Current Science International
Volume: 13 | Issue: 04| Oct. – Dec.| 2024

EISSN:2706-7920 ISSN: 2077-4435
DOI: 10.36632/csi/2024.13.4.44
Journal homepage: www.curresweb.com
Pages: 507-519

The Quality and Bioactive Compounds of Cauliflower Flour-Supplemented Biscuits: Their Potential as a Functional Food for Treating Anaemia in Adolescent Students

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Received: 15 Sept. 2024 **Accepted:** 05 Nov. 2024 **Published:** 10 Nov. 2024

ABSTRACT

Background: Cauliflower leaves are normally redundant and contain high nutritional value. As a result, recovering and using bioactive chemicals from cauliflower leaves is a significant difficulty. The antioxidant capacity of edible parts of cauliflowers is widely studied and has been associated with their flavonoids and phenolic acids contents. The cauliflower leaves powder could be used to treat anaemia or iron deficiency. **Objective:** This study was carried out to produce biscuits made from different ratios (5, 10 and 20%) and different part s of cauliflower (stalk, leaves and flower) flour and wheat flours samples. **Methods:** In this study, replacement of wheat flour with cauliflower stalk flour (CSF), cauliflower flower flour (CFF) and cauliflower leaf flour (CLF) in baked biscuit products and their effect on chemical composition and biochemical compounds contents, Also, effect of cauliflower leaves flour (CLF) supplementation biscuit functional with on blood haemoglobin levels of anaemic adolescent students and mineral content. **Results:** There was a general increase in the protein content of the biscuit with increase in the addition of cauliflower part flour. Biscuits made from 80% wheat flour 20% CLF. powder had high content in chlorophyll A, chlorophyll B and Total chlorophyll, total carotenoids and vitamin C. followed by CLF 10%- biscuit. The control wheat flour had the lowest TPC and CLF 20 biscuit had the highest TPC. The cauliflower flours parts were significantly different from the control wheat flour; however, there was increasing observed with addition of 5% CFF into 95% wheat flour and cauliflower addition of CLF and CSF into 80% wheat flour. All biscuit mixes to CLF which were added at an addition rate of 20% had higher values for the following minerals (Fe, Zn, and Cu), While the lowest value was recorded in the L5% biscuit mixture. The combination of cauliflower powder with fortified ingredients in biscuits can help improve hemoglobin levels in children. Vitamin C from cauliflower, despite some degradation during baking, can still contribute to the overall nutritional profile of the biscuits, enhancing the body's ability to absorb iron. **In conclusion,** cauliflower flour (stalk, leaves and flower) flour can be used as an alternative functional ingredient to partially replace wheat flour in biscuit formulation due to its ability to improve nutritional quality with high biochemical compounds and mineral contents without compromising sensorial palatability. However, baked biscuits with cauliflower powder offer a promising, child-friendly solution to improving iron absorption and combating anaemia. Although some nutrient loss occurs during processing, the combination of cauliflower's inherent nutritional benefits and the popularity of biscuits among children make this an effective strategy to address nutritional deficiencies in a practical, accessible way. In future, it can be applied these results at industrial scale as a functional food.

Keywords: Biscuit, Cauliflower, flour;, Haemoglobin, Anaemia, Student.

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1. Introduction

Globally, consumers are becoming more mindful of the need of eating functional and healthful foods, and they favor foods that offer additional health advantages beyond the bare minimum of nutrients (Baba *et al.,* 2015). As a result, there is a trend to make functional biscuits using wheat flour and functional components, which are health-promoting substances from non-wheat flours (Dewettinck *et al.,* 2008). Refined wheat flour and other substances are used to make biscuits that lack dietary fiber and phytochemicals, two grain components meant to be health-preventive (Fardet, 2010 and Sozer *et al.,* 2014). Wheat flour is a rich source of calories and various nutrients on its own, but because it is refined during processing, it has a poor antioxidant capacity. For this reason, it is necessary to combine it with a substance to increase its antioxidant capacity (Elhassaneen *et al.,* 2016). Brassica crops have been linked to a lower incidence of cancer and cardiovascular disease, among other chronic illnesses. Brassica foods are rich in vitamins, carotenoids, fibre, soluble sugars, minerals, glucosinolates, and phenolic compounds, among other health-promoting phytochemicals (Favela-González *et al.,* 2020).

The primary sources of phenolic compounds in the human diet are vegetables belonging to the Brassicaceae family. They contain derivatives of hydroxycinnamic, caffeic, chlorogenic, ferulic, and synapic acids in addition to flavonols (produced from kaempferol and quercetin) and anthocyanins (found in red cabbage) (Heimler *et al.,* 2006).

Cauliflower leaves are rich in nutrients and typically redundant. However, socioeconomic factors and ignorance affected its utilization to treat different micronutrient deficits (Singh *et al.,* 2019). Because of these plant-based phytochemicals' diverse nutritional, functional, antioxidant, and other medicinal qualities, they can be employed primarily as food sources and also contribute to nutritional security. The leftover cauliflower leaves, which are also a great source of minerals, beta carotene, calcium, iron, and dietary fibre, are not considered to be of any consequence. According to Pankar and Bornare (2018), anaemia, iron deficiency, and other micronutrient deficiencies can possibly be treated with cauliflower leaf powder (CLP). Cauliflower green leaves were dried and found to be very nutrient-dense, containing 43.11 mg of beta-carotene, 60.38 mg of iron, 1.55 mg of copper, 5.86 mg of manganese, and 5.10 mg of zinc per 100g of CLP (Wani *et al.,* 2011). Iron deficiency anaemia ranks ninth out of 26 risk factors included in the Global Burden of Disease (GBD) 2000, accounting for 841,000 deaths and 35,057,000 disability-adjusted life years lost globally (WHO 2011; Stolitzfus 2003). In 2021, the global prevalence of anaemia across all ages was 24.3%, or 1.92 billion (1.89–1.95) prevalent cases, compared to the prevalence of 28.2% in 1990, when there were 1.50 billion (1.48–1.52) prevalent cases.

One of the most important aspects of the entire fight against iron deficiency is controlling it. Support should be given to initiatives that increase the availability, accessibility, consumption, and usage of a sufficient amount, variety, and quality of food for all demographic groups. Iron-rich green leafy vegetables are the most affordable and readily available plant foods. The low availability of iron from non-heme sources, however, is the issue. The total body iron of a healthy adult male is approximately 3.6 grams. Women have roughly 2.4 grams, which is significantly less. According to GBD (2023), one of the leading causes of anaemia years lived with disability (YLDs) in 2021 was dietary iron deficiency. The body efficiently conserves iron. Every day, about 90% is recovered and put to use again (Joshi & Mathur 2010). Cauliflower leaves make up about half of these by-products, which are mostly composed of leaves and stems (Ahmed & Ali 2013). Therefore, it is very difficult to recover and use bioactive compounds from cauliflower leaves.

The amount of flavonoids and phenolic acids in cauliflowers has been linked to their antioxidant ability, which has been extensively researched (Hwang & Lim 2015). However, there hasn't been much focus on bioactive chemicals made from cauliflower by-products (Perna *et al.,* 2019). However, dietary supplementation cannot stop meat oxidation because several of the antioxidant phytochemicals in cauliflower leaves have limited bioavailability (Ferreira *et al.,* 2017 and Elhassaneen *et al.,* 2021).

The aim of this research dealt with the replacement of wheat flour with cauliflower flour. The chemical composition and biochemical compound contents of baked biscuit products made with cauliflower flour (CFF), cauliflower leaf flour (CLF), and cauliflower stem flour (CSF) are examined, as is the impact of adding CSF to biscuits on the mineral content and blood haemoglobin levels of anaemic adolescent students.

2. Materials and Methods

2.1. Material and flour preparation

Fresh white cauliflower (*Brassica oleracea* L. *var. botrytis*) variety was obtained in local distributor in Cairo, Egypt. Each whole cauliflower was separated at different three parts as follows: florets (edible portion), stalks and leaves midribs (non-edible portion) then weighed. All cauliflower samples were washed with subsequent drying in a ventilated oven at 40°C for 16h for stalks and florets samples and 40°C for 12h for leaves samples till moisture of 7-8% to obtain and milled using a Laboratorial disc mill (Quadrumat Junior flour mill or Model Type No: 279002, ©Brabender ® OHG, Duisburg 1979, Germany) to pass through a 60 mesh/inch sieve, until using. Cauliflower of stalk (CSF), Cauliflower of leaves (CLF) and Cauliflower of flower (CFF) flours. Wheat flour (WF) (soft 72%) and other ingredients used in biscuits were obtained from the local markets.

2.2. Formulation of biscuits

Preliminary studies (data not shown) have demonstrated the non-viability of CLF used for producing biscuits with little acceptable sensory characteristics. Biscuits were made with 3 different concentrations (0, 5, 10 and 20%) for each of (CSF), (CFF) and (CLF), as seen in table (1). Whereas, the control sample made by 100% (WF) as a soft 72%. The formulation of biscuits was obtained by fitting the original formulation described by Perez and Germani (2007). The percentage of cauliflower flour was increased relative to CSF, CFF and CLF by facilitating the function of connecting dough. All dry ingredients were mixed together first, then the Fern butter, to form dough was added water gradually to the point of connecting to open the dough. Once this was obtained, types of dough were made in circular shapes of 5 cm in diameter to the biscuits. The biscuits were ordered directly in rectangular pans, and baked in an oven preheated or in a conventional oven at 160 ^{0}C for 20min, or until fully baked. The biscuits were left to rest to be packed in sealed plastic bags. Table (1) shows the formulation used to make the biscuits.

Table 1: Replacing the wheat flour with cauliflower flour with different ratio.

Ingredients (grams)	Replacing the Wheat Flour with Cauliflower Flour			
	WF	CSF	CLF	CFF
*Cauliflower flour (CSF), (CLF) and CFF	0	5	10	20
*Wheat flour (WF) soft, 72%	100	95	90	80
Sugar	57	57	57	57
Butter	28	28	28	28
Baking Powder	1	1	1	1
Salt	1	1	1	1
Vanilla	1	1	1	1
Water	7	7	7	7

*Cauliflower of Stalk flour (CSF), Leaves flour (CLF) and Flower flour (CFF) and Wheat Flour (WF).

2.3. Analytical Methods

2.3.1. Chemical composition

The chemical composition was determined in of the different samples was determined according to the methods of the A.O.A.C., (2007). Moisture, total fat content using the Soxhlet extraction method, crude protein ($N \times 6.25$) using the Kjeldahl method and total ash of samples were determined as described in (A.O.A.C., 2007). Carbohydrate content was estimated by subtracting the sum of percentages of moisture, crude fat, crude protein and ash contents.

The carbohydrate content was calculated according to FAO/WHO, (1998) was calculated by the following equation no.1:

Carbohydrate (%) = 100 - (% of moisture + ash + crude protein + fat) --------------------------------- (1)

2.3.2. Calorific value

Calories content: The total calories of the samples were calculated according to James, (2013) via the following equation:

EISSN: 2706-7920 ISSN: 2077-4435 **DOI: 10.36632/csi/2024.13.4.44**

Total calories (Kcal/100 g) = (Fat × 9 Kcal) + (Protein × 4 Kcal) + (Carbohydrate × 4 Kcal) ------ (2)

2.3.3. Mineral Determination

Iron, zinc and copper contents of CLF, wheat flour and biscuits containing 5%, 10% and 20% CLF, samples were determined in the digested solution according to the method described by Jackson (1973). Minerals concentration (ppm or mg/Kg) of Fe, cu and Zn was determined using a Unicom SP 1900 atomic absorption spectrophotometer (FMD3) according to the method of A.O.A.C. (2019) method, No 980.03.

2.3.4. Bioactive compounds (Vitamin C, Total Polyphenol Content, Carotenoids, chlorophyll (A, B and AB) and Antioxidant Activity Concentration determination

2.3.4.1. Vitamin C Content determination

Vitamin C was analyzed using the A.O.A.C. method (2006). The titrant was prepared with 50 mg of 2, 6-Dichloroindophenol Na salt and 42 mg of sodium bicarbonate ($NaHCO_3$) in 50 mL of water. The solution was diluted to 200 mL with distilled water. The extracting solution was prepared with 15 g of metaphosphoric acid and 40 mL of acetic acid and then diluted to 500 mL with distilled water. Solutions were stored in amber bottles at 4°C. A 100 mL aliquot sample was added to 100 mL of the extracting solution and then filtered using a No.1 filter paper (Whatman, Maidstone, England). The solution was then titrated with the titrant until the solution turned bright pink for at least 5 sec. A standard curve was created using pure ascorbic acid (Sigma Aldrich, St. Louis, MO). Vitamin C retention was calculated using Eq.3.

Retention % = (Ascorbic acid (mg)/ 100 mL juice after treatment) / (Ascorbic acid (mg)/ 100 mL juice before treatment) ×100---(3)

2.3.4.2. Total phenolic content determination

The total phenolic contents were determined according to the Folin-Ciocalteu procedure by Zilic *et al.* (2012). Briefly, the extract (100 µL) was transferred into a test tube and the volume was adjusted to 3.5 mL with distilled water and oxidized with the addition of 250 µL of Folin-Ciocalteu reagent. After 5 min, the mixture was neutralized with 1.25 mL of 20% aqueous sodium carbonate (Na_2CO_3) solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic contents were determined by means of a calibration curve prepared with gallic acid and expressed as milligrams of gallic acid equivalent (mg GAE) per g of sample. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve. The calibration curve was prepared with gallic acid (GA) (Sigma-Aldrich, Germany), and the results were expressed as mg GA/100 g of dry weight. Three replicates of extract were analyzed.

2.3.4.3. Determination of radical DPPH scavenging activity

Total free radical scavenging capacity of the extracts from different samples were estimated according to the previously reported method Brand-Williams *et al.* (1995) and Hwang and Thi (2014) with slight modification using the stable DPPH radical, which has an absorption maximum at 515 nm. A solution of the radical is prepared by dissolving 2.4 mg DPPH in 100 ml methanol. A test solution (5 µl) was added to 3.995 ml of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 min in the dark. Absorbance of the reaction mixture was measured at 515 nm spectrophotometrically. Absorbance of the DPPH radical without antioxidant, i.e. blank was also measured. All the determinations were performed in triplicate. The capability to scavenge the DPPH radical was calculated by the following equation no.4:

DPPH Scavenged (%)= $((A_B–A_A)/A_B)×10$ -- (4)

where, AB is absorbance of blank at t= 0 min; AA is absorbance of the antioxidant at t= 30 min. A calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

2.3.4.4. Carotenoids, Chlorophyll a and b Content Determination

Carotenoids and chlorophyll a and b concentrations were measured according to Lichtenthaler and Wellburn (1983). A 0.1 g sample was weighted on an analytical scale and ground with sand and magnesium carbonate in a mortar. The extraction of carotenoids and chlorophyll a and b was carried out with 25 mL of 80% acetone. The solution was transferred into a centrifuge tube, covered with aluminum foil, and set aside in the dark for half hour and then centrifuged for 10 min. The absorbance of the resulting extracts was measured at wavelengths of 470 nm, 646 nm and 663 nm using a 4054-UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England).

2.4. Patients and Methods

A total of 60 eligible volunteer students moderately anaemic males aged 12 – 15 years old were recruited into the study for 12 weeks. Exclusion criteria included extreme dietary habits such as vegetarianism, severely low fat intake and extreme levels of physical activity. The volunteers were randomly assigned to one of two main groups' males and females these were sub grouped as follows:
Group a (n = 30) consumed about 8 regular plain wheat flour biscuits per day for 12 weeks.
Group b (n = 30) consumed about 8 biscuits supplemented with cauliflower flour per day for 12 weeks.

2.4.1. Methods

This was a randomized, parallel treatment, study carried out at the Nutrition and Food Science Department, National Research Centre, Dokki, Cairo, Egypt.

2.4.2. Blood sampling and biochemical analysis

Blood samples were withdrawn to test the haemoglobin concentration (Walker, 1990) at start and after 12 weeks of the intervention period.

2.4.3. Statistical analysis

SPSS software version 21.0.0 (SPSS Inc., IBM) was used to analyze the obtained results. Mean and SD were calculated for each variable. A paired t-test was used to assess variations in the mean between the groups from the baseline to 12 weeks of intake for the determination of absolute change. A p-value ≤ 0.05 is considered to be statistically significant.

3. Results and Discussions

3.1. Effect of cauliflower stem flour (CSF), (CLF) and (CFF) supplementation biscuit functional on chemical composition

Table (2) displays the biscuits' moisture, ash, crude fiber, fat, protein, and carbohydrate compositions. The moisture percentage of the samples varied from 4.54 to 5.22% across all samples. A product's capacity to withstand storage is positively correlated with its original moisture level. This suggests that sample L5, which contains the least amount of moisture, may have a longer shelf life. But according to Kure (1998), the moisture content of all the biscuit samples was low and might not have a negative impact on the biscuits' quality.

Between samples F5 and F10 and samples L10 and S10, the biscuit samples' fat contents varied from 19.05% to 21.22%. Agu (2007) provided a similar report on wheat/African bread fruit biscuits. The biscuits' protein content varied from 4.375 to 24.12%. With an increase in the amount of cauliflower flour used, the biscuit's overall protein level increased. According to the results, biscuit samples with a 20% (CFF) substitution had a high protein content and could be used as a substitute protein source in cases of protein deficit.

The biscuits' carbohydrate content varied between 49.76% and 68.29%. In general, the biscuits' carbohydrate content dropped as the amount of cauliflower flour increased. For example, adding 5%, 10%, and 20% (CFF) decreased the amount of carbohydrates to 52.23%, 52.27%, and 49.76%, compared to the control's 68.29%. But there was a small increase as more cauliflower flour was added. Cutting back on carbohydrates may help reduce the danger of consuming too much sugar. The biscuit's high protein, ash, and fat content may be the cause of the decrease in carbs. This observation was consistent with previous Messiaen (1992) reports.

Curr. Sci. Int., 13(4): 507-519, 2024
EISSN: 2706-7920 ISSN: 2077-4435 **DOI: 10.36632/csi/2024.13.4.44**

Table 2: Chemical composition or proximate (%) in cauliflower and wheat flour biscuits.

Sample	Moisture	Ash	Fat	Protein	Carbohydrate	K. Calories*
****Cont.**	4.72	1.38	21.22	4.375	68.29	1386.231
F5	4.85	1.59	19.58	21.75	52.23	4720.056
F10	4.57	1.59	19.35	22.22	52.27	4819.908
F20	5.13	1.94	19.05	24.12	49.76	4972.198
L5	4.54	1.51	20.51	8.37	65.07	2362.966
L10	4.73	1.73	20.38	8.19	64.97	2311.87
L20	4.56	2.05	20.15	8.05	65.19	2280.661
S5	5.22	2.19	20.55	14.75	58.29	3624.237
S10	4.94	2.26	20.31	14.17	58.32	3488.424
S20	4.94	2.51	20.08	14.05	58.43	3464.374

*K.calories equations = Fat*9 + Protein*4+ Carbohydrate*4

** Cont. 100% wheat flour, F5 biscuits contain 5% Cauliflower flowers flour and 95% wheat flour, F10 biscuits contain 10% Cauliflower flowers flour and 90% wheat flour, F20 biscuits contain 20% Cauliflower flowers flour and 80% wheat flour, L5 biscuits contain 5% cauliflower leaves flour and 95% wheat flour L10 biscuits contain 10% cauliflower leaves flour and 90% wheat flour, L20 biscuits contain 20% cauliflower leaves flour and 80%, S5 biscuits contain 5% cauliflower steaks flour and 95% wheat flour S10 biscuits contain 10% cauliflower steaks flour and 90% wheat flour, S20 biscuits contain 20% cauliflower steaks flour and 80%.

3.2. Effect of (CSF), (CLF) and (CFF) supplementation biscuit functional on Bioactive compounds content (Vitamin C, total Chlorophyl and total carotenoids)

Carotenoids, also known as total carotenoids, are plant pigments found in fruits and vegetables as microcomponents (Bognar, 1998). These are a class of fat-soluble, aliphatic-alicyclic, yellow-to-red compounds that are extensively found in nature and give edible fruits and vegetables their red, orange, and yellow hues (Bauernfeind, 1981). Results in table (3) illustrate that CLF had the highest content in chlorophyll A, chlorophyll B and total chlorophyll, total carotenoids and vitamin C it was 34.03, 34.30, 68.34, 0.51 and 11.02 %, respectively (on dry weight basis) followed by CSF it was 2.24, 0.85, 1.39, 1.01 and 6.21%, respectively. CSF had highest content in total carotenoids followed by CLF, 1.01 and 0.51 %, respectively. Also, CLF had 11.02 % of vitamin C. Biscuits L20 made from 80% wheat flour 20 CLF had high content in chlorophyll A, chlorophyll B and total chlorophyll, total carotenoids and vitamin C. followed by L10 biscuit.

Results in table (3) illustrate that leaves flour powder (CLF) had the highest content in chlorophyll A, chlorophyll B and total chlorophyll it was 34.03, 34.30, 68.34, 0.51, 11.02 %, respectively (on dry weight basis) followed by CSF it was 2.24, 0.85, 1.39 %, respectively. Stem flour powder had highest content in total carotenoids followed by Leave flour powder, 1.01 and 0.51 %, respectively. Also, CLF had 11.02 % of vitamin C. Biscuits L20 had high content in chlorophyll A, chlorophyll B and total chlorophyll, total carotenoids and vitamin C, followed by F10 biscuit.

Table 3: Bioactive compounds content (%) (Vitamin C, total Chlorophyl and total carotenoids) in cauliflower flour and wheat flour biscuits.

Sample	Chlorophyll A	Chlorophyll B	Total chlorophyll	Total carotenoids	Vitamin C
*CFF	0.42	0.13	0.56	0.08	4.97
CLF	34.03	34.30	68.34	0.51	11.02
CSF	2.24	0.85	1.39	1.01	6.21
Cont.	0.05	0.02	0.04	0.01	0.56
F5	0.19	0.03	0.16	0.01	1.37
F10	0.12	0.03	0.09	0.13	1.69
F20	0.09	0.03	0.12	0.06	1.56
L5	2.50	0.51	3.01	0.89	2.00
L10	4.01	0.82	4.83	1.71	2.37
L20	8.22	1.46	9.67	3.51	2.62
S5	0.18	0.00	0.17	0.05	1.25
S10	0.09	0.43	0.52	0.11	1.88
S20	0.28	0.93	1.21	0.13	2.26

* Cont. 100% wheat flour, F5 biscuits contain 5% Cauliflower flowers flour and 95% wheat flour, F10 biscuits contain 10% Cauliflower flowers flour and 90% wheat flour, F20 biscuits contain 20% Cauliflower flowers flour and 80% wheat flour, L5 biscuits contain 5% cauliflower leaves flour and 95% wheat flour L10 biscuits contain 10% cauliflower leaves flour and 90% wheat flour, L20 biscuits contain 20% cauliflower leaves flour and 80%, S5 biscuits contain 5% cauliflower steaks flour and 95% wheat flour S10 biscuits contain 10% cauliflower steaks flour and 90% wheat flour, S20 biscuits contain 20% cauliflower steaks flour and 80%.

3.3. Effect of (CSF), (CLF) and (CFF) supplementation biscuit functional on Bioactive compounds content (antioxidant activity and total phenol)

Table (4) displays the DPPH of flower stick and leaf flours and cookies. In CFF and CLF, the DPPH ranged from 92.17 to 80.15%, while in L20 and control biscuits, it ranged from 35.86 to 68.27%. There was a notable difference in the DPPH between the control and other biscuits. According to González-Montelongo *et al.,* (2010), the DPPH inhibition for plant materials typically follows a similar order to the TPC. For instance, the DPPH rises as the concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increases. CFF, however, had the greatest DPPH. It increases the DPPH of the biscuit samples compared to the control samples when added to wheat flour.

The biscuits made with CSF 5% (S5) had the lowest DPPH, while the biscuits made with CLF 20% (L20) had the highest DPPH. According to Baba *et al.,* (2015) and Sharma and Gujral (2014), baking and microwave roasting are two processing processes that boost baked goods' antioxidant activity. The total phenolic content must be examined since phenolic compounds (PCs) may directly contribute to the antioxidant effect. Table (4) shows the total phenolic content (TPC) of the cauliflower parts flours and biscuit made from flowers, sticks, and leaves. TPC varied between 10.50 and 38.90 mg/g in the control sample and L20 biscuit, and between 19.40 and 58.20 mg/g in CSF and CLF. The TPC was highest for L20 biscuits and lowest for control wheat flour.

Although the components of cauliflower flour differed greatly from those of the control wheat flour, an increase was noted when 5% CFF was added to 95% wheat flour (F5) and when 20% CLF or CSF was added to 80% wheat flour. Because of the polymerization of polyphenols and the decarboxylation of phenolic acids that take place during heat treatment, baked goods significantly lower levels of phenolic compounds, according to Krystyjan *et al.* (2015). Furthermore, Gelinas and McKinnon (2005) postulated that the concentration of phenolic compounds may be somewhat influenced by Maillard reactions. The current study's results are consistent with those of Elhassaneen *et al.* (2016), who found that adding 5% potato peel powder and prickly pear peel increased the biscuits' TPC from 110.23 to 143.28 and 192.79 mg/100 g of sample.

Table 4: Bioactive compounds content (antioxidant activity and total phenol) in cauliflower and wheat flour biscuits.

Samples	Antioxidant activity (%)	Total phenol (mg gallic acid / gram)
***CFF**	92.17	34.20
CLF	80.15	58.20
CSF	84.32	19.40
Cont.	35.86	10.50
F5	52.37	15.80
F10	64.20	20.30
F20	65.37	21.70
L5	58.55	23.70
L10	65.90	30.40
L20	68.27	38.90
S5	47.54	18.50
S10	59.85	19.40
S20	62.90	19.90

*Cont. 100% wheat flour, F5 biscuits contain 5% Cauliflower flowers flour and 95% wheat flour, F10 biscuits contain 10% Cauliflower flowers flour and 90% wheat flour, F20 biscuits contain 20% Cauliflower flowers flour and 80% wheat flour, L5 biscuits contain 5% cauliflower leaves flour and 95% wheat flour L10 biscuits contain 10% cauliflower leaves flour and 90% wheat flour, L20 biscuits contain 20% cauliflower leaves flour and 80%, S5 biscuits contain 5% cauliflower steaks flour and 95% wheat flour S10 biscuits contain 10% cauliflower steaks flour and 90% wheat flour, S20 biscuits contain 20% cauliflower steaks flour and 80%.

3.4. Effect of cauliflower leaves flour (CLF) supplementation biscuit functional on mineral content

Table (5) shows the mineral contents of cauliflower leaves, wheat flour and wheat biscuits replaced with different proportions of C. L. flour. The results generally showed that the element content in CLF C. L. flour was high content in Fe, Zn and Cu was 433.19, 37.92 and 5.73 mg/Kg respectively compared with wheat flour was 29.06, 8.11 and 2.02 mg/Kg respectively. All biscuit mixes to CLF which were added at an addition rate of 20% L20 had higher values for the following minerals (Fe, Zn, and Cu), where was (121.51, 12.76, 0.321 and 3.07 mg/Kg) respectively, While the lowest value was recorded in the L5 biscuit by (50.21, 8.37 and 2.35 mg/Kg) respectively.

High intake of zinc can hinder copper absorption, leading to copper deficiency. Conversely, maintaining an appropriate ratio of zinc to copper is critical for optimal health. In addition, zinc and iron compete for absorption in the intestine. Consuming large amounts of zinc can inhibit iron absorption, which may lead to iron deficiency. It is important to maintain a balance between zinc and iron intake, especially for individuals at risk of iron deficiency.

Table 5: Mineral Contents (ppm or mg/kg) in Cauliflower leaves and Wheat Flour Biscuits.

Mineral elements	Minerals Concentration (ppm or mg/Kg)		
	Fe	Zn	Cu
*CLF	433.19	37.92	5.73
WF	29.06	8.11	2.02
L5	50.21	8.37	2.35
L10	88.54	10.34	2.56
L20	121.51	12.76	3.07

* WF wheat flour, cauliflower leaves flour (CLF), L5 biscuits contain 5% cauliflower leaves flour and 95% wheat flour L10 biscuits contain 10% cauliflower leaves flour and 90% wheat flour, L20 biscuits contain 20% cauliflower leaves flour and 80%.

3.5. Effect of cauliflower leaves flour (CLF) supplementation biscuit functional on blood haemoglobin levels of anaemic adolescent students

Anaemia, particularly iron deficiency anaemia, is a common nutritional disorder among children, affecting their growth, development, and overall well-being (Ahmed, 2012). One innovative approach to combat this issue is the incorporation of nutrient-rich foods, like cauliflower, into common dietary staples such as biscuits (Kowsalaya and Vidhaya 2004). This strategy not only provides children with essential nutrients but also introduces healthier food options in an appealing and accessible form.

Cruciferous vegetables like cauliflower are prized for their abundance of vital vitamins and minerals (Shivapriya *et al.,* 2012). Vitamin C, an essential component that improves the absorption of non-heme iron (the kind of iron present in plant-based meals), is especially abundant in it (Elif *et al.,* 2022). Because of this, when eaten with foods high in iron, cauliflower is a great dietary option for enhancing iron absorption. Cauliflower leaves flour (CLF) is also a good ally in the fight against anaemia since it includes folate, which is essential for the development and maturation of red blood cells (Bassett and Sammán 2010).

Biscuits are widely consumed and enjoyed by children, making them an ideal vehicle for incorporating nutrient-dense ingredients like cauliflower. By transforming cauliflower into a powder and blending it into biscuit recipes, it becomes possible to deliver key nutrients in a form that is both convenient and palatable. Biscuits also have a longer shelf life and are easy to distribute, which is especially beneficial in areas where fresh produce might not always be available.

The combination of CLF as fortified ingredients in biscuits can help improve hemoglobin levels in children. Vitamin C from cauliflower, despite some degradation during baking, can still contribute to the overall nutritional profile of the biscuits, enhancing the body's ability to absorb iron. This, in turn, may boost haemoglobin synthesis and improve oxygen transport in the blood, as showed in figure (1) and Table (6).

While the heating process during baking can reduce the vitamin C content of cauliflower, the use of modern baking techniques, such as lower temperature baking or fortifying the biscuits with additional vitamin C, can mitigate this loss (Hayet *et al.,* 2021). Moreover, the presence of other nutrients like folate in cauliflower can help support red blood cell production, offering further benefits in addressing anaemia.

Table 6: Comparison of haemoglobin (g/ dl) of boys and girls control and test.

Group	Boys Student		Girls Student	
	Group One Control	Group Two Test	Group One control	Group Two Test
Mean	9.48	10.15*	9.02	10.05*
SD	0.355	0.41	0.886	0.366
SEM	0.065	0.075	0.162	0.067
N	30	30	30	30

*Significant

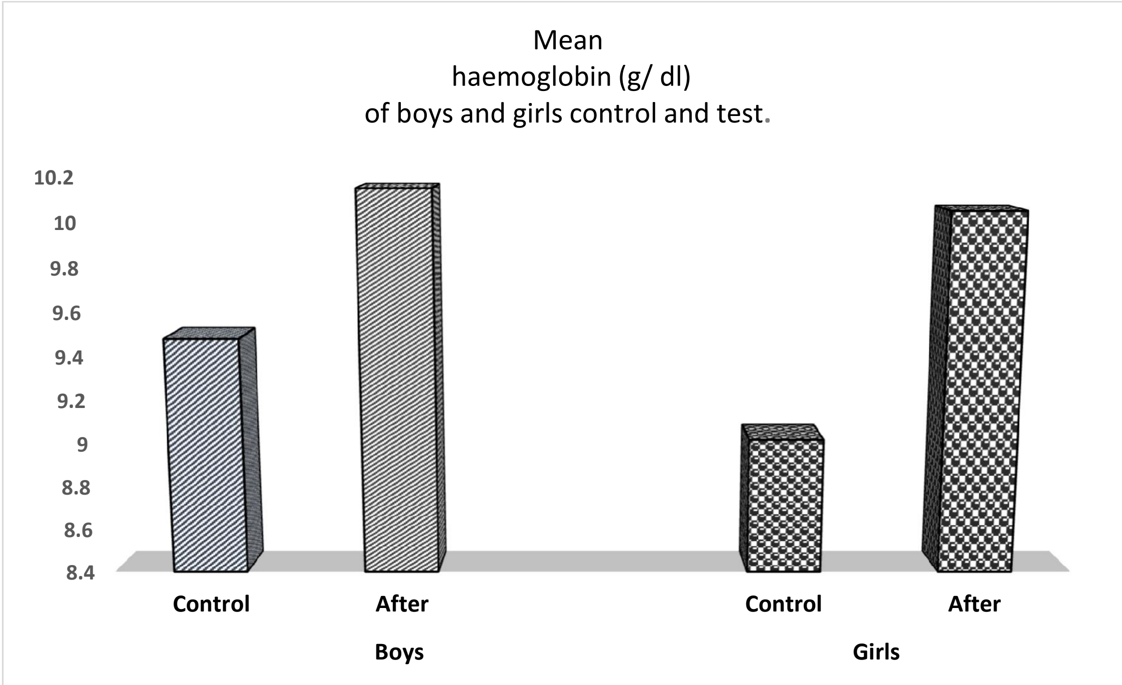

Fig. 1: Mean of haemoglobin (g/ dl) of boys and girls control and test.

4. Recommendations

For this approach to be effective, it is essential that the biscuits are part of a balanced diet that includes other iron-rich foods. The biscuits should also be consumed regularly to provide consistent nutritional support. In cases where vitamin C loss is a concern, pairing the biscuits with fresh fruits high in vitamin C could further enhance iron absorption.

Baked biscuits with cauliflower leaves flour offer a promising, child-friendly solution to improving iron absorption and combating anaemia. Although some nutrient loss occurs during processing, the combination of cauliflower's inherent nutritional benefits and the popularity of biscuits among children make this an effective strategy to address nutritional deficiencies in a practical, accessible way.

5. Conclusions

The supplementary of cauliflower (stalk, Leaves and flower) flour in baked products components is adequate for baking process, since it is possibly be used as a part component for wheat flour substituted as well as being a functional component in composed bakery products because of its able to improve the nutritional quality of cauliflower supplementation biscuit without jeopardizing the palatability.

With an increase in the amount of cauliflower flour used, the biscuit's overall protein level increased. Biscuits made from 80% wheat flour 20% CLF (L20) had high content in chlorophyll A, chlorophyll B and Total chlorophyll, total carotenoids and vitamin C followed by L10 biscuit. L20 biscuit had the highest TPC but The control biscuit was the lowest. L20 Biscuit which were added at an addition rate of 20%(CLF) had higher values for the following minerals (Fe, Zn, and Cu), While L5 biscuit recorded lowest value. The combination of cauliflower leaves flour (CLF) with fortified ingredients in biscuits can help improve hemoglobin levels in children. Vitamin C from cauliflower, despite some degradation during baking, can still contribute to the overall nutritional profile of the biscuits, enhancing the body's ability to absorb iron.

cauliflower flour (stalk, leaves and flower) flour can be used as an alternative functional ingredient to partially replace wheat flour in biscuit formulation due to its ability to improve nutritional quality with high biochemical compounds and mineral contents without compromising sensorial palatability. However, baked biscuits with cauliflower powder offer a promising, child-friendly solution to improving iron absorption and combating anaemia. Although some nutrient loss occurs during processing, the combination of cauliflower's inherent nutritional benefits and the popularity of biscuits

 DOI: 10.36632/csi/2024.13.4.44

among children make this an effective strategy to address nutritional deficiencies in a practical, accessible way. In future, it can be applied these results at industrial scale as a functional food.

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EISSN: 2706-7920 ISSN: 2077-4435 **DOI: 10.36632/csi/2024.13.4.44**

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