**ORIGINAL PAPER** 



# Potential of milling byproducts for the formulation of health drink and detox tea-substitute

Manali Chakraborty<sup>1</sup> · Savita Budhwar<sup>1</sup> · Suneel Kumar<sup>2</sup>

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# Abstract

Due to pandemic situation, a sudden demand of healthy and immune booster products has risen to get rid of infections like Covid-19. The aim of this study is to develop novel health drink and beverages using plant-based byproducts like orange peel, milling byproducts (chickpea husk, rice bran, broken rice, wheat bran). Byproducts were processed by using different culinary processes such as, soaking, blanching, roasting, natural air-drying. Proximate composition along with minerals, antioxidants, Vitamin-C of formulated Health Drink Powder (HDP) and Detox Tea-substitute (DTS) were estimated. Most acceptable variants among the formulated products were estimated through sensory profiling where, HDP1  $(7.79 \pm 0.01)$  and DTS2  $(8.18 \pm 0.11)$  showed higher acceptability scores among others. Crude protein present in HDP and DTS were  $(19.27 \pm 0.01)\%$ and  $(18.21 \pm 0.19)\%$  respectively. Calcium was higher in HDP  $(81.21 \pm 4.03 \text{ mg}/100 \text{ g})$ , whereas phosphorus was higher in DTS  $(211.52 \pm 0.22 \text{ mg}/100 \text{ g})$ . Total phenolic contents of both the products were around 4 mg GAE/g. Vitamin C level was higher in HDP ( $60.23 \pm 0.11$  mg/100 g). Shelf life study and microbial load assessment indicated longer storage life of the formulated products. The Total Plate counts (Log CFU/g) were  $2.12 \pm 0.01$  and  $2.08 \pm 0.12$  found to be in freshly prepared HDP1 and DTS2 respectively under evaluation. The yeast and mold counts (Log CFU/g) was observed after 75th day and 60th day in HDP1 ( $2.09 \pm 0.05$ ) and DTS2 ( $2.01 \pm 0.11$ ) respectively (stored room temperature). The overall acceptability of these novel formulations as determined by sensory evaluation throughout the storage duration was satisfactory. According to the estimated data it can be concluded that the selected byproducts can be utilized as significant plant-based sources to formulate value-added functional products without affecting its sensory quality and to enhance nutritional status of consumer.

**Keywords** Milling byproducts  $\cdot$  Tea-substitute  $\cdot$  Food formulation  $\cdot$  Health drinks powder  $\cdot$  Health-booster  $\cdot$  Plant-based byproducts

# Introduction

Sudden appearance of COVID-19 pandemic situation made everyone realized the importance of good immunity for health. Nowadays to balance health and the hectic lifestyle, people are becoming more reliant upon the concept of the

 Savita Budhwar savitadahiya@cuh.ac.in
 Manali Chakraborty manali11073@cuh.ac.in

> Suneel Kumar suneelkumar@cuh.ac.in

<sup>1</sup> Department of Nutrition Biology, Central University of Haryana, Jant-Pali, Mahendergarh, Haryana 123029, India

<sup>2</sup> Department of Physics and Astrophysics, Central University of Haryana, Jant-Pali, Mahendergarh, Haryana 123029, India plant-based products due to their beneficial aspects [1]. Value added ready-to-eat food products are getting attention among consumers. Therefore, food professionals are trying their best to incorporate plant sources in product formulation to accomplish the demand.

Tea and coffee are believed to be the most consumed beverages. Caffeine is a psychoactive drug which effects brain or state of mind regardless of any age. 100 mg/day of caffeine can show positive effect on several cognitive functions, although more 400 mg/day can become lethal to body, especially upon the immune system [2]. Due to its psychoactive actions, people consume it most as stress-buster or energy elevator without noticing the amount of caffeine intake. Thus, researchers are studying different beverage formulation without caffeine. Consumers also showed interest about the caffeine-free beverages. Legumes are being utilized to develop non-dairy drinks [3]. Beverages and health drinks originated from grain, cereals and legumes have fascinated consumers because of their significant properties and caffeine free nature with a bit different flavor [4]. Cereal-based health drink products are also familiar among consumers [5]. Not only the grains, food experts are now utilizing plantbased byproducts such as milling byproducts, fruit peels etc. to formulate beverages. Presence of higher amount of fiber in these byproducts make them highly eligible for enhanced digestion [6]. Dietary fiber intake can lower the risk of cardiovascular diseases, diabetes, obesity, definite gastrointestinal diseases and enhance gut health resulting in improved immune system [7]. Cereal byproducts viz., wheat bran and rice bran are already in use for health friendly drinks beside food formulation [8]. But legume byproducts viz., chickpea byproduct is yet to reach that mark.

Citrus fruit like orange is common ingredient for producing juice or drinks and generates large amount of wastes like seed, pulp, peels. Studies revealed that these byproducts are potential source of antioxidants, Vitamin-C, immunomodulatory components and many other beneficial bioactive compounds like flavonoids, phenolic, carotenoids, and limonoids [9]. Polymethoxyflavones (PMFs) are a group of flavones present in peel which showed the broad spectrum of immune-modulation [10], anti-obesity [11], anti-inflammation [12]. Orange peel has been used in immune-booster formulations by food experts due its positive effects on immune system as well as heart diseases [13].

Although there are health drinks or beverages available based on only cereal byproducts or orange peel, there is no formulation available as per knowledge to find out the probable contribution of chickpea husk as potential ingredient for beverage manufacturing process or to study the combinations of pretreated cereal-legume byproducts as potential plant-based sources in the caffeine-free drink production and overall quality of those beverages.

*Hence the present study has aimed for the initiative to study:* 

(i) Use of processed milling byproducts viz., chickpea husk, broken rice, rice bran, wheat bran in Health Drinks Powder (HDP) and orange peel incorporated DTS production

 (ii) Nutrient composition, anti-oxidant activity, in vitro digestibility, sensory attributes, and shelf life of prepared products.

# **Materials and methods**

The milling byproducts of chickpea, rice, and wheat were procured from the local dhal mill of Mahendergarh city, Haryana (India). Acquired orange peel from local market was processed to remove its bitterness by repeated blanching followed by drying in hot air oven at 55 °C for 6 h. The dried peels were ground in a laboratory grinder and sieved through a 200  $\mu$ m sieve to obtain fine powder. Milling byproducts were also processed using various culinary methods viz., soaking, blanching, roasting, natural air-drying. Washed byproducts were soaked in water separately for an hour. Later, they were blanched followed by natural air drying at room temperature ( $42 \pm 2$  °C) for 6 h. The experiment was carried out in the Department of Nutrition Biology, Central University of Haryana, India.

# **Experimental design**

As mentioned before, milling byproducts of rice and wheat are more popular in food industry rather than chickpea husk, the current study focused more on the chickpea husk. Therefore, for both the formulations i.e., HDP and DTS, combinations without having chickpea husk is considered as 'control'. In case of HDP, HDP0 does not contain any chickpea husk and same pattern was followed to select control (DTS0) in DTS formulation.

# **Formulation of HDP**

Dried byproducts were combined according to the percentages given in the Table 1 for the variants of HDP. Byproduct mixture was roasted on low flame for 1–2 min. Further

Table 1 Ingredient composition to formulate HDP

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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

Percentage composition of formulated composite flour

jaggery was added to it with continuous stirring to the mixture and roasted on low flame until the jaggery blended properly. The mixture was converted into fine powder using laboratory grinder. Cardamom powder, clove powder, and fennel seed powder were also added to the mixture as required for the flavor (Fig. 1). The final mixture was sieved and stored in air-tight container for further use.

# Formulation of DTS

Byproducts were taken for the variants of DTS according to the combination percentages given in Table 2. Byproduct



Fig. 1 Formulation procedure of HDP

Table 2	Ingredient	composition	to	formulate	DTS
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mixture was first roasted on low flame for a while followed by partial blending in the grinder. Partial blending produced broken parts of the byproducts and gave a similar texture of dried tea leaf. Dried orange peel (taken amount given in Table 2) was roasted along with jaggery in different container and subjected to grinding at low speed. Then the jaggery-blended orange peel fractions were mixed to the partially blended byproduct combination and gave a proper mix followed by light roasting for 1–2 min. Small dip-pouches were filled with the mixture and stored to use as dip using hot water to consume further (Fig. 2).

# **Sensory evaluation**

The appearance, color, texture, flavor and overall acceptance of cereal bran and legume husk based products were determined using a 9-point hedonic rating scale by ten panel members at the Central University of Haryana, where one point indicates lowest acceptance and nine points is for highest acceptance of the products [14]. Product information were given to them before the evaluation. They were told to rinse out their mouths with water in between samples to minimize any residual effect.

# Characterization of the formulated product

# **Proximate analysis**

The proximate analysis was evaluated according to AOAC [15] in triplicate. The Kjeldahl method was used for the analysis of the crude protein. For the estimation of crude fat, soxhlet extraction method was used. The carbohydrate content was found out by using the following formula: (100-the sum of fat, protein, moisture, fiber). Soluble and insoluble dietary fiber of defatted samples were analyzed by the enzymatic method established by Furda [16].

# Sugar and starch

The products were extracted with 25 ml ethanol (80%) and obtained sugar extract dilution for Total soluble sugar

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



Fig. 2 Formulation procedure of DTS

estimation [17]. The absorbance at 625 nm using UV–VIS spectrophotometer was recorded after one ml of the diluted solution was reacted with freshly prepared Anthrone reagent. For reducing sugar [18] 1 ml of sugar extract along with Copper Reagent Mix (Copper reagent A and Copper reagent B) was heated for 20 min in boiling water bath. UV–VIS spectrophotometer was used at 520 nm after further addition of 1 ml Arsenomolybdate reagent. Extracted sugar residue was reacted with Perchloric acid for starch estimation. Further extracted residue was subjected to glucose estimation using the same method followed regarding total soluble sugar estimation. From the obtained data, starch was calculated by multiplying glucose value with 0.9 (*Starch = Glucose* × 0.9).

# **Mineral analysis**

The mineral analysis was done to evaluate the content of iron, and phosphorus on extracted dry ashed samples using a UV–VIS spectrophotometer according to the AOAC [15] method. For calcium was determined following the titration method.

# Determination of antioxidant activity

Sample extracted for the determination of the antioxidant activity was kept in 4 °C in the dark for further use. DPPH radical scavenging activity [19] was determined at 517 nm wavelength using UV–VIS spectrophotometer. The hydrogen atom donating ability of the extracts was analyzed by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) that is violet/purple to yellowish in the presence of antioxidants.

# Total phenolic activity estimation

Total phenolic activity [20] was estimated through Gallic acid calibration standards and was determined by using UV–VIS spectrophotometer method at 750 nm.

# In vitro digestibility estimation

In vitro protein digestibility was examined [21]. The Kjeldahl method was used to estimate the available protein after in vitro digestion. In vitro Starch digestibility [22] was analyzed by using pancreatic amylase.

# Antinutrient evaluation

After the treatment with nitric acid (0.5 M) and centrifugation, the extracted sample was used to check phytic acid level in the products [23] at 465 nm against iso-amyl alcohol blank. Trypsin inhibitor activity (TIA) was determined [24] by sample extraction with trichloroacetic acid (TCA) at 10,000 rpm for 10 min to further check the TCA soluble proteins in the supernatant [25].

# **Determination of Vitamin C**

Vitamin C in the food sample was analyzed by the titration method with 2,6-dichlorophenol-indophenol dye until observed a faint pink endpoint [15]

# Shelf life evaluation of the formulated products

All the analysis which were used to check the shelf life of the products as highlighted were done at the interval of 0, 15, 30, 45, 60, 75, and 90 days.

Sensory evaluation of stored food products The sensory profiling of the stored products was done by the same panelists.

**Determination of free fatty acid generated during storage** Free fatty acid level in the food products were determined according to the standard method of AOAC [15]. Food samples were dissolved by adding 50 ml of neutralized Isopropyl alcohol and titrated against 0.25 N NaOH for 30 s for the quantification.

**Peroxide value analysis** According to the method of AOAC [15], 30 ml of acetic acid and chloroform mixture was added to dissolve the sample. After 1 min of occasional starring with 0.5 ml saturated potassium iodide solution, 30 ml distilled water was added and titrated slowly against 0.01 N sodium thiosulphate followed by another titration after addition of 0.05 ml starch solution until release of all iodine from chloroform layer.

**Microbial load determination** Growth of yeast, mold, and other viable microorganisms was analyzed to check microbial load and microbiological safety level of food [26]. Total plate count was done at incubation temperature 30 °C for 72 h. Yeast and Mold Count was carried out by incubating plates at 25 °C for 3–5 days.

# Statistical analysis

The data obtained were subjected to analysis of variance for a completely random design using MS Office Excel (2016). All experiments were evaluated in triplicates. The data were presented as means  $\pm$  standard error. Assessment of the statistical significance was carried out by using oneway analysis of variance (ANOVA) followed by Tukey HSD test where differences between means were taken as statistically significant if p < 0.05.

# **Result and discussion**

# Sensory evaluation of prepared products

Figure 3 showed the overall acceptability of HDP and DTS. According to the scores, the sensory acceptance got slightly effected ( $p \le 0.05$ ) due to higher substitution percentage of chickpea husk flour and orange peel powder in composite

flour formulation. In case of HDP, increased flour substitution showed significant differences ( $p \le 0.05$ ) between treatments of the products. Overall acceptability score of HDP1 was  $7.79 \pm 0.01$  whereas HDP4 showed acceptability score  $7.26 \pm 0.18$ . Increased flour substitution in case of DTS exerted significant differences ( $p \le 0.05$ ) on the colour, but other attributes were enhanced until DTS2 ( $8.18 \pm 0.11$ ). After that, increased substitution declined the product's sensory quality that is  $8.03 \pm 0.05$  in case of DTS4. It might happen due to bitter taste of orange peel, as higher percentage of the peel may abolish the taste and flavour. A study by Mishra and Chandra [27] also showed same pattern of observation in the sensory attributes of formulated biscuits from rice bran and soy flour. With increased substitution of rice bran, sensory scores of the product got low. With addition of wheat bran in product formulation, another study by Sozer et al. [28] also showed the same observation. Sensory evaluation of the formulated food products was conducted among panelists because the major aim of the current study was to develop a product that would be acceptable in the market and can be used as a supplement of health beneficial nutrients to minimize the occurrence of malnutrition. From the obtained data, it seemed that the use of byproducts in food production can break the stigma associated with edible agricultural byproduct consumption and might be easier to grow attention among consumers worldwide to accept the product. Bose and Shams-Ud-Din [29] also concluded the same, based upon the obtained nutrient availability and sensory score of pretreated chickpea husk based crackers in their study. Based upon the sensory evaluation, most preferred formulations were stored and further analysis was done to check the shelf life which are HDP1 and DTS2.

# Detailed study of the nutritional attribute of the formulated products

Nutrient composition of the preferred compositions was done. HDP1 and DTS2 were subjected for the determination of proximate composition, mineral content, macro-nutrients, antioxidant activities and shelf life evaluation.

#### **Proximate compositions**

The nutrient compositions of the formulated products were presented in tabular form. Substitution with composite flour showed enhanced nutrient composition of formulated HDP1 and DTS2 (Table 3). In the legume-cereal husk incorporated products, crude protein of the products was in the range of 18–19% ( $p \le 0.05$ ). Moisture content of HDP1 and DTS2 were around 4–4.5%. HDP1 showed lesser moisture [(4.19±0.02)%]. The moisture content of the product is directly linked with its shelf life as it measures the probability and vulnerability of microbial contamination. The







**Table 3** Proximate composition of formulated products per 100 g (%,dry matter basis)

Proximate composition	HDP1	DTS2
Moisture	$4.19 \pm 0.02^{b}$	$4.47 \pm 0.06^{a}$
Crude protein	$19.27 \pm 0.01^{\circ}$	$18.21 \pm 0.19^{a}$
Crude fat	$2.98\pm0.01^{\rm b}$	$3.09 \pm 0.04^{a}$
Crude fiber	$4.95\pm0.06^{\rm b}$	$5.07 \pm 0.11^{a}$
Ash	$5.02 \pm 0.12^{a}$	$4.98 \pm 0.12^{a}$
Total carbohydrate	$63.59 \pm 0.11^{a}$	$64.18 \pm 0.09^{b}$

Data presented are proximate compositions of developed Products

Significantly different, p < 0.05 (Statistical analysis has been done row wise)

Assigned letters (viz., a, b, and c) highlight significant (P  $\leq 0.05$ ) differences. Means that are not significantly different are assigned a common letter

estimated moisture content was found to be acceptable enough to store for a longer duration at appropriate conditions as low moisture content between 1 and 5% indicates less perishable. The estimated data also showed less carbohydrate level with husk flour addition. Crude fat (%) was found lesser in the products ranging from 2 to 3%. Lower fat content due to use of milling byproduct in frankfurters was perceived by Choi et al. [30]. They observed reduced fat contents from 30 to 12% supplemented with 2% rice bran fiber. The higher level of fiber content present in these products makes them appropriate for improved digestion. Due to lesser carbohydrate content in the formulated recipes, it might be helpful for weight management along with improved blood sugar, blood pressure. Incorporation of such low-carb containing snack food options in the diet can lower the triglyceride level and improve the quality of health status rather than the snack products available in the market. Total carbohydrate in HDP1 and DTS2 was  $(63.59 \pm 0.11)\%$  and  $(64.18 \pm 0.09)\%$  which is much lower than the health drinks available in market. Generally, 100 g of malt-based market health drinks possess carbohydrate content around 85 g, of which almost 32 g is sugar [31]. Low carbohydrate was also found in the study by [30] in rice bran utilization in food production.

### Sugar and starch level

Non-reducing sugar in HDP1 and DTS2 was around 1.5 g/100 g. Estimated reducing sugar level in the

Table 4Analyzed nutrient composition of formulated products per100 g

Nutrient composition	HDP1	DTS2
Sugars and starch content		
Non-reducing sugar (g/100 g)	$1.42 \pm 0.21^{a}$	$1.57 \pm 0.19^{b}$
Reducing sugar (g/100 g)	$2.79\pm0.08^{\rm a}$	$3.11 \pm 0.01^{b}$
Total soluble sugar (g/100 g)	$4.21 \pm 0.05^{a}$	$4.68 \pm 0.01^{b}$
Starch (g/100 g)	$9.90 \pm 0.01^{b}$	$11.01 \pm 0.05^{a}$
Minerals (mg/100 g)		
Iron	$5.29\pm0.08^{\rm b}$	$6.87 \pm 0.05^{a}$
Calcium	$81.21 \pm 4.03^{b}$	$78.96 \pm 4.36^{a}$
Phosphorus	$139.58 \pm 0.20^{b}$	$211.52 \pm 0.22^{a}$
Antinutrient		
Trypsin inhibitor activity (TIU/ mg)	$1.95 \pm 2.01^{b}$	$2.97 \pm 2.06^{a}$
Phytic acid (mg/100 g)	$197.01 \pm 1.32^{b}$	$201.25 \pm 1.38^{a}$
Antioxidants		
Total phenolic content (mg GAE/g)	$4.01 \pm 0.05^{b}$	$4.04 \pm 0.05^{a}$
DPPH Radical Scavenging Activ- ity (%)	$56.09 \pm 0.05^{b}$	$57.12 \pm 0.06^{a}$
In vitro digestibility of Starch and Pro	otein	
In vitro digestibility of Starch (mg maltose released/g of product starch)	$28.01 \pm 0.07^{b}$	$28.19 \pm 0.07^{a}$
In vitro digestibility of protein (%)	$70.09 \pm 0.15^{b}$	$70.11 \pm 0.16^{a}$
Dietary fiber		
Soluble dietary fiber(g/100 g)	$2.96\pm0.12^{\rm b}$	$3.29 \pm 0.12^{a}$
Insoluble dietary fiber (g/100 g)	$7.05 \pm 0.11^{b}$	$7.21 \pm 0.11^{a}$
Total dietary fiber (g/100 g)	$10.01 \pm 0.01^{b}$	$10.5 \pm 0.06^{a}$
Vitamin		
Vitamin C (mg/100 g)	$60.23 \pm 0.11^{a}$	$57.36 \pm 0.09^{b}$

Data presented are nutrient compositions of developed products

Significantly different, p < 0.05 (Statistical analysis has been done row wise)

Assigned letters (viz. a, b, and c) highlight significant ( $p \le 0.05$ ) differences. Means that are not significantly different are assigned a common letter

formulations was almost 3 g/100 g (Table 4). Starch content in the formulations was in the range of 9–11 g/100 g and this present amount might be due to addition of broken rice in the ingredient mixture. Though starch in broken rice is much higher than the examined data of the formulations. Loss of starch may occur due to roasting and heat treatment during the processing of the products [32].

# Minerals

Level of ash content (%) indicates enhanced mineral proportion. Ash content (%) was significantly moderate in all the formulations, around 5%. Generally, health drinks available in market, show lower ash content (1-2%), hence also the mineral contents. But Iron in HDP1 and DTS2 was  $5.29 \pm 0.08$  mg/100 g and  $6.87 \pm 0.05$  mg/100 g respectively. Calcium was higher in HDP1  $(81.21 \pm 4.03 \text{ mg}/100 \text{ g})$ than DTS2 (78.96  $\pm$  4.36 mg/100 g). Phosphorus was more in DTS2  $(211.52 \pm 0.22 \text{ mg}/100 \text{ g})$  than HDP1  $(139.58 \pm 0.20 \text{ mg}/100 \text{ g}) \text{ (p} \le 0.05)$ . Enhanced protein, fiber, and ash content due to legume fortification already was testified by several studies [33]. These higher levels of proximate nutrients along with minerals such as ash, phosphorus, calcium, and iron contents (Table 4) of the formulations due to milling byproduct comprising flour substitution can also indicate to the fact that foods formulated using these agricultural byproducts are high in essential micronutrients. Rice bran in the formulation contributed a potent role in available iron level [34]. This byproducts-based formulated product in the current study could be beneficial to prevent the micronutrient deficiencies worldwide. Primarily found micronutrient deficiencies such as iron, calcium, phosphorus, folate deficiencies result in impaired growth and intellectual which can affect pregnant mother and child with an increased risk of morbidity and mortality [35]. The developed novel products can not only be an addition to a healthy diet but also can contribute to micronutrient requirements to enhance health status.

# **Antinutrient factors**

Antinutrient levels were comparatively higher in these novel formulations due to presence of legumes. Trypsin inhibitor activity was found  $1.95 \pm 2.01$  TIU/mg along with  $197.01 \pm 1.32$  mg/100 g of phytic acid content in HDP1 and  $2.97 \pm 2.06$  TIU/mg Trypsin inhibitor activity along with  $201.25 \pm 1.38$  mg/100 g of phytic acid content (Table 4) 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 [36]. Only thing that matters in case of antinutrient in body or consuming food is the level present or consumed. Despite presence of high level of antinutrient in chickpea husk [37], products showed negligible level in comparison to source. This decreased level is believed due to the washing, cooking, fermentation and processing of the husk. Although heating affects vary moderately upon phytic acid content, trypsin inhibitor activity significantly decreased [38, 39].

#### Phenolic compounds and antioxidant properties

According to research data, in vitro evaluation of DPPH scavenging activities of the Chickpea husk indicated potential antioxidant properties [37] along with rice bran [40] and wheat bran [41]. Thus determination of antioxidant activity of these husk based products was necessary to analyze the effects of cooking and other processing method [42]. 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  $4.01 \pm 0.05$  mg GAE/g and  $4.04 \pm 0.05$  mg GAE/g. there was no significant difference ( $p \le 0.05$  between the phenol content of the products. DPPH Radical Scavenging activity of HDP1 was lower  $[(56.09 \pm 0.05)\%]$  than that of DTS2  $[(57.12 \pm 0.06)\%]$  (Table 4). 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 [37].

The postharvest conditions cause changes in different phytochemical substances viz., phenol content [43–45]. Antioxidant properties of these phenols are solely dependent upon to the hydroxyl groups and aromatic rings. Many ongoing studies have recorded a significant and positive correlation ( $p \le 0.05$ ) between the available phenolic contents and the antioxidant activity of food [44]. Available phenol content in food not only helps to maintain the quality, inclusion of phenol rich foods in diet also showed a strong evidence of lower risk of developing diseased conditions such as, cardiovascular diseases, cancer, diabetes, neurological issues. It is crucial to understand the composition and activity of the antioxidants and to assess the content present in the food beverages consumed [44]. Along with chickpea husk flour, wheat bran flour might also have significant involvement in the availability of antioxidant activity in the formulated recipes [46]. Food formulation whether in home or industry always includes few primary steps such as peeling, washing, and chopping. Based upon the formulation plan ingredients were processed through boiling, frying (deep or shallow), traditional oven or microwave baking and others. These several processing methods showed an effect on the phenol activity of foods prepared. According to some research studies phenol activity seemed to be increased after cooking along with the increased antioxidant activity varying significantly between the used ingredients and followed methods during the preparation. Researchers believed that the disruption of the cell membrane due to heat generated during cooking results in the release of membrane-bound phytochemicals leading to increased bioavailability of the phenols and antioxidants. Though there are also cases where thermal processing did not affect phenol activity, rather the activity was decreased [44]. The enhanced antioxidant content in formulations might contribute to the minimization of the risk of cardiovascular diseases, diabetes relatively than the consumption of market foods. Incorporation of such novel foods in diet can be a bonus to uphold the taste with health.

#### 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 4). Gluten of wheat creates a difficult condition for enzymatic hydrolysis due to presence of proline fractions [47]. Lack of gluten in legume husk and small proportion of incorporation of wheat bran in composite flour development might improve the in vitro protein digestibility (70%). Improved in vitro digestibility of starch and protein was also observed in a study [48] due to incorporation of germinated legume grains lacking gluten protein. Therefore, it might be established that the lesser consumption of higher gluten-rich foods can improve digestion.

# **Dietary fiber**

Substitution of composite flour in product formulation showed significant quantity of soluble dietary fiber (around 3 g/100 g). Whereas, it has been found that market food generally shows fiber somewhat like 1 g/ 100 g or less, especially the 100% wheat products. Insoluble dietary fiber (g/100 g) level was also elevated (around 7 g/100 g) in these recipes. Total dietary fiber (g/100 g) of the formulated products was almost 10 g/100 g (Table 4). Similar enhanced level of dietary fiber was also observed in a study after substitution of chickpea husk fiber [37]. Commercially available high fiber products are generally made utilizing wheat bran or wheat grain. Some ongoing studies are also there to prepare high fiber product with wheat bran and mango peel powder [49]. Due to a much higher level of fiber (83.45 g/100 g) in Chickpea husk studies are going on to formulate high fiber food products using chickpea husk flour [37]. Formulated value-added products in this study could contribute to fiber requirement to much extent. The available fiber can help maintaining metabolic health conditions viz., lower cholesterol, better glycemic control [50, 51]. Throughout the large intestine, if fiber remains intact without being affected by fermentation, it can provide a laxa-tive effect [52].

# Vitamin C level in formulations

It has been found that the formulated recipes contain remarkable amount of Vitamin C. HDP1 showed  $60.23 \pm 0.11$  mg/100 g and DTS2 contains  $57.36 \pm 0.09$  mg/100 g of Vitamin C (Table 4). 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 [13], blanching and roasting during the formulation of the recipes may be responsible of Vitamin C loss and

> 7.4 7.2

> > Oth day

Colour

15th day

Appearance

30th day

Flavor

45th day

Taste

60th day

75th day

■ Texture ■ Overall acceptability

comparatively lower level of presence in products than the orange peel [53]

# 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 4 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/1000 g and 12 meq peroxide/1000 g peroxide value on initial day and 90th day regarding the assessment of storage stability (Table 5). Free fatty acid value also increased in the range 0.3–0.8 mg



**Fig. 4** The effect of storage on hedonic quality characteristics analysis of Products incorporated with composite flour

90th day

 Table 5
 Shelf life determination of novel products

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Day intervals	0th day	15th day	30th day	45th day	60th day	75th day	90th day
HDP1							
Peroxide value (meq peroxide/1000 g	$2.04\pm0.01^{\rm e}$	$2.75\pm0.08^{\rm b}$	$4.92\pm0.04^{\rm c}$	$6.88\pm0.11^a$	$8.89\pm0.01^{\rm e}$	$10.98 \pm 0.04^{\circ}$	$12.20 \pm 0.03^{d}$
Free fatty acid (mg KOH/100 g)	$0.32\pm0.08^{\rm b}$	$0.41\pm0.10^a$	$0.52\pm0.12^a$	$0.59\pm0.11^a$	$0.67\pm0.16^{\rm c}$	$0.72\pm0.08^{\rm b}$	$0.79 \pm 0.02^{d}$
DTS2							
Peroxide value (meq peroxide/1000 g)	$2.11\pm0.01^{\rm e}$	$3.95\pm0.08^{\rm b}$	$4.99\pm0.04^{\rm c}$	$6.84 \pm 0.11^{a}$	$8.79\pm0.01^{\rm e}$	$10.78 \pm 0.04^{\circ}$	$12.02 \pm 0.03^{d}$
Free fatty acid (mg KOH/100 g)	$0.34 \pm 0.08^{b}$	$0.47\pm0.10^a$	$0.59\pm0.12^a$	$0.63\pm0.11^a$	$0.75\pm0.16^{\rm c}$	$0.80\pm0.08^{\rm b}$	$0.84\pm0.02^{\rm d}$

Data presented are significantly different, p<0.05 (Statistical analysis has been done row wise)

Assigned letters (viz., a, b, c, d) highlight significant ( $p \le 0.05$ ) differences. Means that are not significantly different are assigned a common letter

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

HDP1	
Total plate count	
Oth day	$2.12\pm0.01$
15th day	$2.19 \pm 0.05$
30th day	$2.26 \pm 0.12$
45th day	$2.31 \pm 0.01$
60th day	$2.35 \pm 0.09$
75th day	$2.41 \pm 0.21$
90th day	$2.47 \pm 0.08$
Yeast and mold count	
0th day	-ve
15th day	-ve
30th day	-ve
45th day	-ve
60th day	-ve
75th day	$2.09 \pm 0.05$
90th day	$2.11 \pm 0.14$
DTS2	
Total plate count	
Oth day	$2.08 \pm 0.12$
15th day	$2.15 \pm 0.05$
30th day	$2.23 \pm 0.02$
45th day	$2.29 \pm 0.22$
60th day	$2.31 \pm 0.19$
75th day	$2.39 \pm 0.20$
90th day	$2.45 \pm 0.04$
Yeast and mold count	
Oth day	-ve
15th day	-ve
30th day	-ve
45th day	-ve
60th day	$2.01 \pm 0.11$
75th day	$2.14 \pm 0.04$
90th day	$2.20\pm0.07$

-ve: not detected

 Table 7
 Estimation of antioxidant activity in the products after 90th day of the storage

After 90th day of the storage				
Antioxidants	HDP1	DTS2		
Total phenolic content (mg GAE/g)	$3.85 \pm 0.12^{b}$	$4.09 \pm 0.01^{a}$		
DPPH radical scavenging activity (%)	$55.81 \pm 1.02^{b}$	$56.95 \pm 0.14^{a}$		

Assigned letters (a, b) highlight significant ( $p \le 0.05$ ) differences. Means that are not significantly different are assigned a common letter

KOH/100 g in these novel formulations. The range of increased peroxide value was quite moderate and the increased levels, recorded on 90<sup>th</sup> day were quite acceptable as the peroxide value should not be above 10–20 meq/kg fat [54] to avoid rancidity flavor. Interpretation of the data indicated higher storage stability of the products as ready-to-eat food.

# Microbiological safety of food

The assessment of the microbial count of the products is presented in Table 6. 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  $2.12\pm0.01$ and  $2.08\pm0.12$  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  $2.09\pm0.05$  after 75th day and  $2.01\pm0.11$  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 byproducts viz., bran of cereals and pulses were found microbiologically safe and healthy enough to incorporate in diet [55–57].

# Antioxidant activity of the stored products

Based upon the estimated data (Table 7), the antioxidant activity of the stored products (after 90th day) was lower than that of freshly prepared products. Total phenol contents were  $3.85 \pm 0.12$  mg GAE/g and  $4.09 \pm 0.01$  mg GAE/g for HDP1 and DTS2 respectively. Whereas, DPPH Radical Scavenging Activity was ( $55.81 \pm 1.02$ )% and ( $56.95 \pm 0.14$ )% for HDP1 and DTS2 respectively.

Based upon the evaluated scores and the overall analysis, it can be concluded 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 byproducts. Available antinutrient present in the foods was less (within the tolerable level) due to cooking and processing of composite flour. This current study discloses a novel potential research area of value-added product formulation by processing byproducts of cereal and legumes in addition to replacement or substitution of usually utilized flours. A healthy body is always less prone to any sort of infections and fatal diseases. Incorporation of these herbs and spices, underutilized greens and their byproducts 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.

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Code availability Not applicable.

# Declarations

Conflict of interest Authors disclose no conflict of interest.

**Ethical approval** This research article does not contain any studies with human participants or animals performed by any of the authors.

**Consent to participate** All authors gave their consent to participate in this study.

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