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Formulation and nutritional analysis of bio-fortified pearl millet-derived biscuits enriched with carrot and cauliflower leaves powder

Rajni, Asha Kawatra, Sonia, Reema Devi, Neha and Charan Singh

Abstract
Pearl millet, carrot, and cauliflower leaves showcase an impressive nutritional profile, holding promise in the battle against malnutrition. This research delves into the incorporation of bio-fortified pearl millet infused with powdered carrot and cauliflower leaves to elevate the nutritional value of baked goods, specifically biscuits. The study explores different ratios (5%, 10%, 15%, and 20%) of carrot and cauliflower leaves powder blended into pearl millet-based biscuits. A thorough nutritional analysis covering proximate composition (moisture, crude fat, protein, ash, and crude fiber) was undertaken, complemented by a sensory assessment using a 9-point hedonic scale by semi-trained judges. The findings indicate a significant enhancement in nutritional attributes across all carrot and cauliflower leaves powder-fortified biscuits, notably a substantial increase in crude protein content and a heightened presence of dietary fiber. The sensory evaluation revealed that the 5% supplemented biscuits received favorable reviews from the panel of judges. Subsequently, there was a moderate-to-slight preference observed for biscuits developed in trial groups Type-II, Type-III, and Type-IV.

Keywords: Pearl millet, carrot, cauliflower, biscuit, proximate

1. Introduction
Addressing malnutrition is a complex undertaking, and a potent strategy involves the bio-fortification of cereals and millets. This method involves genetically enhancing key crops to augment their nutrient content, primarily by selecting cereal and millet varieties naturally enriched with specific micronutrients. Additionally, combating malnutrition encompasses fortification practices, achievable through both agricultural techniques and manual interventions (Rathore *et al*., 2016) [13]. Pearl millet (*Pennisetum glaucum*), extensively cultivated in semi-arid regions of Africa and India, emerges as a nutritional powerhouse. It boasts 11.65 grams of high-quality protein, 2.63 grams of crude fiber, 80 mg of calcium, 18 mg of iron, 7 mg of zinc, and 2 mg of fat-soluble vitamin E. These exceptional attributes categorize it as a "nutri-cereal" (Sehgal & Kawatra, 2003; Abdalla *et al*., 2009; Longvah *et al*., 2017; Kulthe *et al*., 2016) [15, 1, 7, 5]. Pearl millet stands out for its elevated energy content, lower starch levels, a low glycemic index (GI) of 55, and its gluten-free nature (Nambiar *et al*., 1930) [10]. Its amino acid composition, crucial for protein quality, surpasses that of sorghum and maize and is comparable to wheat, barley, and rice (Rai *et al*., 2008) [12]. Additionally, pearl millet exhibits notable antioxidant activity, measuring approximately 1.33 milligrams in terms of ascorbic acid equivalence.
India, renowned for its diverse climatic conditions, showcases a rich variety of locally cultivated and consumed edible green leafy vegetables. A novel approach has emerged, integrating by-products from food processing industries into food preparation (Balasundram *et al*., 2006) [2]. Often underestimated, carrot and cauliflower leaves stand out as rich sources of antioxidants and bioactive compounds (Mutahar *et al*., 2012; Youssef *et al*., 2013) [9, 20]. Carrot leaves (*Daucus carota* L.) are particularly noteworthy for their abundant protein and essential vitamins such as vitamin C and β-carotene. They also contain various minerals, including sodium, phosphorus, potassium, calcium, magnesium, manganese, zinc, and iron (Pereira *et al*., 2003) [21]. Additionally, these leaves serve as sources of omega-3 and omega-6 fatty acids. Similarly, cauliflower (*Brassica oleracea* var. botrytis) leaves encompass inherent protein, β-carotene, iron, and calcium. In particular, dried carrot leaf powder exhibits substantial nutrient content, containing 20.27 gm of protein, 1432 mg of calcium, 36.30 mg of iron, and 8.8 mg of zinc content (Goneim *et al*., 2011) [4].

In contrast, cauliflower leaves showcase 28 gm of protein, 16 gm of crude fiber, 30640 µg of β-carotene, and 196 mg of polyphenol content per 100 grams of the sample, positioning them as superior sources of nutrition (Reema, 2002) [14]. This current study focuses on the readily available, cost-effective, and nutritionally rich aspect of pearl millet-based supplemented biscuits enriched with carrot and cauliflower leaves powder. The aim is to leverage their potential to enhance the product quality by improving taste and increasing macro and micronutrient content.

2. Material and Methods

The bio-fortified pearl millet variety HHB-299 was obtained from the Bajra Section of the Department of Genetics and Plant Breeding at CCS Haryana Agricultural University. Additionally, the leaves of carrot and cauliflower, along with other ingredients, were sourced from the local market. To enhance the nutritional profile and prolong the shelf life of bio-fortified pearl millet flour, a blanching procedure was implemented. Initially, the pearl millet grains underwent thorough cleaning and were blanched for a duration of 30 seconds. After the blanching process, the grains were carefully dried in an oven at a controlled temperature of 50ºC. Concurrently, freshly harvested carrot and cauliflower leaves were meticulously selected and separated from their stems. The leaves underwent a thorough washing under running water to eliminate any impurities and were subsequently blanched and dried separately using a mechanical drier. Following the drying process, the leaves were finely ground into powder and employed in the development of the product. Biscuits in the control group were prepared using a combination of pearl millet flour and all-purpose flour in equal measures (50:50). Additionally, variant biscuits were formulated, incorporating carrot and cauliflower leaves at different proportions of 5%, 10%, 15%, and 20%, categorized as Type-I, Type-II, Type-III, and Type-IV, respectively, as indicated in Table 1. After production, all the biscuits were securely stored in sealed packaging for subsequent analysis, encompassing evaluations of sensory attributes and nutritional composition (Fig. 1).

2.1 Sensory Evaluation of Biscuits

The sensory quality attributes of the biscuits were evaluated by a panel of ten semi-trained judges using a nine-point hedonic scale. Panelists were presented with samples of the buns and asked to rate the acceptability of the product on a scale of 1 to 9, where 9 indicated "extremely like" and 1 indicated "extremely dislike."

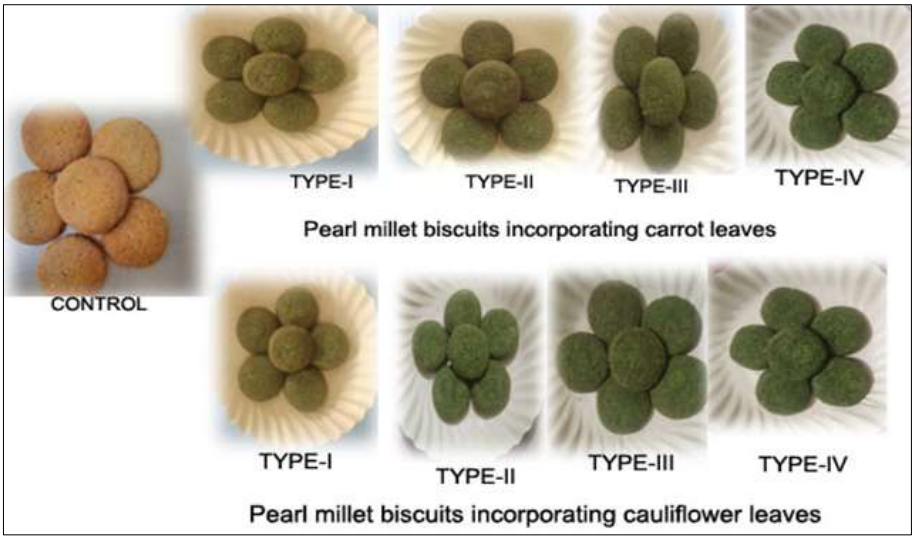

Fig 1: Carrot and Cauliflower leaves incorporated pearl millet biscuits

Table 1: Formulation of ingredients for product development

Ingredients	Control	Type-I	Type-II	Type-III	Type-IV
Pearl millet flour: Refined flour	50:50	47.5: 47.5	45:45	42.5:42.5	40:40
Carrot leaves powder(g)	-	5	10	15	20
Cauliflower leaves powder(g)	-	5	10	15	20
Ghee (g) + Milk (ml)	40+60	40+60	40+60	40+60	40+60
Sugar(g)	40	40	40	40	40
Baking powder	a pinch	a pinch	a pinch	a pinch	a pinch

2.2 Proximate Analysis of Biscuits

The analysis of the developed supplemented biscuits adhered to established standard methodology. The proximate composition, encompassing moisture, crude fat, crude protein, crude fiber, and ash content, was assessed following the guidelines of the AOAC (Association of Official Analytical Chemists) 2000. For determining crude protein, the KEL PLUS Automatic Nitrogen Estimation System was employed, with a conversion factor of 6.25 utilized to convert nitrogen values to crude protein content. The estimation of crude fat was conducted using the Automatic SOCS plus Solvent Extraction System.

2.3 Statistical Analysis

The collected data from both the nutritional analysis and sensory evaluation of developed buns were statistically analyzed using parameters such as mean and standard error. The ANOVA (analysis of variance) technique was employed

for the statistical analysis, following the standard method outlined by Sheoran and Pannu (1999) [22].

3. Result and Discussion

3.1 Sensory Characteristics of Biscuits

As per the assessment conducted using a 9-point hedonic scale, focusing on attributes such as color, appearance, aroma, texture, taste, and overall acceptability of the developed biscuits enriched with carrot and cauliflower leaves powder (Fig. 2), the results indicated that the 5% supplementation of experimental biscuits (Type-I) garnered the highest preference among the judges, followed by Type-II, Type-III, and Type-IV. Control biscuits secured the highest mean acceptability scores across all sensory attributes, falling into the category of 'liked very much.' The findings of the current study closely align with those of earlier research, where the sensory evaluation of biscuits enriched with cauliflower leaves powder demonstrated that an incorporation level of 6% received the highest sensory scores from the judges (Singh & Verma, 2022) [12].

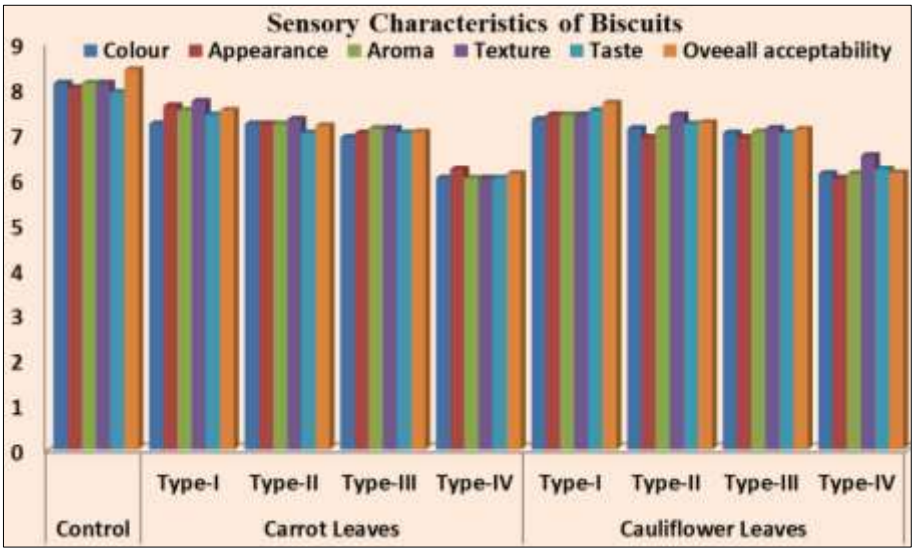

Fig 2: Sensory characteristics of bio-fortified pearl millet based biscuits incorporating carrot and cauliflower leaves powder

This is further substantiated by Singh *et al*. (2018) [18], who reported positive sensory evaluation results for various food products supplemented with dehydrated cauliflower leaves, indicating their overall acceptability in the market. The incorporation of dehydrated carrot and cauliflower leaves powder into various products proves to be a successful strategy, yielding health-promoting and wellness-enhancing outcomes. In the experimental biscuits, cauliflower leaves supplemented biscuits exhibited higher mean scores for overall acceptability across the four variations compared to those enriched with carrot leaves powder. Conversely, the average scores for overall acceptability within all variations of biscuits incorporating both carrot and cauliflower leaves powder ranged between 6.10 and 7.66. Mean sensory scores of the supplemented biscuits across all characteristics ranged from 'liked moderately to slightly.' The findings of the current study align with the conclusions of Wani and Sood (2014) [19], suggesting that the sensory evaluation favored biscuits composed of 90 percent malted wheat flour and 10 percent cauliflower leaf powder, which exhibited the highest acceptability and demonstrated favorable storage attributes.

3.2 Proximate Evaluation of Biscuits

The nutritional evaluation of biscuits developed from bio-fortified pearl millet (HHB-299) with incorporation of carrot and cauliflower leaves powder in four variation highlighted different nutrient contents depending on their respective compositions.

3.2.1 Moisture content

The initial moisture content in the control biscuit was determined to be 4.35% on a fresh weight basis. However, upon the addition of carrot and cauliflower leaves powder, a significant decrease in moisture content was observed, particularly at supplementation levels of up to 20%. The highest moisture content of 4.35% was recorded in the control (50:50 pearl millet flour and refined flour), while the lowest was 2.28% in Type-IV (80:20:: pearl millet flour + refined flour: carrot leaves powder) biscuits. The observed reduction in moisture content could be attributed to the natural moisture-absorbing properties of carrot and cauliflower leaf powder. This phenomenon aligns with the findings reported by Kumar and Barmanray (2007) [6] in their study on button mushroom-fortified biscuits.

3.2.2 Crude protein

The analysis unveiled a significant increase in the crude protein content of the developed biscuits through the progressive addition of carrot and cauliflower leaves powder. The control biscuits demonstrated a crude protein content of 9.37%, while the supplemented biscuits, ranging from 5 to 20% inclusion of carrot and cauliflower leaves powder, exhibited a range of crude protein content varying between 10.87% and 15.34%, respectively. Singh *et al*., (2018) [18], in their investigation focusing on the crude protein content of biscuits fortified with cauliflower leaf powder, found that during the initial storage phase, treatment T_8 (70:30 malted wheat flour: cauliflower leaves powder) exhibited the highest protein content at 9.51%, followed by T_7 (80:20 malted wheat flour: cauliflower leaves powder) at 9.44%, and T_6 (90:10 malted wheat flour: cauliflower leaves powder) at 9.43%, respectively. In their earlier work in 2005, Singh *et al*., utilized dried cauliflower (*Brassica oleracea*) leaves in the preparation of namakpara, kurmura, biscuit, and cake, noting

that the protein content was highest in kurmura (12.25%) and lowest in biscuits (7.42%). The present findings align with those of Verma *et al*., (2001) [23], who documented the utilization of cauliflower greens and Bengal gram greens in the preparation of nutritious snacks. Their study revealed that the protein content of various food items such as biscuits, chikki, chiwda mix, and khatta mitha chiwda derived from cauliflower greens, Bengal gram, and others ranged from 8.49, 8.14, 6.74, and 11.06 g/100 g, respectively.

3.2.3 Crude fat

The control biscuits, devoid of the addition of carrot and cauliflower leaves powder, exhibited an average crude fat content of 25.70%. In contrast, the crude fat content in biscuits supplemented with leaves powder at 20% ranged from 20.27% to 25.28%, respectively (Table 2). Among the experimental biscuits, the highest crude fat content was observed in Type-I biscuits supplemented with 5% of cauliflower leaves powder, while the lowest was recorded in Type-IV biscuits enriched with 20% of carrot leaves powder. Singh *et al*., 2018 [18], scrutinized the impact of cauliflower leaf addition on sweet and salty biscuits, discovering that the crude fat content decreased as the proportion of cauliflower leaf increased. The observed range of crude fat content was 41.61% to 44.62% at the 5, 10, and 15% levels of cauliflower leaf powder incorporation. The observed decrease in fat content in the malted samples could potentially be attributed to the sprouting process, which tends to enhance the activity of lipase enzymes, ultimately resulting in reduced fat content (Wani & Sood, 2014) [19]. These findings align with the results reported by Singh *et al*. (2018) [18], who observed a similar decrease in crude fat content during the storage of biscuits supplemented with varying levels of jaggery.

Table 2: Proximate composition of bio-fortified pearl millet based carrot and cauliflower leaves powder supplemented biscuits

Treatments	Moisture*	Crude Protein	Crude Fat	Ash	Crude Fibre
Control	4.35±0.17	9.37±1.66	25.70±0.60	1.60±0.03	1.90±0.15
Carrot leaves Biscuits Type-I	2.46±0.03	10.87±0.33	24.55±0.10	1.83±0.08	2.15±0.05
Type-II	2.37±0.14	11.64±0.14	23.46±0.37	1.96±0.12	2.30±0.04
Type-III	2.33±0.03	12.43±0.15	22.32±0.13	2.14±0.12	2.34±0.11
Type-IV	2.28±0.06	14.01±0.52	20.27±0.05	2.40±0.26	2.53±0.12
Cauliflower leaves Biscuits Type-I	2.83±0.03	12.39±0.13	25.28±0.09	1.90±0.11	2.35±0.07
Type-II	2.75±0.03	13.51±0.13	24.70±0.78	2.10±0.15	2.40±0.05
Type-III	2.67±0.06	14.02±0.51	23.94±0.49	2.30±0.09	2.50±0.06
Type-IV	2.58±0.06	15.34±0.28	23.88±0.11	2.46±0.10	3.07±0.19

Values are mean ±SD of three independent determinations
* Moisture was analysed on fresh weight basis

3.2.4 Ash content

The examination of the data presented in Table 2 emphasized a significant impact of the treatments on the ash content (percentage) of the biscuits. The total ash content in the experimented biscuits, with the addition of carrot leaves powder at 5, 10, 15, and 20% levels, was recorded as 1.83%, 1.96%, 2.34%, and 2.53%, respectively. Similarly, in the case of cauliflower leaves supplementation, the ash content was found to be 1.90%, 2.10%, 2.30%, and 2.46%, respectively (Table 2). According to Singh *et al*. (2018) [18], the ash content in the biscuits supplemented with 30 percent cauliflower leaves powder was found to be 1.59%. In contrast, the control biscuits were observed to have the lowest ash content, measuring at 1.03%. These findings indicate a notable influence of the cauliflower leaves powder supplementation on the ash content of the biscuits, underscoring the potential impact of this addition on the nutritional composition of the final product.

3.2.5 Crude fibre

Table 2 depicts a significant increase in crude fiber across various treatments in the developed biscuits as the supplementation levels of leaves powder increased. Specifically, Type-IV biscuits, enriched with 20% carrot leaves powder, revealed a crude fiber content of 2.53%, while those supplemented with cauliflower leaves powder showed 3.07% (Table 2). In comparison, the control biscuit demonstrated the lowest crude fiber content at 1.90%, in contrast to the experimented biscuits. Singh *et al*. (2018) [18] reported that the highest crude fiber content, reaching 13.62%, was observed in T_8 (70:30 malted wheat flour: cauliflower leaves powder). On the other hand, the lowest crude fiber content, at 6.12%, was recorded in T_1 (100:00 wheat flour: cauliflower leaves powder). Notably, the incorporation of cauliflower leaf powder led to an increase in the fiber content across these treatments.

4. Conclusion

The present study underscores the nutritional excellence of bio-fortified pearl millet, further heightened by the integration of carrot and cauliflower leaves powder, resulting in an overall nutrient-dense composition. Organoleptic assessments conducted by judges revealed that the control biscuits exhibited a higher mean overall acceptability compared to the experimental biscuits. Biscuits supplemented with carrot and cauliflower leaves powder displayed elevated levels of crude protein and crude fiber when compared to the control. Particularly, the inclusion of cauliflower leaves powder led to the highest crude protein content among the supplemented biscuits. In conclusion, the incorporation of carrot and cauliflower powder in pearl millet biscuits represents a promising approach to enhance their nutritional profile, catering to the preferences of health-conscious consumers and contributing to the advancement of nutritionally fortified baked goods.

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