respectively.

Proximate composition	Bengal gram husk	Wheat bran	Rice bran	Broken rice	Moong bean husk
Moisture (%)	5.60±0.11	6.77±0.10	4.66±0.06	12.02±0.08	7.09±0.03
Ash content (%)	4.05±0.12	4.20±0.11	4.79±0.03	0.49±0.05	3.79±0.16
Crude fat (%)	1.36±0.21	2.32±0.15	17.88±0.31	0.85±0.24	2.21±0.12
Crude protein (%)	4.12±0.21	11.63±0.06	12.96±0.01	6.01±0.16	7.75±0.16
Crude fibre (%)	40.01±0.22	8.43±0.03	10.01±0.32	0.22±1.03	18.70±0.35
Total carbohydrate (%)	44.86±0.26	66.65±0.02	49.7±0.58	80.41±0.61	60.46±0.09

Table 3. 4: Proximate composition of by-products of cereals and pulses (%, dry weight basis)

3.3.3. Nutrient composition of the milling by-products

Phosphorus levels of the milling by-products of rice i.e., rice bran and broken rice were 1184.56±0.08 mg/100g and 116.55±0.12 respectively. Wheat bran showed 988.01±0.03 mg/100g phosphorus according to the estimated data. The same of the legume husks i.e., Bengal gram husk and moong bean husk were 250±0.11 mg/100g and 359.11±0.37 mg/100g.

Iron was higher in rice bran (29.31±0.14 mg/100g) than other by-products. Broken rice and wheat bran showed 2.66±0.08 mg/100g and 10.15±0.10 mg/100g iron. Bengal gram husk and moong bean husk showed 6.05±0.15 mg/100g and 24.05±1.06 mg/100g iron according to the data.

Calcium obtained in Bengal gram husk was the highest that is 899.01±0.23 mg/100g and that of moong bean husk was 398.05±0.02 mg/100g. Wheat bran, rice bran, and broken rice contained 71.06±1.66 mg/100g, 69.32±0.08 mg/100g, and 12.99±0.45 mg/100g calcium.

Minerals	Bengal gram husk	Wheat bran	Rice bran	Broken rice	Moong bean husk
Phosphorus (mg/100g)	250±0.11	988.01±0.03	1184.56±0.08	116.55±0.12	359.11±0.37
Iron (mg/100 g)	6.05±0.15	10.15±0.10	29.31±0.14	2.66±0.08	24.05±1.06
Calcium(mg/ 100g)	899.01±0.23	71.06±1.66	69.32±0.08	12.99±0.45	398.05±0.02

Table 3. 5: Available Minerals composition of milling by-products of cereals and pulses

In vitro starch digestibility of rice bran seemed to be the highest which was 82.32±0.61 mg maltose released/ g and that of the other milling by-product of rice i.e., broken rice was 51.20±0.11 mg maltose released/ g. Bengal gram husk showed the least in vitro starch digestibility (1.95±0.10 mg maltose released/ g) and the digestibility of wheat bran was 2.49±0.55 mg maltose released/ g.

In vitro protein digestibility of Bengal gram husk was (13.98±0.25) % and that of wheat bran was (24.12±0.09) %. Milling by-products of rice i.e., rice bran and broken rice showed (84.05±0.11) % and (56.33±0.98) %.

Antinutrient present in the by-products was estimated and according to the obtained data, Bengal gram husk exhibited the highest **trypsin inhibitor activity** and **phytic acid** which were 289.22±1.56 TIU/ mg and (980.12±0.79) % respectively. Phytic acid levels present in rice bran and broken rice were (7.69±0.26) % and (96.33±0.02) % respectively. Wheat bran showed higher phytic acid level than rice by-products which was (54.77±0.58) %. Trypsin inhibitor activity in wheat bran was 55.33±0.04 TIU/mg and that of rice by-products (Rice bran and broken rice) was in the range of 10-17 TIU/mg.

Total phenolic content broken rice was the least (0.56±0.01 mg GAE/ g) whereas that of rice bran was 1.16±0.01 mg GAE/ g. Wheat bran possessed 3.25±0.01 mg GAE/g of total phenol and Bengal gram husk contained 14.21±0.25 mg GAE/ g of the same which was the highest among all. **DPPH RSA** of Bengal gram husk and rice bran was in around

79%. But that of broken rice was the lowest i.e., (16.54±1.65) %. Wheat bran exhibited (55.32±0.55) % DPPH RSA.

Sugar and starch estimation was determined to study the level of reducing and non-reducing sugar along with available starch in the by-products. Rice bran contained higher level of total sugar content (11.29±0.05 g/100g) than other by-products. Reducing and non-reducing sugar of the same were 5.06±0.06 g/100g and 6.23±0.09 g/100g respectively. 23.91±0.01 g/100g Starch was present in rice bran. Broken rice contained 1.72±0.20 g/100g of total sugar where 0.41±0.03 g/100g was reducing sugar and 1.31±0.05 g/100g was non-reducing sugar. Starch present in the same was 75.36±0.12 g/100g which was the highest among other by-products. The least starch was present in wheat bran (1.05±0.07 g/100g). However, Bengal gram husk possessed 38.9±0.28 g/100g starch. Total sugar level of wheat bran was 3.01±0.11 g/100g where 2.02±0.25 g/100g was reducing sugar and 0.99±0.01 g/100g was non-reducing sugar. In case of Bengal gram husk, total sugar content, reducing sugar and non-reducing sugar were 6.95±0.23 g/100g, 2.00±0.01 g/100g, 4.95±0.15 g/100g respectively.

Nutrient composition	Bengal gram husk	Wheat bran	Rice bran	Broken rice
In vitro starch digestibility (mg maltose released/ g)	1.95±0.10	2.49±0.55	82.32±0.61	51.20±0.11
In vitro protein digestibility (%)	13.98±0.25	24.12±0.09	84.05±0.11	56.33±0.98
Trypsin inhibitor activity (TIU/ mg)	289.22±1.56	55.33±0.04	10.85±2.01	17.01±0.01
Phytic acid (%)	980.12±0.79	54.77±0.58	7.69±0.26	96.33±0.02
Total phenolic content (mg GAE/ g)	14.21±0.25	3.25±0.01	1.16±0.01	0.56±0.01
DPPH RSA (%)	79.20±2.12	55.32±0.55	79.89±0.99	16.54±1.65
Total sugar content (g/100 g)	6.95±0.23	3.01±0.11	11.29±0.05	1.72±0.20
Reducing sugar content(g/100 g)	2.00±0.01	2.02±0.25	5.06±0.06	0.41±0.03
Non- reducing sugar content (g/100 g)	4.95±0.15	0.99±0.01	6.23±0.09	1.31±0.05
Starch (g/100 g)	38.9±0.28	1.05±0.07	23.91±0.01	75.36±0.12

Table 3. 6: Nutrient Composition of milling by-products of cereals (Rice, Wheat) and pulses (Bengal gram)

Dietary fibre	Bengal gram husk	Wheat bran	Broken rice	Rice bran	Moong bean husk
Soluble dietary fibre(g/ 100g)	6.81±0.06	9.97±0.55	0.61±0.23	2.01±0.54	1.81±0.55
Insoluble dietary fibre (g/ 100g)	75.98±0.25	30.01±2.03	0.72±0.01	21.30±0.02	54.29±1.53
Total dietary fibre (g/ 100g)	82.79±0.09	39.98±0.12	1.33±0.68	23.31±1.59	56.10±0.56

Table 3. 7: Nutrient Composition of milling by-products of cereals (Rice, Wheat) and pulses (Bengal gram, Moong bean)

Dietary fibre of the milling by-products of cereals and legumes was estimated comprising the data of soluble dietary fibre, insoluble dietary fibre, and total dietary fibre. The highest level of total dietary fibre was found in Bengal gram husk (82.79±0.09 g/100g) followed by moong bean husk (56.10±0.56 g/100g). Soluble and insoluble dietary fibre present in Bengal gram husk were 6.81±0.06 g/100g and 75.98±0.25 g/100g respectively. Moong bean husk contained 1.81±0.55 g/100g and 54.29±1.53 g/100g of soluble and insoluble dietary fibre respectively. The lowest level of fibre was found in broken rice which was 1.33±0.68 g/100g of total dietary fibre (0.61±0.23 g/100g soluble dietary fibre and 0.72±0.01 g/100g insoluble dietary fibre). Rice bran possessed 23.31±1.59 g/100g of total dietary fibre where 2.01±0.54 g/100g and 21.30±0.02 g/100g were of soluble and insoluble dietary fibre respectively. 39.98±0.12 g/100g total dietary fibre was present in wheat bran, consisted of 9.97±0.55 g/100g and 30.01±2.03 g/100g of soluble and insoluble dietary fibre respectively.

3.3.4. Proximate composition of fruit by-products (peels)

Moisture content of orange peel [(10.33±0.02) %] was higher than that of banana peel [(9.55±0.01) %]. Estimated data showed higher ash content in banana peel which was (12.90±0.05) % and that in orange peel (5.11±0.23) %. Crude fat levels present in orange peel and banana peel were (1.73±0.05) % and (0.79±0.01) % respectively. Orange peel [(12.23±0.12) %] possessed more crude fibre than banana peel [(7.64±0.11) %]. However, crude protein present in orange peel [(6.91±2.15) %] was higher than that of banana peel [(1.96±1.96) %].

Proximate composition	Orange peel	Banana peel
Moisture (%)	10.33±0.02	9.55±0.01
Ash content (%)	5.11±0.23	12.90±0.05
Crude fat (%)	1.73±0.05	0.79±0.01
Crude fibre (%)	12.23±0.12	7.64±0.11
Crude protein (%)	6.91±2.15	1.96±1.96

Table 3. 8: Proximate composition of fruit by-products (peels) (%, dry weight basis)

3.3.5. Nutrient composition of fruit by-products (peels)

Minerals estimated showed higher phosphorus in banana peel that was 210.65±0.86 mg/100g and that of orange peel was 20.98±0.09 mg/100g. Iron and calcium were also higher in banana peel i.e., 47.06±0.36 mg/100g and 54.15±0.01 mg/100g respectively. Orange peel contained 19.91±0.45 mg/100g of Iron and 1.59±0.12 mg/100g of Calcium. Total sugar levels found in orange peel and banana peel were 46.21±0.23 g/100g and 47.88±0.66 g/100g respectively. Starch was higher in banana peel (4.05±0.01 g/100g) and that of orange peel was almost negligible (0.251±0.05 g/100g). Banana peel was found to be dietary fibre rich.

Nutrient composition	**Orange peel**	**Banana peel**
Phosphorus (mg/100g)	20.98±0.09	210.65±0.86
Iron (mg/100 g)	19.91±0.45	47.06±0.36
Calcium (mg/ 100 g)	1.59±0.12	54.15±0.01
Total sugar content (g/100 g)	46.21±0.23	47.88±0.66
Starch (g/100g)	0.251±0.05	4.05±0.01
Total Dietary fibre (g/ 100g)	40.17±0.02	62.64±0.32
Soluble dietary fibre (g/ 100g)	18.52±0.09	12.65±0.22
Insoluble dietary fibre(g/100g)	21.65±0.05	49.99±0.19
DPPH RSA (mg/ml)	2.33±0.03	89.05±0.01
Total phenolic content (mg GAE/g)	2.56±.0.25	158.98±0.68

Table 3. 9: Nutrient composition of Fruit by-products (peels)

Obtained data showed 62.64±0.32 g/100g total dietary fibre in banana peel where 12.65±0.22 g/100g and 49.99±0.19 g/100g were of soluble and insoluble dietary fibre respectively. Orange peel possessed 40.17±0.02 g/100g of total dietary fibre consisting of 18.52±0.09 g/100g soluble dietary fibre and 21.65±0.05 g/100g insoluble dietary fibre. DPPH RSA was found to be 2.33±0.03 mg/ml in orange peel and that of banana peel was 89.05±0.01 mg/ml. Total phenolic content of banana peel was higher than that of orange peel (2.56±.0.25 mg GAE/g). Banana peel contained 158.98±0.68 mg GAE/g total phenol.

Estimated data showed enriched nutrient composition of the milling by-products. These by-products were further processed and utilized for the formulation of value added products followed by evaluation of sensory parameters and characterization of the developed novel products.

3.3.6. Sensory evaluation of the formulated value added products (Instant Mix and Bran *Paneer*)

Sensory evaluation of the products was carried out to understand the organoleptic acceptance of the formulations among consumers. Besides enhancing nutrient availability, valorisation process of the milling by-products during product development also results in improved sensory profile. According to the study by Mishra and Chandra (2012), with increased addition of rice bran in the formulation, sensory quality of the developed product became low. Sozer *et al.,* 2014 also depicted the same observation during formulation of product using wheat bran. In this current study, among the variants of IM-I (Figure 3.6) and BP (Figure 3.7), the sole use of cereal by-products (0% chickpea husk flour) i.e., IM-I0 (8.39±0.02) and BP0 (7.99±0.011) showed highest overall acceptability. As the current study primarily focuses on the impact of chickpea husk along with selected cereal by-products, to carry out further experiments the chosen variants comprising both chickpea husk and cereal by-products with highest overall acceptability were BP1 (7.85±0.02) (5.93% of chickpea husk flour) and IM-I1 (8.24±0.01) (5.94% of chickpea husk flour). The preferred formulations (IM1 and BP1) were stored for further analysis. Bose and Shams-Ud-Din (2010) in their study also concluded that according to the obtained data of analysed pre-treated chickpea husk based product, husk based products can be rich in nutrient availability and high sensory score.

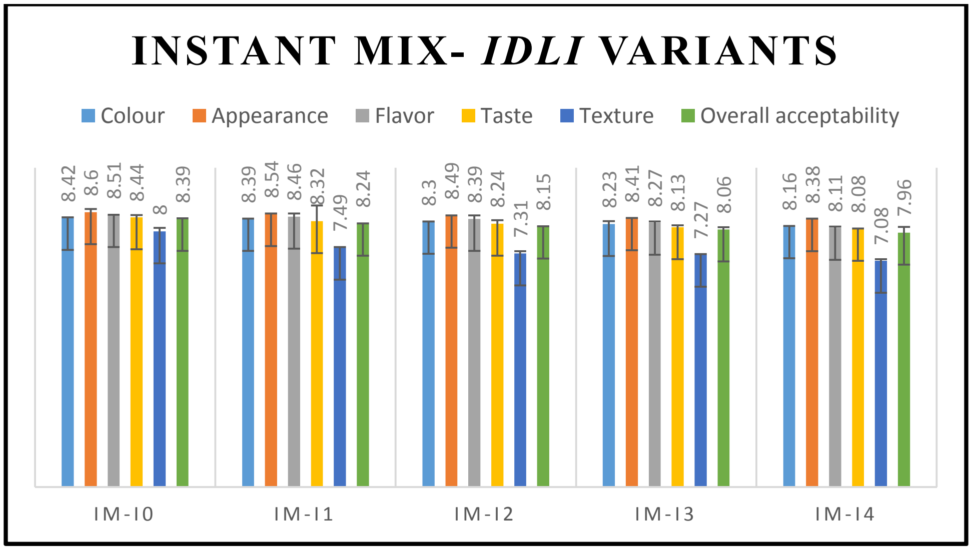

Figure 3. 6: Sensory evaluation of prepared value added milling by-product based product- Instant Mix- *idli*

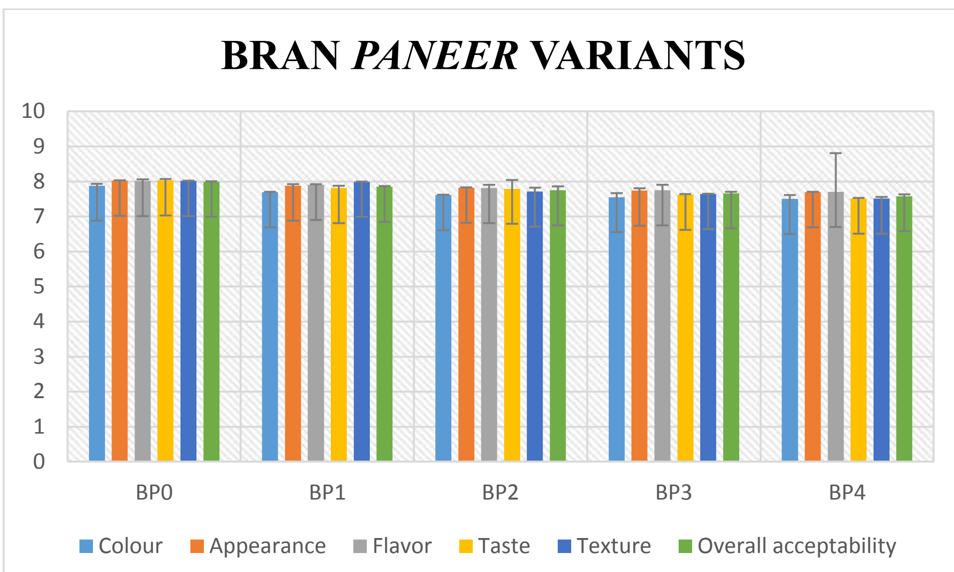

Figure 3. 7: Sensory evaluation of the formulated nutrient rich milling by-product based Bran *Paneer*

3.3.7. Microbiological safety of developed products (Instant Mix and Bran *Paneer*)

Microbial counts of food products are also crucial to food safety along with the production, processing of the products and their preservation for storage. In this current study, microbial analysis of freshly prepared BP1 and the *idli* batter from the IM1 was done (Table 3.10) to evaluate safety of these by-product-based fermented products. The *Total Plate*

Counts (Log CFU/g) were found to be 3.86±0.01 and 2.88±0.01 in freshly prepared BP1 and IM1 *Idli* Batter respectively**.** Yeast-mold counts in fresh preparations were negative. But the counts (Log CFU/g) increased to 5.03±0.02 and 9.03±0.02 next day in BP1 and IM1 *Idli* Batter (stored at freezer; 4°C) respectively. All fresh formulations were observed to be retaining the edibility quality. In a study, contribution of by-products of rice and corn on the formulation of a fermented food product (tarhana) was evaluated (Aktaş and Akın, 2020). For the successful study of the formulated product, microbial count determination was carried out to monitor the prevalence of bacterial growth. Another study also evaluated the microbial counts of functional low-fat yogurt formulated from barley bran and *Lactobacillus acidophilus* (Hasani *et al.,* 2017). Both the studies concluded successful formulation and improved food properties of the developed fermented food using milling by-products (Aktaş and Akın, 2020; Hasani *et al.,* 2017).

Bran Paneer (BP1)	**Total Plate Count**	3.86±0.01
	Yeast and Mold Count	nd (Fresh preparation)
		5.03±0.02 (After 24 hours)
Instant Mix (IM1) Batter (*Idli* Batter)	**Total Plate Count**	2.88±0.01
	Yeast and Mold Count	nd (Fresh preparation)
		9.03±0.02 (After 24 hours)

Table 3. 10: Microbial load (Log CFU/g) of freshly formulated products [nd: not detected]

3.3.8. Detailed study of the nutritional attributes of the formulated products (Instant Mix and Bran *Paneer*)

BP1, IM1 and *Idli* prepared using the preferred Instant Mix (IM-I-1) were analysed to evaluate the nutritional value. Analysis of both IM1 and IM-I-1 were done to understand the effect of fermentation on IM1 and to get a comparative analysis of the product (IM1) and result (IM-I-1).

3.3.8.1. Proximate compositions

Moisture content of food product is a crucial aspect to understand the storage stability of the product (Harhar *et al.,* 2010). The moisture contents of BP1 and IM-I-1 were higher, ranging in between 54-62%. But IM1 showed lesser moisture (4.71±0.06%) indicating storage for a longer duration. However, the moisture contents of BP1 and IM-I-1 were higher than that of IM1. Thus it can be said that the later had higher shelf life. Substitution with by-products showed enhanced nutritional attributes of BP1, IM1, and IM-I-1 **(**Table 3.11). Crude protein (%) in all the products ranged in between 17-18%. Protein is essential for the proper functioning of biological activities viz., growth and development of organisms (Friedman and Brandon, 2001). Crude fat (%) was found less in the products ranging from 2% to 4%, that is lesser than the products available in market. Higher moisture and protein level with low fat content in BP1 was established. In a study the same pattern of lower level of fat with higher moisture in soy protein isolate paneer (Kumar *et al.,* 2011) was observed. Moisture and protein contents of BP1 were found to be higher in comparison to Moisture (54.8±0.45%) and protein (17.7±0.38%) contents of high fat paneer (control), prepared in this study done by Kumar et al. (2011). Total carbohydrate Percentage was 14.92±0.11 in BP1 and 20.32±0.01 in IM-I-1. Singh Sibian & Singh Riar (2021), in their study regarding product formulation, stated that protein, fibre, and ash content was enhanced after legume fortification. However, carbohydrate level of the legume fortified product was lower than the control product. In this current study by-products were processed by various domestic cooking methods viz., soaking, blanching, roasting before product formulation. According to Liu *et al.,* 2020, the study stated decrease in carbohydrate level of food prepared by using domestic cooking methods.

3.3.8.2. Sugar and starch

Reducing sugar level of the products was around 3g/100g. The reducing sugar levels of BP1 and IM1 were 2.95±0.08 g/100g and 3.01±0.01 g/100g respectively. IM1 was fermented before cooking of *idli* (IM-I-1). After the preparation of *idli* using IM1, slight decline was found in the reducing sugar level of the *idli*s (IM-I-1) which was 2.91±0.01 g/100g (Table 3.12). The total soluble sugar levels of IM1 and IM-I-1 were 4.28±0.01 g/100g and 3.98±0.01 g/100g respectively. Starch content was found to be higher in IM1

(29.01±0.05 g/100g) and IM-I-1 (28.51±0.06 g/100g) than BP1 (10.90±0.01 g/100g). This might occur due to higher percentage of broken rice used in this formulation (IM1 and IM-I-1). Though starch was higher in IM1, after fermentation of the instant mix to prepare *idli* (IM-I-1), starch of IM-I-1 decreased. The same pattern was observed in a study done by Kumari et al. (2020) to analyze the effect of buckwheat incorporated *idli*. They mentioned that during fermentation, starch breaks down to reducing sugar and results into decreased starch level in the product.

Proximate composition	**Bran Paneer (BP1)**	**Instant Mix (IM1)**	**Instant Mix- *Idli* (IM-I-1)**
Moisture	62.19±0.02[b]	4.71±0.06[a]	54.01±0.01[c]
Crude protein	18.99±0.01[c]	17.01±0.19[a]	18.61±0.11[b]
Crude fat	2.08±0.01[b]	3.99±0.04[a]	3.09±0.04[a]
Crude fibre	4.91±0.06[b]	8.07±0.11[a]	8.01±0.02[c]
Ash	1.82±0.12[a]	4.90±0.12[a]	3.97±0.05[b]
Total carbohydrate	14.92±0.11[a]	69.39±0.09[b]	20.32±0.01[c]

Table 3. 11: Nutrient composition of the Products per 100g (%, dry matter basis) [Data presented (means of triplicate± Standard error) are significantly different, $p < 0.05$ (Statistical analysis has been done row wise)]

3.3.8.3. Minerals

Ash was moderate in all the formulations ranging from 1%-5% (Table 3.12) compared to market foods (1-2%). Iron (mg/100g) was comparatively lesser in BP1 (3.09±0.08) than IM1 (14.87±0.05) and IM-I-1 (15.46±0.08). On the contrary, Calcium content (mg/100g) was higher in BP1 (150.01±4.03), whereas IM1 (71.86±4.36) and IM-I-1 (80.04±0.10) seemed to possess lesser Calcium level. Higher calcium content in BP1 might be due to presence of milk in the recipe. Phosphorus (mg/100g) was more in IM1 (201.42±0.22) and IM-I-1 (209.92±0.14) than BP1 (131.08±0.20). The higher levels of minerals (Table 3.11) found in these legume husk fortified cereal bran food products can also indicate to the fact that these formulations can be considered as micronutrient-enriched formulations (Sohail *et al.,* 2017). Moreover, in case of IM1 and IM-I-1, it has been observed than IM-I-1 possesses enhanced mineral contents than IM1. This might happen due to lactic acid fermentation of the IM1 during the processing of *idli* batter. According to the studies, fermentation contributes to the improvement of the micronutrient availability (Scheers *et al.,* 2016). Even there are studies showed that the lactic acid fermentation of cereal-legume by-products is one of the proficient ways to valorise them (Spaggiari *et al.,* 2020). However, other fermented product, BP1 showed lesser nutrient estimations in some cases. This might happen due to the processing of composite flour milk during the preparation of BP1. During the complete squeezing of the blended semi-liquid composite flour, a portion of the blend was stuck in the mesh of the muslin cloth which was unavoidable and the drained liquid was collected as composite flour milk for the formulation. Thus partial utilization of the blend can be a reason, BP1 showed lower mineral levels than IM1 and IM-I-1. This estimated data of minerals point towards the probable way to prevent the micronutrient deficiencies.

3.3.8.4. Antinutrient factors

The antinutrient levels were comparatively lesser in the products as compare to that present in the raw legumes and chickpea husk. This might occur due to processing and fermentation of the by-products during the preparation of products. Trypsin inhibitor activity was found around 1.05 TIU/mg along with 127 mg/100g of phytic acid content in BP1 and 1.57 TIU/mg Trypsin inhibitor activity along with 171 mg/100g of phytic acid (Table 3.12) content in IM-I-1. IM1 showed bit higher Trypsin inhibitor activity and phytic acid content. Available antinutrient in the range of healthy limit of the body are not supposed to be harmful**.** Although there is no official Recommended Daily Allowance (RDA) of phytic acid, 100-400 mg per day are recommended. According to a study, 498.6-604.9 mg/100 g phytic acid content available in immature wheats at early stages of kernel development, was considered low phytic acid content (Özkaya *et al.,* 2018). Benefits of phytic acid as hypoglycemic, antioxidant and anti-bacterial have been mentioned (Kumar *et al.,* 2021). Another study showed trypsin inhibitor activity in fermented batter of pure black gram dal for *idli* preparation where the highest trypsin inhibitor activity (TIU) found was 20, per g of the sample (moisture free basis) (Amane and Ananthanarayan, 2018). Formulated products showed negligible antinutrient content in comparison to source. Reduced level of anti-nutritive compound was also observed in studies involving domestic cooking methods to get processed plant-based product (Managa *et al.,* 2020).

3.3.8.5. Phenolic compounds and antioxidant properties

According to available literature, various cooking methods improve the antioxidant activity due to application of certain conditions leading to the cell rupture and release of the antioxidants (Myint *et al.,* 2017). Total Phenolic content was around 3-4 mg GAE/g and for DPPH RSA (%) it was 55% (Table 3.11).

Nutrients	**Bran Paneer (BP1)**	**Instant Mix (IM1)**	**Instant Mix-*Idli* (IM-I-1)**
Non-Reducing sugar (g/100 g)	1.12±0.21[a]	1.27±0.19[b]	1.07±0.19[b]
Reducing sugar (g/100 g)	2.95±0.08[a]	3.01±0.01[b]	2.91±0.01[b]
Total soluble sugar (g/100 g)	4.07±0.05[a]	4.28±0.01[b]	3.98±0.01[b]
Starch (g/100 g)	10.90±0.01[c]	29.01±0.05[b]	28.51±0.06[a]
Iron (mg/100g)	3.09±0.08[b]	14.87±0.05[a]	15.46±0.08[b]
Calcium (mg/100g)	150.01±4.03[b]	71.86±4.36[a]	80.04±0.10[c]
Phosphorus (mg/100g)	131.08±0.20[b]	201.42±0.22[a]	209.92±0.14[c]
Trypsin inhibitor activity (TIU/mg)	1.05±2.01[b]	3.97±2.06[a]	1.57±0.06[c]
Phytic acid (mg/100 g)	127.01±1.32[b]	241.25±1.38[a]	171.05±1.07[a]
Total phenolic content (mg GAE/ g)	4.21±0.05[b]	3.04±0.05[b]	3.94±0.11[a]
DPPH Radical Scavenging Activity (%)	55.89±0.05[c]	51.02±0.06[b]	53.92±0.12[a]
In vitro digestibility of Starch (mg maltose released/ g of sample)	28.41±0.07[b]	26.59±0.07[a]	27.91±0.07[a]
In vitro digestibility of Protein (%)	71.19±0.15[b]	70.12±0.16[a]	70.46±0.02[c]
Soluble dietary fibre(g/ 100g)	1.96±0.12[b]	7.29±0.12[a]	7.31±0.01[b]
Insoluble dietary fibre (g/ 100g)	7.75±0.11[b]	10.81±0.11[b]	10.97±0.45[a]
Total dietary fibre (g/ 100g)	9.71±0.01[b]	18.10 ±0.06[a]	18.28 ±0.06[a]

Table 3. 12: Analysed nutrient composition of formulated products per 100g [Data presented (means of triplicate± Standard error) are significantly different, $p < 0.05$ (Statistical analysis has been done row wise)]

BP1 exhibited the highest antioxidant activity among the prepared products. Total phenolic content of BP1 was 4.21±0.05 mg GAE/g and DPPH radical scavenging activity was 55.89±0.05%. IM1 showed the least antioxidant activity. Total phenolic content was 3.04±0.05 mg GAE/g and DPPH radical scavenging activity was 51.02±0.06%. However, after fermentation of IM1, prepared *idli* (IM-I-1) revealed improved activity. Total phenolic content of IM-I-1 was 3.94±0.11 mg GAE/g and DPPH radical scavenging activity was 53.92±0.12%.

Same observation was stated by Hur et al. (2014) regarding the study of plant-based foods. According to their study, antioxidant activity of the plant-derived product gets improved after fermentation. Improved Total phenolic content and antioxidant activity was observed after substitution of chickpea husk in food formulation (Niño-Medina *et al.,* 2019).

3.3.8.6. In vitro protein and starch digestibility

Starch, protein and lipid are considered as vital constituents of a stable food matrix. During the digestion of these elements in the small intestine, essential nutrients are released (Desai *et al.,* 2021). In the current study, in vitro digestibility of starch and protein of the formulated products were monitored. In vitro protein digestion of the products was improved up to 70% (Table 3.12). BP1 showed better in vitro digestibility of protein in comparison with IM1 and IM-I-1. 71.19±0.15% in vitro digestibility of protein was recorded in case of BP1. After fermentation of IM1 regarding the preparation of *idli* (IM-I-1), in vitro protein digestibility of IM1 increased from 70.12±0.16% to 70.46±0.02% (IM-I-1). In vitro digestibility of starch present in BP1 was higher than that of IM1 and IM-I-1. 28.41±0.07 mg maltose released/ g of BP1 whereas the obtained data of IM-I-1 was 27.91±0.07 mg maltose released/ g of sample. According to a study, fermentation makes proteins (Çabuk *et al.,* 2018) easy to digest. Implication of cooking methods also enhance the digestibility (Fu *et al.,* 2020). Same pattern was observed in this current study after application of various domestic cooking methods to prepare the fermented products. Incorporation of legumes in product formulation was also observed in a study (Singh Sibian & Singh Riar, 2021) due to incorporation of legumes. Therefore, it can indicate that these novel formulation BP1 and IM1/ IM-I-1 can be a nutritive diet to boost digestibility.

3.3.8.7. Dietary fibre

Utilization of composite flour in product development showed increase in soluble dietary fibre (around 7 g/ 100g in IM1 and IM-I-1; 1.96 g/100g in BP1). Insoluble dietary fibre (g/ 100g) level was also elevated (around 11g/100g). Total dietary fibre (g/ 100g) of the formulations was almost 9-18g/100g (Table 3.11). Similar observation was also disclosed in a study due to substitution of chickpea husk fibre (Niño-Medina *et al.,* 2019).

3.3.9. Shelf life of the prepared products

Besides estimation of nutrient composition of novel formulated products, determination of the shelf life of those products are equally important. Various enzymatic and microbial reactions take place in the foods and affect their sensory attributes as well as nutritional quality (Gichau *et al.,* 2020). Depending upon the preferences of consumers and their lifestyles, processing of raw materials, product preparation along with improved shelf life (Taormina, 2021). In the current study, the most preferred products (BP1, IM1) were further stored to study the storage stability. Shelf life of IM-I-1 was not evaluated as the major aim of this study was to formulate ready-to-eat IM1 which can be stored for longer time and use accordingly.

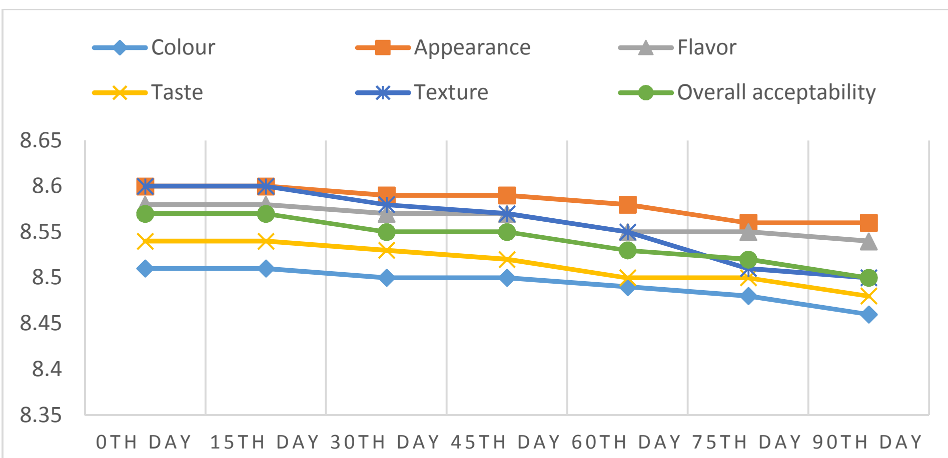

Figure 3. 8: The effect of storage duration on sensory quality characteristics of formulated Product- IM1 (stored at room temperature)

After the preparation of products, BP1 was stored in the freezer at 4°C and IM1 was stored at the room temperature which was 24±2°C during the experiment carried on. Figure 3.8 showed that the sensory quality of the stored product IM1 retained until 90th day from the manufactured date except the BP1 (Figure 3.9). Although milk based products show storage stability up to 10-15 days, being a milk based value added product, BP1 showed spoilage after 14th day of the storage.

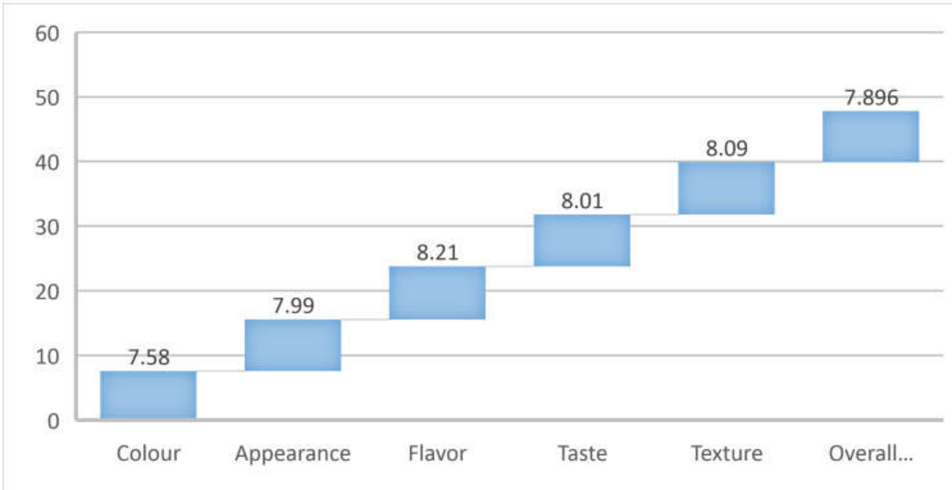

Figure 3. 9: Sensory characteristics of formulated Product- BP1 (stored at 4°C freezer) at 0th day (Product showed spoilage after 14th day of the storage).

Day intervals	0th day	15th day	30th day	45th day	60th day	75th day	90th day
Instant Mix (IM1)							
Peroxide value (meq peroxide/1000g	2.14±0.01^{e}	3.75±0.08^{b}	6.91±0.04^{c}	7.78±0.11^{a}	9.79±0.01^{e}	11.98±0.04^{c}	13.20±0.03^{d}
Free fatty acid (mg KOH/100g)	0.31±0.08^{b}	0.41±0.10^{a}	0.49±0.12^{a}	0.53±0.11^{a}	0.67±0.16^{c}	0.70±0.08^{b}	0.74±0.02^{d}
Bran Paneer (BP1)							
Peroxide value (meq peroxide/1000g	1.01±0.09^{a}	Showed spoilage after 14th day					
Free fatty acid (mg KOH/100g)	0.23±0.08^{b}						

Table 3. 13: Shelf life analysis of products- Peroxide value and free fatty acid analysis. [Data presented (means of triplicate± Standard error) are significantly different, p < 0.05 (Statistical analysis has been done row wise)]

Peroxide value and free fatty acid value of IM1 increased until 90th day. It showed 2.14±0.01 meq peroxide/1000g and 13.20±0.03 meq peroxide/1000g peroxide value on initial day and 90th day regarding the assessment of storage stability. Other products viz., BP1 showed spoilage on 14th day of the storage. Free fatty acid increased from 0.3 to 0.7 mg KOH/100g in IM1 (Table 3.13).

Fermented product viz., paneer generally shows spoilage within few days in room temperature. At room temperature BP showed spoilage after 4 days of the manufacturing date. Whereas, at 4°C storage condition, it showed spoilage after 14th day (**Figure 3.10)**. Hence BP was exposed to gamma radiation to enhance shelf life and the irradiated BP which was stored at room temperature showed spoilage after 9th day without effecting the sensory qualities of the product.

The radiation counts observed after irradiating Bran Paneer to the gamma radiations had been tabulated; Observations were recorded until the counts obtained from samples and background become same.

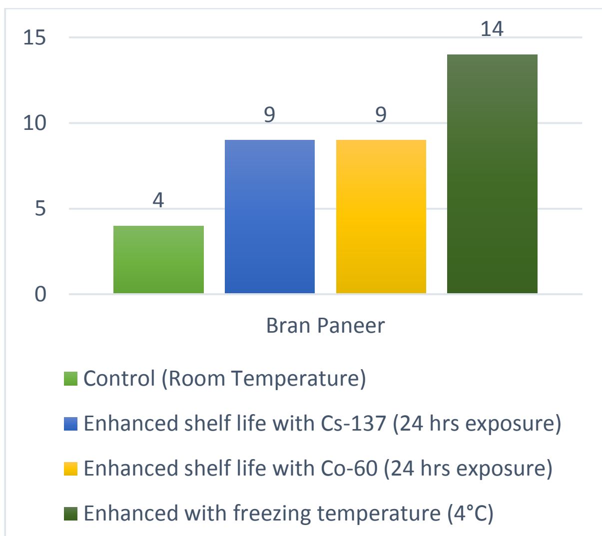

Figure 3. 10: Graphical presentation of shelf life of Bran Paneer

Radiation Counts in Bran *Paneer*									
Duration of exposure	Days								
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
22 hrs.	392	311	265	201	147	94	38	0	0
37 hrs.	368	310	257	210	166	101	41	0	0
48 hrs.	357	307	249	181	123	73	29	0	0

Table 3. 14: Observed Radiation Counts in gamma irradiated Bran Paneer

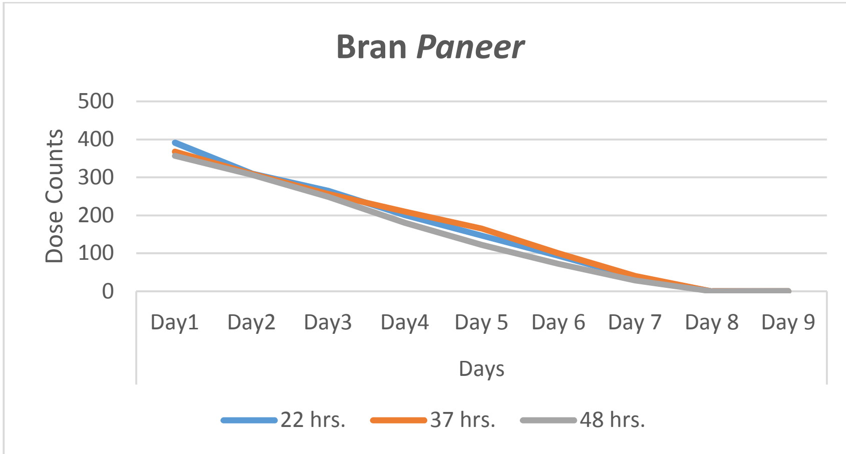

Figure 3. 11: Graphical Representation of Observed radiation counts in Bran *Paneer*

3.3.10. Nutrient analysis of irradiated product

The nutrient analysis had been done when the dose count of the irradiated product became zero to check the edibility of the products. Even after becoming zero dose count of radiation, products showed visual microbial spoilage few days later. Hence it was necessary to check the quality of the products in between the time period of zero dose count to visual microbial growth.

Products (After showing zero count)	Moisture (%)	Ash (%)	Crude fat (%)	Crude protein (%)	Crude fibre (%)	Total carbohydrate (%)
Bran paneer	61.89±0.02	1.81±0.07	1.99±0.23	19.01±0.25	4.92±0.19	10.38±0.29

Table 3. 15: Nutrient composition of food products after gamma irradiation

Products (After showing zero count)	Free fatty acid value (mg KOH/100g)	Peroxide value (meq peroxide/1000g)
Bran paneer	0.25±0.24	1.03±1.14

Table 3. 16: Chemical composition of food products after irradiation to study storage stability

The formulated fermented products showed significant nutrient availability and satisfactory sensory acceptance. A notable change in the levels of mineral content and total carbohydrate of fermented and non-fermented stages of products conveyed the importance of fermented milling by-products. Implication of various domestic cooking methods and thermal processing improved the quality of the products.

Formulated value added products were popularized in collaboration with KrishiVigyan Kendra, Mahendergarh (India). The processing technology to process the milling by-products for the development of novel nutrient rich products was transferred from lab to community to enlighten local women about the probable utilization of milling by-products for food formulation. Women from adaptive villages were trained by organizing Hands-on training program.

Figure 3.12: Popularization of the formulated product in collaboration with KrishiVigyan Kendra

3.4. Summary

We formulated Instant Mix (IM) for Idli (IM-I) and Bran Paneer (BP) variants which were analysed based upon the microbial counts and sensory attributes. Accepted variants (BP1, IM1) by panellists were further subjected for nutrient analysis and shelf-life evaluation. The analysis showed rich proximate composition, minerals viz., Calcium (80-150 mg/100g), iron (3-15 mg/100g), phosphorus (131-209 mg/100g), and antioxidant activity. Lower total soluble sugar concentration (3-4 g/100 g) and antinutrient activities viz., Trypsin inhibitor activity (1-1.5 TIU/mg) and phytic acid level (127-171 mg/100g) were observed which can be beneficial for consumers. The overall acceptability of these legume husks enriched cereal bran-based formulations was found to be satisfactory.

Popularization of the acceptable product and transfer the technology regarding their preparation and utilization was done through training programs to disseminate the knowledge from lab to community for the generation of local entrepreneurship or low scale business. Hence, processing of these by-products through fermentation to develop novel value-added food products with improved nutrient and sensory quality along with acceptable storage stability would increase the possibilities of milling by-product utilization is perceived through this study.