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Production of dry toast slices with high nutritional value and attractive color using red beet, spinach, or carrot pastes

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ABSTRACT

Vegetables are essential dietary components, providing a wealth of nutrients, particularly minerals and fiber. This research aimed to enhance dry toast slices' nutritional value and visual appeal by incorporating red beet, spinach, or carrot paste as natural food colorings. A chemical evaluation of the vegetable pastes was conducted, including chemical composition, mineral, phenolic, flavonoid content, and total pigments (betalain, carotene, and chlorophyll). Additionally, antioxidant, antimicrobial, and cytotoxicity activities were measured. The study investigated the effects of adding 30% vegetable paste to the toast dough. The resulting dry toast slices were evaluated for their chemical composition, mineral content, color, physical properties, and sensory characteristics. The results indicated that spinach paste had a higher fat, protein, and ash content than red beet and carrot paste. Furthermore, it exhibited higher amounts of minerals (except P and Cu) than others. The red beet, spinach, and carrot pastes displayed phenolic compound contents of 200, 88, and 18 mg GAE/100g, respectively, while the antioxidant activity values were 90.35, 80.25, and 75.95 %, respectively. The total pigment contents of the pastes were 450 mg/100g betalain for red beet, 120 mg/100g chlorophyll for spinach, and 75 mg/100g carotenoid for carrot. The inhibition zones of the antimicrobial activity for the vegetable pastes, tested against Gram-positive and Gram-negative bacteria, fungi, and yeast, yielded inhibition zones ranging from 8 to 12 mm. The cytotoxic effects of the vegetables on Caco-2 human cancer cell lines followed the order: red beet > spinach > carrot. Fortification of wheat flour with 30% of each vegetable paste resulted in toast with superior sensory characteristics and increased nutritional value compared to the control sample. This research demonstrates the potential of using red beet, spinach, or carrot pastes as natural food colorings to enhance bakery products' nutritional profile and visual appeal.

1. Introduction

Many previous studies have explored the benefits of consuming vegetables and fruits and their impact on protecting against chronic diseases, especially those that appear to people when they age. These diseases include immune dysfunction, cardiovascular diseases, cataracts, brain-related diseases, and cancer. Foods rich in polyphenols offer more than just basic nutrition; they also contain bioactive compounds that can help prevent or delay the onset of these diseases (Raghunath et al., 2013). The polyphenols have the ability, at low concentrations, to scavenge free radicals (Varga et al., 2024). Vegetables are rich sources of both fiber and polyphenols (Fatima, 2024). Fortifying foods with vegetables adds bioactivity and can help reduce calorie intake from high-calorie diets (Dega and Barbhai 2023).

In many developing countries, vegetables, particularly cooked vegetables, are crucial in diets. These vegetables are often added to bread, pastries, or other starchy foods to enhance their nutritional value. Examples include onion peels, beetroot, spinach, and mushrooms (Ranawana et al., 2016 and Singh 2020). Vegetables and fruits are sometimes incorporated into pasta for added flavor, visual appeal, and increased nutritional content.

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Vegetable pasta typically contains ingredients like tomatoes, spinach, or beets (*Beta vulgaris* L). Beetroot belongs to the family Chenopodiaceae and is known for its bright scarlet color, juice with high nutritional value and medicinal properties (Liliana and Oana-Viorela, 2020). Cultivated worldwide, beetroot finds its way into many diets and food products as a natural coloring agent. It's also valued for its high antioxidant content and abundance of minerals like iron, potassium, and magnesium, all of which contribute to heart and blood vessel health. Additionally, beetroot contains vitamins A, C, and B, fiber and natural colors. It can be eaten raw, cooked, or roasted (Baião et al., 2020 and Atalar et al., 2024). Spinach, a leafy green vegetable distinguished by its chlorophyll pigment, offers a wealth of health benefits due to its abundance of essential vitamins and minerals. Renowned for its richness in both carotenoids and vitamins, spinach is also a good source of minerals like iron, calcium, and phosphorus, making it a popular choice for human consumption (Singh et al., 2023). Cruciferous vegetables and dark-green leafy vegetables, in particular, provide essential nutrients and promote overall health by reducing inflammation and potentially preventing various chronic diseases (Wallace et al., 2020). Carrots a popular root vegetable are rich in carotenoids, flavonoids, vitamins, and minerals. They also contain polyacetylenes, all of which contribute to their nutritional and health benefits. Consumers of all ages are drawn to the vibrant color of carrots as a result of their high content of pro-vitamin A (Sule and Abu 2017 and Pavlović et al., 2024). Like many other colorful vegetables, carrots are considered a valuable source of powerful antioxidants, known to neutralize free radicals.

This study aimed to develop colored dry toast slices with high nutritional value for consumers by incorporating vegetables rich in natural pigments, such as red beets, spinach, or carrots.

2. Materials and Methods

Materials

Vegetables (red beets, spinach, and carrot), and other ingredients (dry yeast, sugar, salt, milk powder, and sunflower oil) were purchased from the local market in Giza, Egypt. The wheat flour (72% extraction rate) was purchased from South Cairo Company of milling, Egypt. The cells of human intestinal carcinoma (Caco-2) were obtained from the American Type Culture Collection (Rockville, MD). All chemicals used were of analytical reagent grade and were purchased from El-Gomhouria Co. for Chemical, Giza, Egypt.

Methods

Preparation of paste of raw materials

Red beet, spinach, and carrot were thoroughly washed under running tap water to remove any dirt and other foreign particles. The vegetables were then boiled in a minimal amount of drinking water until tender. For spinach, tender leaves were selected prior to boiling. Carrots were chopped before boiling. Once the red beet was cool enough to handle, the thin outer skin was scrubbed off by hand under running tap water. Finally, all the cooked vegetables were cut into smaller pieces and mashed into a smooth paste using a Braun hand blender (Germany). Finally, the vegetables paste samples were stored in polyethylene bags at -18°C until analysis.

Preparation of the toast dough

The toast dough was prepared according to the method described by the AACC (2010) with some recipe modifications. The dough formula was prepared by mixing wheat flour 72% extraction (100 g), dry yeast (2 g), sugar (3 g), salt (1 g), milk powder (2 g), and sunflower oil (2 g). Water was then added incrementally until a cohesive dough formed (control sample). The fortified toast dough samples were prepared by incorporating the maximum possible amount of one of the previously prepared vegetable pastes (red beet, spinach, or carrot) into the control dough, without affecting the final shape and texture of the toast. This involved conducting several trials with different vegetable paste concentrations. Based on the evaluation of color, texture, and elasticity, the optimal concentration for all vegetables was determined to be 30% (Figure 1).

Preparation of dry toast slices

The toast bread (control and fortified with the paste of red beet, spinach, or carrot) was prepared by baking the toast dough in Thermo Scientific Precision Compact Oven (Fisher Scientific, USA) at 180 °C for 40 min., then cooled at room temperature. Next, the cooled toast was sliced and dried further in an electric oven at 180°C for 10 minutes (Figure 2). Finally, the dry toast slices were cooled again and stored in polyethylene bags at room temperature until further analysis.

Figure 1. The dough of fortified with the raw material

1) Toast dough made from wheat flour 72% extraction fortified with 30% red beet paste.
2) Toast dough made from wheat flour 72% extraction fortified with 30% spinach paste.
3) Toast dough made from wheat flour 72% extraction fortified with 30% carrot paste.

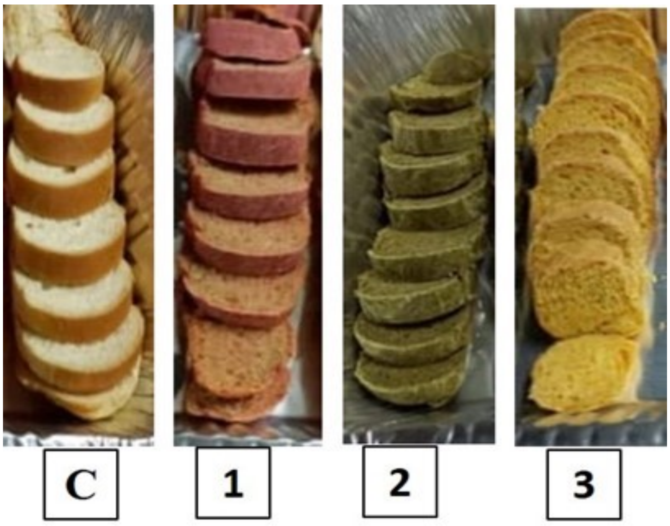

Figure 2. The dry toast slices fortified with the raw material

C) The dry toast slices made from wheat flour 72% extraction (Control).
1) The dry toast slices made from wheat flour 72% extraction fortified with 30% red beet paste.
2) The dry toast slices made from wheat flour 72% extraction fortified with 30% spinach paste.
3) The dry toast slices made from wheat flour 72% extraction fortified with 30% carrot paste.

Analytical methods

Proximate chemical composition of raw materials (wheat flour 72% extraction, red beet paste, spinach paste, and carrot paste) and dry toast slice samples was determined according to AOAC (2016) methods. Carbohydrate content was calculated by difference. Mineral contents (Calcium (Ca), iron (Fe), zinc (Zn), phosphorus (P), sodium (Na), copper (Cu), and selenium (Se)) was measured using an Agilent Technologies (model 4210 MP-AES), atomic absorption spectrophotometer instrument outlined in AOAC (2016).

Phenolic and flavonoid compounds contents of raw material

The total phenolic compound contents of raw materials (wheat flour 72% extraction and the pastes of red beet, spinach, or carrot) were determined colorimetrically using the Folin–Ciocalteu reagent according to the method described by Cosmulescu and Trandafir (2012). Total flavonoid compounds were determined according to the methods of Cosmulescu et al. (2015).

Identification of phenolic acids and flavonoid compounds of raw material

Phenolic and flavonoid compounds of paste of red beet, spinach, or carrot were identified using HPLC following the method of Goupy et al. (1999) and Mattila et al. (2000), respectively.

Total pigments

Determination of total betalain

Betalain content in red beet paste was extracted with distilled water and measured at a wavelength of 535 nm as described by Castellar et al. (2003).

Determination of chlorophyll content

Total chlorophyll content was measured using the spectrophotometric method by Gogoi and Basumatary (2018).

Determination of total carotene

The total carotene content in carrot was calculated consistent according to the method of Nagata and Yamashita (1992).

Antioxidant activity of raw materials

The antioxidant activity of raw materials (wheat flour 72% extraction and the pastes of red beet, spinach, or carrot) was evaluated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical described by Rattanachitthawat et al. (2010).

Antimicrobial activity of raw materials

The paste of red beet, spinach, or carrot samples was evaluated for their antimicrobial activity against pathogenic bacterial strains, one pathogenic fungus strain, and one pathogenic yeast. This method was carried out using the disc diffusion method; the plates were incubated at 37 °C overnight (in the case of bacteria) and 28 °C for 3 days in the case of fungi. The inhibition zones were recorded in mm for replicates prepared for each treatment according to the method of Kotzekidou et al. (2008). The pathogenic microorganism's bacterial strains used in this work (Gram-positive and Gram-negative) were kindly supplied by the Microbiology Department, Faculty of Agriculture Cairo University. These strains are *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 20231, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 33018, and *Escherichia coli* ATCC 25922. These Cultures were maintained on nutrient agar slants at 4ºC. Mold Filamentous food–borne fungus; *Aspergillus niger* was isolated from different spoilage sources like vegetables, fruits, or grains (Rizk et al., 2009). Yeast *Candida albicans* CAIM-22 was obtained from MIRCEN (Microbiology Research Center) Ain–Shams University, Cairo, Egypt.

Cytotoxicity activity of the raw material on human cell lines (MTT Protocol)

The MTT assay, as described by Mosmann (1983), was used to evaluate the cytotoxicity of red beet, spinach, and carrot pastes against the human colorectal adenocarcinoma cell line (Caco-2). The pastes were tested at 31.25 to 1000 µg/ml concentrations. This in vitro bioassay was conducted by the Bioassay-Cell Culture Laboratory at the National Research Center in Cairo, Egypt. The average viability of Caco-2 cells was compared to the control to assess the impact of the vegetable pastes. Cell viability percentages were then plotted against the corresponding paste concentrations. The minimum concentration of each vegetable paste that inhibited Caco-2 cell growth was determined as the effective concentration. This was compared to a positive control (100 µg/ml of a known cytotoxic natural agent causing 100% cell death under the same conditions).

Percentage of viability = absorbance of the sample/ absorbance of control

Percentage of toxicity = 100 – the percentage of viability

Physical properties of dry toast slices

Specific volume

Specific volume, volume, weight, and diameter of the dry toast slices (control and fortified with red beet, spinach, or carrot paste) were determined following the method outlined in AACC (2010).

Color measurement

The color of the dry toast slices (Control and fortified with the paste of red beet, spinach or carrot) was measured using a hand-held Chromameter (model CR-400, Konica Minolta, Japan). The tristimulus values *L** (brightness), *a** (red to green color (redness), and *b** (yellowness- yellow to blue color)) were measured according to the method outlined by McGuire (1992).

Sensory evaluation of dry toast slices

Sensory evaluation of prepared dry toast slices (Control and fortified by paste of red beet, spinach, or carrot) was measured according to Linda et al. (1991). The samples were conducted by twenty semi-trained panelists from the staff members of the Food Science Department, Faculty of Agriculture - Cairo University. Each panelist was provided with the sample in an unlabeled under white lights and asked to cleanse the palate with water before tasting the second sample. Sensory evaluations for the crust color, crumb color, texture, odor, taste, appearance, and overall acceptability (20 marks each), were done to determine consumer acceptability. A numerical hedonic scale ranging from 1 to 20 (where 1 is the most disliked and 20 is the most liked) was used for sensory evaluation.

Statistical analysis

The means of the results are statistically analyzed using one-way analysis of variance (ANOVA) with a significance level of alpha = 0.05. Statistical software (Assistat Version 7.7, Brazil) was used for all analyses, as described by Silva and Azevedo (2009).

3. Results and Discussion

Chemical composition of raw materials

The moisture, fat, protein, ash, fiber, and carbohydrate contents of the raw materials (72% extraction wheat flour, red beet paste, spinach paste, and carrot paste) were analyzed. The results are presented in Table 1. The results presented in Table 1. indicated that there were significant differences ($P \leq 0.05$) among samples of raw materials used under this study in moisture, fat, protein, ash, fiber and carbohydrate contents. Moisture content ranged from 90.48% for spinach paste to 9.54% for wheat flour (72% extraction). The higher moisture content of pastes of red beet, spinach, or carrot can be explained by the prior preparation of the paste (boiling process), which increased vegetable's water content. Spinach paste had a higher fat, protein, and ash content (3.99%, 26.68%, and 19.96%, respectively) than red beet paste (1.70%, 11.85%, and 13.33%, respectively) and carrot paste (1.85%, 7.18%, and 13.12%, respectively). The results also showed that no significant differences were found between wheat flour (72% extraction) and red beet paste in protein contents (11.50%, and 11.85%, respectively), also between red beet paste and carrot paste for ash contents (13.33%, and 13.12%, respectively). The pastes displayed a different trend for fiber content compared to protein and ash. Red beet paste boasted the highest fiber content (20.74%), significantly exceeding the minimal amount found in wheat flour (72% extraction) (0.39%). Conversely, wheat flour (72% extraction) reigned supreme in carbohydrate content (86.43%). Carrot paste followed with a substantial amount (70.13%), while red beet paste contained a moderate level (52.38%).

Table 1. Chemical composition (%) of raw materials (on dry weight basis)

Raw materials	Moisture	Fat	Protein	Ash	Fiber	Carbohydrate
Wheat flour 72% extraction	9.54 ± 0.75^{d}	1.17 ± 0.4^{d}	11.5 ± 0.4^{b}	0.51 ± 0.8^{c}	0.39 ± 1.0^{d}	86.43 ± 2.7^{a}
Red beet paste	86.50 ± 0.22^{c}	1.70 ± 0.3^{c}	11.85 ± 0.3^{b}	13.33 ± 0.6^{b}	20.74 ± 2.4^{a}	52.38 ± 3.6^{c}
Spinach paste	90.48 ± 0.23^{a}	3.99 ± 1.3^{a}	26.68 ± 1.5^{a}	19.96 ± 2.5^{a}	9.45 ± 1.6^{b}	39.92 ± 2.2^{d}
Carrot paste	87.40 ± 0.10^{b}	1.85 ± 0.1^{b}	7.18 ± 01.3^{c}	13.12 ± 0.5^{b}	7.72 ± 0.9^{c}	70.13 ± 1.5^{b}

Values are mean of three replicates ± SD, number in the same column followed by the same letter is not significantly different at 0.05 level. Carbohydrate was calculated by difference.

Interestingly, spinach paste had the lowest carbohydrate content (39.92%) among all the ingredients. Table 1 showed the significant differences in chemical composition between wheat flour (72% extraction) and the red beet, spinach, and carrot pastes. The pastes generally contain higher levels of moisture, fat, protein, ash, and fiber compared to wheat flour. This translates to a more complementary nutritional profile when these pastes are incorporated into toast. These results are in agreement with the results of Kale et al. (2018) where they reported that the moisture, fat, protein, ash, fiber, and carbohydrates of red beets are 87.4%, 2.3%, 10.7%, 11.7%, 15.1% and 60.2%, respectively (on dry weight basis). These results can be compared with the results of Shehatta et al. (2024) who showed that the chemical composition of Egyptian red beets was 84.95% for moisture, 10.8 % for fat, 9.9% for protein, 9.9% for ash, 16.6% for fiber, and 52.8% for carbohydrates (on dry weight basis). Also, these results are in agreement with the results of Arscott and Tanumihardj (2010) who reported that the moisture, fat, protein, ash, fiber, and carbohydrate contents of spinach are 89.2%, 3.7%, 26.9%, 15.7%, 20.4%, and 33.3%, respectively (on dry weight basis). While, carrots contained 85.4%, 1.4%, 6.8%, 6.8%, 19.2%, and 65.8%, respectively (on dry weight basis). Meanwhile, Kavitha and Ramadas (2013) found that fat, protein, ash, fiber, and carbohydrate contents of spinach based on dry weight were 3.99%, 26.68%, 19.96%, 9.45%, and 39.92%, respectively.

Minerals contents of raw material

Maintaining vital functions and good health is closely related to the presence and availability of minerals. Deficiencies can contribute to various health problems, including Alzheimer's disease, high blood pressure, and heart-related conditions (Hoffman, 2017). The results presented in Table 2 showed the mineral contents of the raw materials (wheat flour 72% extraction and the pastes (red beet, spinach, or carrot)). In the present study, the spinach paste recorded high contents of all the determined minerals (except P and Cu) compared to other samples. Wheat flour 72% extraction showed the lowest contents of Ca, Fe, P, and Na, while it showed the highest contents of Cu (0.4mg/100g). Carrot paste showed the highest contents of P (533mg/100g), followed by spinach paste (490 mg/100g), and red beet paste contained 340 mg/100g. Kale et al. (2018) found that the mineral contents of beetroot were iron 0.75, copper 0.09, sodium 72.58 and zinc 0.21 mg/100g. Sharma et al. (2012) reported that carrots are a good source of carbohydrates and minerals like Ca, P, Fe, and Mg. These results are in agreement with the results of Arscott and Tanumihardj (2010) who reported that the calcium, iron and sodium contents of spinach and carrot were 99, 2.7 and 79mg/100g, respectively and 33, 0.3 and 69mg/100g, respectively. Schlering et al. (2020) found that the calcium and iron content of spinach were 88.34 mg/100g and 2.16mg/100g, respectively. Jyoti and Jood (2020) and El-Sayed (2020) reported that the dehydrated spinach powder has the potential to serve as a valuable source of calcium, iron, and fiber in the diet of the population in developing countries.

Table 2. Minerals content (mg/100g) of raw materials (on dry weight basis)

Raw materials	Ca	Fe	Zn	P	Na	Cu	Se
Wheat flour 72% extraction	14±2.95^{d}	1.2±0.21^{d}	0.45±0.02^{b}	100±4.21^{d}	3 ±3.61^{d}	0.4±0.01^{a}	0.1±0.02^{c}
Red beet paste	27±3.20^{c}	1.8±0.14^{c}	0.35±0.04^{c}	340±5.84^{c}	53±3.44^{b}	0.11±0.02^{b}	0.2±0.03^{b}
Spinach paste	97±3.70^{a}	4.7±0.23^{a}	0.53±0.01^{a}	490±3.01^{b}	89±2.10^{a}	0.13±0.02^{b}	1.0± 0.02^{a}
Carrot paste	50±2.45^{b}	2.2±0.11^{b}	0.2±0.03^{d}	533±2.80^{a}	40±4.50^{c}	0.02±0.01^{c}	0.1±0.02^{c}

Values are mean of three replicates ± SD, number in the same column followed by the same letter is not significantly different at 0.05 level.

Phenolic and flavonoid compounds content of raw materials and its antioxidant activity

The results presented in Table 3 showed the total phenolic and flavonoid contents of the raw materials (wheat flour 72% extraction and the pastes (red beet, spinach, or carrot)). The analysis revealed interesting results regarding the presence of health-promoting compounds in the toast ingredients. Red beet paste emerged as the champion in terms of phenolic compounds, boasting the highest concentration at 200mg GAE/100g. In contrast, carrot paste took the crown for flavonoid content, containing the most significant amount (170mg Quercetin/100g) compared to the other samples. Notably, all the pastes (red beet, spinach, and carrot) surpassed wheat flour (72% extraction) in their content of both phenolic and flavonoid compounds. These results are in agreement with the results of Ninfali et al. (2005) who reported that the total phenolic compounds of raw beetroot, spinach and carrot were 154.1, 89.4 and 14.6 mg CAE/100g, respectively and Schlering et al. (2020) who found that the total phenols and flavonoids content of spinach were 72.95 and 67.93 mg/100g, respectively. Koubaier et al. (2014) found red beet (*B. vulgaris* L.) extract had appreciable amounts of phenolic compounds (1350 mg GAE/100g). This increment may be due to the difference in the type of sample or method of preparation or other environmental or genetic factors, consistent with Ganjewala et al. (2009) who decided, that the composition is influenced by different origins, environmental, and seasonal factors. Ahmad et al. (2020) found that incorporating spinach in chicken sausages increased the total flavonoid content. There is a close relationship between the levels of color pigments and phenolic compounds (Pietta, 2000 and Elham et al., 2006). The antioxidant activity of the raw materials (wheat flour 72% extraction and the pastes (red beet, spinach, or carrot) can be attributed to the presence of polyphenols and flavonoids. It was found that red beet paste exhibited the highest antioxidant potential (90.35%), followed by spinach paste (80.25%), carrot paste (75.95%), then wheat flour, 72% extraction (47%). It can also be noted that these results are consistent with the percentages of phenolic compounds present in the same pastes (red beet, spinach, or carrot). The significant decrease in antioxidant activity of wheat flour 72% extraction (47%) may be due to the extremely low content of phenolic compounds. These results were consistent with Koubaier et al. (2014) who reported that red beetroot extract can greatly eliminate DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radicals. The crude betalain extract from red beetroot showed highest DPPH radical scavenging activity of 97%. Also, they confirm a direct relationship between the degree of coloration (increasing the concentration of pigments) and increasing of the efficiency of antioxidants. Kaliyaperumal and Radhika (2020) reported that the *Spinach oleraceae* contained many nutrients and some bioactive compounds which makes it a good leafy vegetable and has an antioxidant value that can be used as a therapeutic drug. Ahmad et al. (2020) found that sausage made by adding spinach or carrots to the sausage formula showed high antioxidant properties.

This is due to the presence of phenolic and flavonoid compounds in a high percentage of the added vegetables. The extent of antioxidant activity depends on the levels of color pigments (Halliwell et al., 2005). Khan et al. (2021) and Fatima (2024) reported that the polyphenols and flavonoids play an important role in protecting against cardiovascular diseases (CVDs), due to their antioxidant, anti-atherosclerotic, and anticoagulant effects. They also noted that consuming 100 mg of flavonoids daily through the diet may reduce the risk of morbidity and mortality from coronary heart disease (CHD) by approximately 10%.

Table 3. Phenolic and flavonoid compounds content of raw material and its antioxidant activity

Raw materials	Phenolic compounds (mg GAE\100g)	Flavonoid compounds (mg Quercetin \100g)	Antioxidant activity(%)
Wheat flour72% extraction	0.90	1.7	47
Red beet paste	200	33	90.35
Spinach paste	88	28	80.25
Carrot paste	18	170	75.95

Identification of phenolic compounds of raw materials

The presented results in Table 4 showed the phenolic compounds of red beet, spinach, or carrot pastes. From these findings, it was possible to see that the red beet paste sample included 11 phenolic compounds, whereas the spinach and carrot paste samples contained 12 compounds. The main phenolic component present in red beet paste and carrot paste was found to be pyrogallol at concentrations of 170.34 and 172.5 µg/g, respectively, whereas ellagic acid and pyrogallol were the main components in the spinach paste with concentrations of 273.05 and 129.95 µg/g, respectively. From the results in the same Table, it was found that the concentrations of the rest of the phenolic compounds other than pyrogallol in the red beet paste (10 compounds) did not exceed 6 µg/g. Whereas for spinach paste, it was found that the concentration of the rest of the phenolic compounds other than ellagic acid and pyrogallol (5 compounds) did not exceed nine µg/g, and the concentration of the other 5 compounds ranged from 33.81 µg/g (catechin) to 10 µg/g (Ferulic acid). While the rest of the phenolic compounds other than pyrogallol in the carrot paste (9 compounds) did not exceed 4.5 µg/g and two compounds showed concentrations of 13 and 10.69 µg/g (catechol and ellagic acid, respectively). The results of the phenolic compound content of red beets are almost consistent with the results of Shehata et al. (2024). Ahmad et al. (2020) reported that sausages containing spinach or carrots (at a ratio of 40 vegetables to 60 chicken meat) recorded 63.82% or 45.28% DPPH, respectively. The high DPPH values suggest a strong presence of antioxidant compounds in both the pastes and (presumably) the toast samples. These antioxidants have the ability to neutralize free radicals, which are unstable molecules that can damage fats and contribute to various health issues. Phenolic compounds, particularly abundant in the pastes, play a crucial role in safeguarding the digestive system. They help prevent damage caused by reactions within the stomach and intestines, potentially improving gut health (Halliwell, 2007). Notably, red beet paste likely contains gallic acid, a potent antioxidant with minimal toxicity. This quality makes it a desirable additive in processed foods, as it effectively inhibits the formation of harmful peroxides (Queiroz et al., 2019).

Identification of flavonoid compounds of raw materials

The results of the identification of flavonoid compounds in the pastes of red beet, spinach, and carrot are shown in Table 5. The results presented in Table 5 indicated that the paste of spinach had a high level of quercetin (5235.76 µg/g), followed by rutin (2607.40µg/g), rosmarinic (2044.70µg/g), naringenin (1393.50µg/g), apigenin-7-glucose (1302.03µg/g), and quercetrin (1017.03µg/g) compared to the pastes of red beet or carrot.

The concentration of the flavonoid compounds in the red beet paste ranged between 2.84µg/g for apigenin and 81.59 µg/g for quercetrin. The highest concentration of flavonoid compounds in the carrot paste was quercetin (122.91µg/g), and apigenin showed the lowest value (1.74µg/g). These results are consistent with the results of Shehata et al. (2024). The toast ingredients might also benefit from the synergistic effect of specific flavonoid compounds present. Catechin and quercetin, for example, can work together to boost overall antioxidant activity. However, the link between these flavonoids and the synthesis of dextran molecules is not entirely clear. Dextrans are complex sugars produced by bacteria, and their connection to the direct effects of flavonoids needs further exploration. Also, it was found that the presence of dextran and gallic acid is more efficient as an antioxidant agent (Queiroz et al., 2019). Sharma (2006) confirmed that flavonoids have many health benefits for humans, as they play a necessary role antioxidants and scavenge of free radicals. They work to improve blood circulation, especially for people who have Alzheimer's disease. They also showed an anti-cancer, anti-tumor, and anti-microbial role.

Table 4. Identification of phenolic compounds of raw materials (µg/g)

Phenolic compounds	Red beet paste	Spinach paste	Carrot paste
Pyrogallol	170.34	129.95	172.5
Gallic acid	0.90	0.93	0.97
Catechol	ND	10.75	13.0
4-Amino-benzoic acid	0.47	ND	0.56
Catechein	5.90	33.81	ND
Chlorogenic acid	ND	15.92	4.40
P-OH-benzoic acid	3.55	5.81	1.18
Caffeic acid	0.78	8.39	1.58
Vanillic acid	1.30	6.88	1.42
Caffeine	2.03	13.11	3.03
Ferulic acid	1.50	10.0	1.70
Ellagic acid	4.56	273.05	10.69
Coumarin	0.27	2.68	0.43

ND: Not detected

Table 5. Identification of flavonoid compounds of raw materials (µg/g)

Flavonoid compounds	Red beet paste	Spinach paste	Carrot paste
Rutin	49.35	2607.40	119.7
Rosmarinic	13.97	2044.70	24.89
Quercetrin	81.59	1017.03	54.28
Apigenin-7-glucose	71.01	1302.03	54.67
Naringenin	26.26	1393.50	42.21
Kaempferol	10.20	ND	8.25
Quercetin	ND	5235.76	122.91
Apigenin	2.84	ND	1.74

ND: Not detected

Pigments content of raw materials

The total pigment contents of the pastes of red beet, spinach, and carrot are shown in Table 6. From these results, it could be observed that the carrot paste had the lowest pigment content (75 mg/100g) of total carotenoid, while the red beet paste had the highest betalain pigment content (450 mg/100g).Whereas the spinach paste contained a moderate amount of chlorophyll pigment (120 mg/100g). Koubaier et al. (2014) reported higher pigment content in beetroots, with values around 53 mg betanin equivalent per gram of extract and 46 mg vulgaxanthin equivalent per gram of extract. This difference in pigment content compared to other studies might be attributed to the estimation method used. Koubaier et al. (2014) employed High -Performance Liquid Chromatography (HPLC), which identified three pigment fractions: one at 448 nm and two at 538 nm. In contrast, this present research utilizes a spectrophotometric method at a single wavelength (535 nm).

Also, Shehata et al. (2024) found that the contents of betaxathin and betacyanin (betalain pigments) of the red beetroot (*Beta vulgaris*) were 8.27 and 4.63 mg/g, respectively, and decreased by about 83.22% and 88.09%, respectively as a result of the drying method at 60 °C. Rekha et al. (2013) found that adding red beets, spinach, and carrots to pasta dough at rates of up to 15 %, 20 %, and 42 % on a fresh weight basis, respectively, resulted in the retention of significant levels of the pigments betalain, chlorophyll, and carotene, which have nutritious and beneficial properties for human health, in addition to the attractive coloring of pasta produced in natural colors. EL-Qudah (2009) reported that raw carrots contain large amounts of a variety of carotenoids, which have an important role in human health. Carrots contain α-carotene and beta-carotene in proportions of 47.1 and 47.5 µg/g, respectively.

Table 6. Total pigments of raw materials (mg/100g)

Raw materials	Total carotenoid	Total chlorophyll	Total betalain
Red beet paste	---	---	450
Spinach paste	---	120	---
Carrot paste	75	---	---

The antimicrobial activity of the raw materials

The antimicrobial activity of the red beet, spinach and carrot was studied against Gram-positive (*Staphylococcus aureus,* and *Bacillus cereus*) and Gram-negative (*Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli*) bacteria, mold filamentous food–borne fungus (*Aspergillus niger*) and yeast (*Candida albicans*). The antimicrobial activity was assessed by evaluating the inhibition zone (mm) and the obtained results are shown in Table 7. It could be observed that the red beet, spinach and carrot samples showed varying degrees of antimicrobial activity against all strains tested. The inhibition zones were in the range of 8 – 12 mm.

Table 7. The antimicrobial activity of the raw materials

Raw materials	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Aspergillus niger*	*Candida albicans*
	Inhibition zone (mm)						
Red beet	10	ND	8	10	ND	10	10
Spinach	9	ND	8	11	ND	10	9
Carrot	11	10	9	11	ND	11	12

ND: Not detected

From the obtained results, it could be observed that carrot in general showed slightly increase in the inhibition zones for all the used microorganisms compared to red beet or spinach with exception of *Bacillus cereas* which showed similar inhibition zone. Spinach sample showed slightly lower inhibition zones for *Psudomonas aeruginosa* and *Candida albicans* compared to other two raw materials and it showed similar inhibition zones of that of red beet for *Staphylococcus* and *Aspergillus niger*. This study aligns with previous research by Ahmad et al. (2020). Their findings demonstrated that incorporating vegetables like spinach or carrots into chicken sausage (at a 40:60 vegetable-to-meat ratio) reduced the total microbial count compared to control sausages without vegetables. While the exact mechanisms need further investigation, the presence of these vegetables might contribute to this effect. Another relevant study by Oyetayo and Ariyo (2013) explored the relationship between phenolic compounds and microbial growth in mushrooms. They observed variations in the total phenolic content of mushroom extracts (ranging from 0.1µg/g to 2.63µg/g) over a storage period. Interestingly, they found a correlation between higher phenolic content and a stronger inhibition of microbial growth. This inhibition was measured by the size of the inhibition zone, which ranged from 5.33mm to

20.33mm. Koubaier et al. (2014) confirm a direct relationship between the degree of coloration (increasing the concentration of pigments) and increasing resistance to pathogens. Phenolic compounds have antimicrobial activity. The reason for the lack of growth of microorganisms may be due to the role played by these phenolic compounds in decomposing cell membranes and their ability to prevent the synthesis of proteins, in addition to the lack of synthesis of enzymes that degrade proteins (Cowan, 1999).

The cytotoxicity of the red beet, spinach, and carrot on the Caco-2 human cell types

The red beet, spinach, and carrot were designated *in vitro* for their cytotoxicity activity on the Caco -2 human cancer cell lines through the employment of the MTT assay. The percentage of viable cells and the IC_{50} value were measured and the obtained results are shown in Table 8. These results indicated that as the concentration of raw materials (red beet, spinach, or carrot) increased the viability percentages decreased, while toxicity activity percentages were increased. Decreasing of the red beet, spinach, and carrot concentrations from 1000 µg/ml to 31.25 µg/ml led to an increase the viability percentage from 14% to 99.5%, from 32.75% to 99.9%, and from 48.5% to 99.61%, respectively. While the toxicity activity percentages decreased from 86.2% to 0.55%, from 67.2% to 0.11%, and from 51.9% to 0.38%, respectively. As indicated in Table 8 it was found that the maximum percentage of toxicity (86.2, 67.2, and 51.9%) was observed with the maximum concentration (1000 µg/ml) of the used raw material, respectively. Moreover, the IC_{50} [the concentration causing death of 50% of the human tumor cell line (Caco-2 human cancer cell lines)] was found to equal 433.05, 830.04, and 960.13µg/ml for the used raw material, respectively. From these results, it could be concluded that the strength of the cytotoxic effect of red beets, spinach, and carrots on the Caco-2 human cancer cell lines was in this order: red beets, followed by spinach, then carrots. Koubaier et al. (2014) confirm a direct relationship between the degree of coloration (increasing the concentration of pigments) and improving viral defense.

Table 8. The cytotoxic IC_{50} values of the red beet, spinach and carrot according to the MTT assay on the Caco-2 human cell types

Raw materials	Raw material conc. (µg/ml)	Viability (%)	Toxicity (%)	IC_{50} (µg/ml)
Red beet	1000	14	86.0	433.05
	500	36.2	63.8	
	250	88	12.0	
	125	96.9	3.1	
	62.5	99.38	0.62	
	31.25	99.5	0.5	
Spinach	1000	32.75	67.25	830.04
	500	85.7	14.3	
	250	98.53	1.47	
	125	99.5	0.5	
	62.5	99.7	0.3	
	31.25	99.9	0.1	
Carrot	1000	48.5	51.5	960.13
	500	94.0	6.0	
	250	98.4	1.6	
	125	98.4	1.6	
	62.5	99.9	0.1	
	31.25	99.61	0.39	

IC_{50}: Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs.

Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on chemical composition of dry toast slices (dry weight bases)

The dry toast slices (control and fortified with a paste of each of RBP (red beet), SP (spinach), or CP (carrot)) at levels of 30% of each were analyzed for their moisture, fat, protein, ash, fiber and carbohydrate contents and the obtained results are shown in Table 9. The results presented in Table 9 indicated that there were significant differences ($P \leq 0.05$) between samples of the dry toast slices (control and fortified with paste of red beet, spinach, or carrot) in moisture, fat, protein, ash, fiber and carbohydrate contents, with the exception of samples of dry toast slices fortified with red beet and the slices fortified with carrot for fat content, dry toast slices fortified with spinach and the slices fortified with carrot for protein content, dry toast slices fortified with red beet and the slices fortified with carrot for fiber content and dry toast slices fortified with red beet and the slices fortified with spinach for carbohydrate content. The moisture content ranged from 7.98% for the dry toast slices fortified with carrot paste to 9.20% for the dry toast slices fortified with spinach paste. The dry toast slices fortified with red beet paste had a higher content of both protein (10.3%) and ash (3.7%) when compared to the dry toast slices fortified with either spinach (9.23 and 2.6 %, respectively) or carrots (9.55 and 1.9 %, respectively). Whereas, the dry toast slices fortified with spinach paste had a higher content of both fat (8.18%) and fiber (1.18%) when compared to the dry toast slices fortified with either red beet (6.48 and 0.88 %, respectively) or carrots (6.30 and 0.9%, respectively). Also, the dry toast slices fortified with carrot paste had a higher content of carbohydrate (73.37%) when compared to the dry toast slices fortified with either red beet (69.48%) or spinach (69.61%). Rekha et al. (2013) found that adding red beet, spinach, and carrot to pasta dough at rates of up to 15 %, 20 %, and 42 % on a fresh weight basis, respectively, did not improve the protein content and crude fiber content with the addition of vegetable puree. Ingle et al. (2017) improved the nutritional qualities of cookies with the incorporation of different levels of beetroot powder by up to 20%. They found that the proximate composition of cookies enriched with beetroot powder up to 20% led to an increase protein content from 7.39 to 9.12 %, crude fiber from 0.95 to 1.90 %, and ash from 0.93 to 1.89%.

Table 9. Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on chemical composition (%) of dry toast slices (on dry weight basis)

Samples	Moisture	Fat	Protein	Ash	Fiber	carbohydrate
Control	8.11 ± 0.10^c	9.68 ± 0.2^a	11.15 ± 0.12^a	0.75 ± 0.12^d	0.47 ± 0.05^c	77.95 ± 1.32^a
RBP	8.86 ± 0.20^b	6.48 ± 0.1^c	10.30 ± 0.22^b	3.70 ± 0.23^a	0.88 ± 0.02^b	69.48 ± 0.02^c
SP	9.20 ± 0.12^a	8.18 ± 0.3^b	9.23 ± 0.30^c	2.60 ± 0.11^b	1.18 ± 0.01^a	69.61 ± 0.02^c
CP	7.98 ± 0.20^d	6.30 ± 0.2^c	9.55 ± 0.44^c	1.9 ± 0.10^c	0.90 ± 0.03^b	73.37 ± 2.55^b

- Control: Toast made from wheat flour 72% extraction.
- RBP, SP and CP represent toast samples made from wheat flour 72% extraction that fortified by 30% of each of paste of red beet, spinach and carrot, respectively.
- Carbohydrates were calculated by difference. Values are mean of three replicates ± SD, number in the same column followed by the same letter is not significantly different at 0.05 level.

Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on minerals content of dry toast slices (dry weight bases)

The effect of fortification of wheat flour 72% extraction with pastes of each of red beet, spinach or carrot at levels of 30% of each on mineral contents of the produced RBP (red beet paste), SP (spinach paste), or CP (carrot paste) toast was studied and the obtained results are shown in Table 10.

Table 10. Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on minerals content (mg/100g) of dry toast slices (dry weight bases)

Samples	Ca	Fe	Zn	P	Na	Cu	Se
Control	10±3.74^{d}	0.38±0.20^{d}	0.07±0.03^{c}	52±5.23^{d}	10±2.40^{d}	0.02±0.03^{c}	0.04±0.01^{b}
RBP	30±2.90^{c}	0.98±0.10^{c}	0.22±0.01^{b}	106±4.32^{c}	20±4.55^{c}	0.09±0.01^{a}	0.05±0.01^{a}
SP	59±3.74^{b}	1.20±0.12^{a}	0.30±0.02^{a}	190±2.30^{a}	43±2.90^{a}	0.08±0.02^{a}	0.04±0.02^{b}
CP	64±2.50^{a}	1.07±0.01^{b}	0.23±0.01^{b}	118±3.84^{b}	29±3.51^{b}	0.07±0.01^{b}	0.01±0.02^{c}

- Values are mean of three replicates ± SD, number in the same column followed by the same letter is not significantly different at 0.05 level.
- Control: Toast made from wheat flour 72% extraction.
- RBP, SP and CP represent toast samples made from wheat flour 72% extraction that replaced by 30% of each of paste of red beet, spinach and carrot, respectively.

From the results presented in Table 10 it could be noticed that all the determined minerals were increased due to the fortification process by the three pastes (RBP, SP, and CP) compared to control sample except for Se. The results also indicated that fortification by carrot paste lead to an increase Ca content by about 540% followed by 490% with fortification by spinach paste and 200% with fortification by red beet paste in comparison with control sample. The highest increase in Fe content was observed with fortification by spinach paste (215.78%) followed by fortification with carrot paste (181.57%) and then fortification by red beet paste (157.89%) compared to control sample. However, fortification of wheat flour 72% extraction by spinach paste showed the highest increase percentages for Zn, P and Na being 328.57%, 265.38% and 330%, respectively, while the lowest increase percentages for the same minerals were observed with fortification by red beet paste which were 214.28%, 103.84% and 100%, respectively compared to control sample. Fortification with carrot paste showed an increase in the percentages of Zn, P and Na which were 228.57%, 126.92% and 190%, respectively compared to control sample. Cu content also increased due to the fortification process and the percentages of increase were 350%, 300% and 250% for fortification by paste of each with red beet, spinach, or carrot, respectively compared to control sample. However, fortification by red beet paste showed a slight increase in Se content (25%), while fortification by carrot paste led to decrease Se content by 75% compared to control sample. No change in Se content was observed with fortification by spinach paste. The results of the statistical analysis showed that Ca, Fe, P and Na contents of all samples were significantly different. Zn contents of the dry toast slices fortified with red beet paste or carrot paste samples were not significantly different. The same trend was also observed for Cu contents of the dry toast slices fortified with red beet paste or spinach paste samples, and for Se contents of control and fortification by spinach paste samples. Awuchi et al. (2020) reported that micronutrients (vitamins and minerals) have numerous health benefits including tissue maintenance, bone and teeth formation and health, serving as cofactors and coenzymes to enzyme of various enzyme systems, aiding the regulation and coordination of most body functions, and other biochemical and physiological functions in the body. Dixit et al. (2023) reported that most minerals play important roles in the growth and regulation of various physiological processes such as muscle contraction, oxygen transport, and maintenance of osmotic pressure, bones, and tissues. Few minerals help in the absorption of nutrients, the metabolism of carbohydrates, protein, and fat, and the usage of nutrients by the body cells.

Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on physical properties of dry toast slices

The effect of fortification of wheat flour 72% extraction by paste of red beet, spinach or carrot at levels of 30% of each on physical properties of dry toast was studied and the obtained results are shown in Table 11.

Table 11. Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on physical properties of dry toast slices

Samples	Diameter (cm)	Volume (Cm^3)	Specific volume	Weight (g)
Control	7.5 ± 0.01^a	30 ± 0.2^d	1.4 ± 0.3^c	6.2 ± 0.1^c
RBP	7.4 ± 0.03^a	35 ± 0.1^b	1.8 ± 0.1^a	6.9 ± 0.3^a
SP	7.4 ± 0.03^a	33 ± 0.3^c	1.5 ± 0.2^b	6.5 ± 0.2^b
CP	7.3 ± 0.02^a	36 ± 0.5^a	1.4 ± 0.3^c	6.8 ± 0.2^a

- Values are mean of three replicates ± SD, number in the same column followed by the same letter is not significantly different at 0.05 level.
- Control: Toast made from wheat flour 72% extraction.
- RBP, SP and CP represent toast samples made from wheat flour 72% extraction that replaced by 30% of each of paste of red beet, spinach and carrot, respectively.

The obtained results indicated that diameters values of all the produced toast samples were approximately the same and were not significantly different. The highest volume value was observed for toast sample that produced from wheat flour fortified by carrot paste ($36cm^3$) followed by that fortified with red beet paste ($35cm^3$) then that fortified by spinach paste ($33cm^3$), while control sample showed the lowest volume value ($30cm^3$) and all were significantly different from control and in between. The samples that fortified by red beet paste or carrot paste showed slightly high values for weight (6.9 and 6.8g, respectively) and were not significantly different in between. However, the sample that fortified by spinach paste showed weight value of 6.5 g and control sample showed the lowest weight value (6.2g). This might be due to the high fiber content of the used pastes which bind more water compared to wheat flour 72% extraction. Zaki et al. (2018) reported that water absorption increased as oat flour level increased. This increase is due to the high fiber content of oat flour, which characterized by its high water-holding capacity. The same results also indicated that control sample and fortification by carrot paste showed the same specific volume value being 1.4 for each, while fortification by red beet paste showed the highest specific volume value (1.8) and fortification by spinach paste showed specific volume value of 1.5. Both samples were significantly different in between and from control and the fortified with carrot paste samples. According to Mahmoud et al. (2023) incorporating whole oat flour and roasted flaxseed flour into biscuit dough increase the specific volume, which is a key indicator of biscuit quality. However, the presence of roasted flaxseed flour also leads to a gradual decrease in weight as the substitution level increases. This weight reduction can be attributed to the addition of fiber from the flaxseed flour. Fiber disrupts gluten network formation by diluting gluten proteins and interacting with them.

Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on color parameters of dry toast slices

The color parameters (*L**, *a** and *b**) of toast containing 30% red beet paste, spinach paste, or carrot paste were determined. The results are presented in Table 12. Lightness (*L**) values were highest in the control sample (69.29), indicating a lighter color compared to the three treatments with added vegetables. However, the control sample had lower *a** values (reflecting less green) and *b** values (reflecting less blue) compared to the vegetable-fortified toast. The same results also indicated that fortification by carrot paste resulted in high values for *L** and *b** parameters and low values for *a** parameter in comparison with other two treatments. The lowest values for *L** and *b** parameters were observed with fortification by red beet paste compared to other two treatments. Moreover, the highest *a** values were observed with fortification by spinach paste which reflect more green color compared to other samples and this might be due to its high content of green pigment (chlorophyll).

The higher b^* values which observed with fortification by carrot paste might be due to its high content of yellow pigments (carotenoids). These results are in agreement with Ingle et al. (2017) who reported that the incorporation of beetroot powder in cookies lowered the lightness (L^*) indicating that lightness decreased with the reduction in the proportion of wheat flour because of the loss of the white color of the flour, but it increased redness (a^*) of cookies.

Table 12. Effect of fortification of wheat flour 72% extraction by pastes of each of red beet, spinach

Samples	L^*	a^*	b^*
Control	69.29	2.51	16.76
RBP	46	5	19
SP	47	15	22
CP	56	7	35

-L^* (lightness with L^* = 100 for lightness, and L^* = zero for darkness), a^* [(chromaticity on a^* green (−) to red (+)], b^* [(chromaticity on a blue (−) to yell low (+)].
-Control: Toast made from wheat flour 72% extraction.
-RBP, SP and CP represent toast samples made from wheat flour 72% extraction that replaced by 30% of each of paste of red beet, spinach and carrot, respectively.

Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on sensory characteristics of dry toast slices

Dried toast samples fortified using the paste of each red beet, spinach, or carrot paste at a level of 30% were evaluated for sensory characteristics: crust color, crumb color, texture, odor, taste, appearance, and overall acceptability as shown in Table 13.

Table 13. Effect of fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot on sensory characteristics of dry toast slices

Samples	Crust color (20)	Crumb color (20)	Texture (20)	Odor (20)	Taste (20)	Appearance (20)	Overall Acceptability (20)
Control	19.0±0.03^{b}	19.0±0.03^{c}	19.0±0.20^{a}	19.6±0.01^{b}	19.4±0.02^{b}	19.2±0.02^{b}	19.1±0.01^{b}
RBP	19.6±0.01^{a}	19.4±0.04^{b}	19.0±0.12^{a}	19.8±0.03^{a}	19.7±0.03^{a}	19.8±0.04^{a}	19.5±0.01^{a}
SP	18.9±0.02^{a}	19.2±0.01^{b}	19.0±0.21^{a}	19.4±0.03^{b}	19.5±0.02^{a}	19.6±0.02^{b}	19.2±0.03^{a}
CP	19.8±0.02^{a}	19.6±0.01^{a}	19.0±0.11^{a}	19.8±0.02^{a}	19.8±0.01^{a}	19.8±0.04^{a}	19.6±0.02^{a}

-Values are mean of three replicates ± SD, number in the same column followed by the same letter is not significantly different at 0.05 level.
-Control: Toast made from wheat flour 72% extraction.
- RBP, SP and CP represent toast samples made from wheat flour 72% extraction that replaced by 30% of each of paste of red beet, spinach and carrot, respectively.

The sensory scores for all evaluated characteristics (crust color, crumb color, texture, odor, taste, appearance, and overall acceptability) were generally similar for the three fortified toast samples. These scores were also slightly higher compared to the control sample. The crust color of the three vegetable-fortified toasts (red beet, spinach, and carrot) showed no significant differences between them. However, all three differed significantly from the control (unfortified) toast. This same trend held true for taste and overall acceptability scores, where there were no significant differences among the vegetable additions compared to a significant difference from the control. Texture scores revealed no significant differences between the control and any of the vegetable-fortified samples. The crumb color followed a similar pattern. Neither red beet nor spinach paste additions resulted in significant color differences compared to each other, but both differed significantly from the control and the carrot paste samples.

Odor scores mirrored this trend as well. Red beet and carrot paste additions showed no significant difference in odor, but both differed significantly from the control and the spinach paste samples. The same trend was observed for odor and appearance, however, these results revealed that fortification of wheat flour 72% extraction by paste of each of red beet, spinach or carrot at a replacement level of 30% led to the production toast having higher scores for all the sensory characteristics than the control sample and these products also had more nutritional value compared to the control sample. Ingle et al. (2017) concluded that the cookies prepared with the addition of 10% beetroot powder were more acceptable as compared to other treatments. The color and appearance of cookies are a function of reducing sugars, as these reducing sugars during baking caramelize to produce the brown color of cookies. The light brown color of the cookies was achieved with 10% beetroot powder.

4. Conclusion

This study investigated that red beet, spinach, or carrot pastes contain essential nutrients and high mineral content (P, Na, Ca, and Fe). These pastes have many important active components such as polyphenols and flavonoids. The study also showed that these pastes could scavenge free radicals with high efficiency, which indicates that these pastes have excellent properties as antioxidants. By measuring the ability of the paste of red beet, spinach, or carrot on the toxicity of the human tumor cell line (Caco-2 human cancer cell lines), it is clear that it can produce a lethal effect on cancer cells. It also has a great ability to inhibit many pathogenic microbes. Also, the results indicated the possibility of producing dry toast slices with high acceptance and attractive colors. The study also recommends that the use of natural food colors in dried food products (such as slices of dry toast) contributes to reducing the waste of fresh food raw materials that may occur due to spoilage during growth, harvesting, storage, manufacturing, distribution, and preparation for consumption while maintaining the quality of the products and public health, as well as reducing microbial spoilage of fresh raw materials. It is also nutritional and health-wise attractive to many categories of consumers, especially children, hotel and resort guests, and on special occasions. It also meets some profitable nutritional and economic needs, thus preserving the product with an attractive color for a longer period than it remained fresh.

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References

AACC (2010). Approved Methods of the AACC. (10th Ed.). American Association of Cereal Chemists, Paul, Minnesota, USA. 2010.

Ahmad, S., Jafarzadeh, S., Ariffin, F. and Zainul Abidin, S. (2020). Evaluation of Physicochemical, Antioxidant and Antimicrobial Properties of Chicken Sausage Incorporated with different Vegetables. Ital. J. Food Sci., 32: 75-90

AOAC. (2016). Association of Official Analytical Chemists international Official Methods of Analysis 20th ed. Washington, DC, USA.

Arscott, S.A. and Tanumihardj, S.A. (2010). Carrots of Many Colors Provide Basic Nutrition and Bioavailable Phytochemicals Acting as a Functional Food. Comprehensive reviews in food science and food safety, 9(2):223-239

Atalar, I., Konar, N., Dalabasmaz, S., Kantemur, P. S. and Han, E. (2024). Drying and characterization of red beet color liquid process waste to develop a novel bulking agent. Journal of Cleaner Production, 442: 141030

Awuchi, C.G., Igwe, V.S., Amagwula, I.O. and Echeta, C. (2020). Health Benefits of Micronutrients (Vitamins and Minerals) and their Associated Deficiency Diseases: A Systematic Review. International Journal of Food Sciences, 3 (1):1-32.

Baião, D.S., da Silva, D.V.T., and Paschoalin, V. M. F. (2020). Beetroot, A Remarkable Vegetable: Its Nitrate and Phytochemical Contents Can be Adjusted in Novel Formulations to

Benefit Health and Support Cardiovascular Disease Therapies. Antioxidants, 9(10), 960 https://doi.org/10.3390/antiox9100960

Castellar, R., Obon, J., Alacid, M. and Fernández-López, J. (2003). Color Properties and Stability of Betacyanins from Opuntia Fruits. J. of Agricultural and Food Chemistry, 51: .2776-2772 http://dx.doi.org/10.1021/jf021045h

Cosmulescu, S. and Trandafir, I. (2012). Anti-Oxidant activities and total phenolics contents of leaf extracts from 14 cultivars of walnut (*Juglans regia* L.). Journal of Hort. Science and Biotechnology, 87:504-508.

Cosmulescu, S., Trandafir, I., Nour, V. and Botu, M. (2015). Total phenolic, flavonoid distribution and antioxidant capacity in skin, pulp and fruit extracts of plum cultivars. Journal of Food Biochemistry, 39(1): 64-69. Doi.org/10.1111/ jfbc.12112

Cowan, M.M. (1999) Plant Products as Antimicrobial Agents. Clinical Mi-crobiology Reviews. 12(4): 564 – 582.

Dega, V. and Barbhai, M.D. (2023) Exploring the underutilized novel foods and starches for formulation of low glycemic therapeutic foods: a review. Nutrition and Food Science Technology, 10–2023
https://doi.org/10.3389/fnut.2023.1162462

Dixit, V., Kamal, S.W.J., Chole, P.B., Dayal, D., Chaubey, K.K., Pal, A. K., Xavier, J., Manjunath, B.T., and Bachheti, R. K. (2023). Functional Foods: Exploring the Health Benefits of Bioactive Compounds from Plant and Animal Sources. Journal of Food Quality, Volume 2023, Article ID 5546753, 22 pages
https://doi.org/10.1155/2023/5546753

Elham, G, H. Reza, K. Jabbar, S. Parisa and J. Rashid (2006). Isolation and structure characterisation of anthocyanin pigments in black carrot (*Daucus carota* L). Pak J. Biol. Sci., 9(15): 2905-2908.

EL-Qudah, J.M. (2009). Identification and Quantification of Major Carotenoids in Some Vegetables. Am. J. Applied Sci., 6 (3): 492-497.

El-Sayed, S.M. (2020). Use of spinach powder as functional ingredient in the manufacture of UF-Soft cheese. Heliyon, 6: e03278

Fatima, T. (2024). Nutraceutical Potential and Health Benefits of Different Polyphenol-Rich Vegetables Stalk Extracts by Exploring Anti-oxidant Activities. Lat. Am. J. Pharm., 43(1): 225-233

Ganjewala, D., Sam, S. and Khan, K. (2009). Biochemical compositions and antibacterial activities of *Lantana camara* plants with yellow, lavender, red and white flowers. Eur. Asian J. BioSci., 3: 69 – 77.

Gogoi, M. and Basumatary, M. (2018). Estimation of the chlorophyll in seven citrus species of Kokrajhar district, BTAD, Assam, India. An Inter. J. Society for Tropical Plant Res., 5(1): 83- 87. DOI:https://doi.org/10.22271/tpr.

Goupy, P., Hugues, M. and Boivin, P. (1999). Anti-oxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J. Sci. Food Agric., 79: 1625-1634.

Halliwell, B. (2007). Dietary polyphenols: Good, bad, or indifferent for your health? Cardiovascular Research, 73:341–347 .

Halliwell, B., J. Rafter and A. Jenner (2005). Health promotion by flavonoids, tocopherols, tocotrienols and other phenols: direct or indirect effects? Antioxidant or not? Am. J. Clin. Nutr., 81: 268S-276S.

Hoffman, R. (2017). Micronutrient deficiencies in the elderly – could ready meals be part of the solution? J. Nutr. Sci., 6 (2): 1–4.

Ingle, M., Thorat, S.S., Kotecha, P.M., and Nimbalkar, C.A. (2017). Nutritional assessment of beetroot (*Beta vulgaris* L.) powder cookies. Asian J. Dairy & Food Res., 36(3): 222-228.

Jyoti and Jood, S. (2020). Physicochemical and nutritional composition of composite flour enriched with spinach leaves powder for development of value-added baked products. International Journal of Chemical Studies, 8(4):1841-1844.

Kale, R.G., Sawate, A.R., Kshirsagar, R.B., Patil, B.M. and Mane, R.P. (2018). Studies on evaluation of physical and chemical composition of beetroot (*Beta vulgaris* L.). International Journal of Chemical Studies, 6(2): 2977-2979

Kaliyaperumal, S. and Radhika, M. (2020). Study of phytochemical analysis and antioxidant activity of *Spinach oleracea* L plant leaves. The Pharma Innovation Journal, 9(7): 01-04

Kavitha, V. and Ramadas, V.S. (2013). Nutritional composition of raw fresh and shade dried form of spinach leaf (*Spinach oleracea*). An International Journal, 1(8):767-770

Khan, J., Deb, P. K., Priya, S., Medina, K. D., Devi, R., Walode, S. G., and Rudrapal, M. (2021). Dietary flavonoids: Cardioprotective potential with antioxidant effects and their pharmacokinetic, toxicological and therapeutic concerns. Molecules 26(13): 4021.

Kotzekidou, P., Giannakidis, P. and Boulamatsis, A. (2008). Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in chocolate, Swiss Soc. Food Science and Technology, 41:119-127. https://doi.org/10.1016/j. lwt.2007.01.016

Koubaier, H.B.H., Snoussi, A., Essaidi1, I., Chaabouni, M.M., Thonart, P. and Bouzouita, N. (2014). Betalain and Phenolic Compositions, Antioxidant Activity of Tunisian Red Beet (*Beta Vulgaris* L. Conditiva) Roots and Stems Extract. International Journal of Food Properties, 17: 1934–1945.

Liliana, C. and Oana-viorela, N. (2020). Red beetroot: Composition and Health Effects-A Review journal of Nutritional Medicine and Diet Care ISSN; 2572-3278 DOI;10.23937/2572-3278.1510043

Linda, M.P., Deborah, A.M., Gail, B., and Larmond, E. (1991). Laboratory methods for sensory analysis of food. Research Branch Agriculture, Ottawa, Canada, 1-99.

Mahmoud, H.M.A., Hussein, A.M.S., Hegazy, N. A., Ammar, A.S.M., and Riyad, Y.M. (2023). Preparation and Quality Characteristics of Biscuits Fortified with Oat and Flaxseed. Egypt. J. Chem., 66(8): 411 - 423

Mattila, P., Astola, J. and Kumpulainen, J. (2000). Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. J. Agric. Food Chem., 48: 5834-5841.

McGuire, R.G. (1992). Reporting of objective color measurements. HortScience, 27: 1254-1255.

Mosmann, T. (1983) Rapid colorimetric assays for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods.65:55-63.

Nagata, M. and Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaish, 39(10):925 -928. Doi.org/10.3136/nskkk1962.39.925

Ninfali, P., Mea, G., Giorgini, S., Rocchi, M. and Bacchiocca, M. (2005). Antioxidant capacity of vegetables, spices and dressings relevant to nutrition. British Journal of Nutrition, 93:257–266

Oyetayo, V.O., and Ariyo, O.O. (2013). Antimicrobial and Antioxidant Properties of Pleurotus ostreatus (Jacq: Fries) Cultivated on Different Tropical Woody Substrates. Journal of Waste Conversion, Bioproducts and Biotechnology, 1 (2): 28–32.doi: 10.5147/jwcbb.2013.0121

Pavlović, N., Marjanović, M., Mladenović, J., Stevović, V., Petrović, M., Živković, I., and Zdravković, J. (2024). Nutritional quality of organically grown carrot (*Daucus carota* L.) and effect of processing. Journal of Central European Agriculture, 25(1): 223-233

Pietta, P. G. (2000). Flavonoids as antioxidants. J. Nat. Prod., 63(7): 1035-1042.

Queiroz, M.F.; Sabry, D.A.; Sassaki, G.L.; Rocha, H. A. O. and Costa, L. S. (2019). Gallic acid-dextran conjugate: green synthesis of a novel antioxidant molecule. Antioxidants, 8:478-491. doi:10.3390/antiox8100478

Raghunath, M., Sreeramulu, D., Reddy, C.V., Chauhan, A.K., and Balakrishna, N., (2013). Natural antioxidant activity of commonly consumed plant foods in India: Effect of domestic processing. Oxidative Medicine and Cellular Longevity, 2013, 369479.

Ranawana, V., Campbell, F., Bestwick, C., Nicol, P., Milne, L., Duthie, G., and Raikos, V. (2016). Breads fortified with freeze-dried vegetables: Quality and nutritional attributes. Part II: Breads not containing oil as an ingredient. Foods, 5:62.

Rattanachitthawat, S., Suwannalert, P., Riengrojpitak, S., Chaiyasut, C. and Pantuwatana, S. (2010). Phenolic content and antioxidant activities in red unpolished Thai rice prevents oxidative stress in rats. J. Medicinal Plants Res., 4 (9): 796-801.

Rekha, M.N., Chauhan, A.S., Prabhashankar, P., Ramteke, R.S. and Venkateswara Rao, G. (2013). Influence of vegetable paste on quality attributes of pastas made from bread wheat (*T.Aestivum*). Cyta. Journal of Food, 11: 142-149

Rizk, M.A, Khalil, M.S., Abu-Zaid, A. and Magdi, M. (2009). Evaluation of antimicrobial activity of stored fermented soybean products .Bull. Fac. Sci. Cairo Univ., 77(C): 1-17.

Schlering, C., Zinkernagel, J., Dietrich, H., Frisch, M. and Schweiggert, R. (2020). Alterations in the Chemical Composition of Spinach (*Spinacia oleracea* L.) as Provoked by Season and Moderately Limited Water Supply in Open Field Cultivation. Horticulturae, 6(2):25-47.

Sharma, D.K. (2006). Pharmacological properties of flavonoids including flavonolignans-Integration of petrocrops with drug development from plants. J. Scient. Ind. Res., 65(6): 477-484.

Sharma, K.D., Karki, S., Thakur, N.S. and Attr, S. (2012). Chemical composition, functional properties and processing of carrot—a review. j. Food Sci. Technol., 49(1): 22-32.

Shehata, E.A., Abo-Raya, S.H., and Baioumy, A.A. (2024). Impact of different drying treatments on the biochemical and antioxidant activity properties of Egyptian red beetroot. Food Systems, 7(1):151-156. https://doi.org/10.21323/2618-9771-2024-7-1

Silva, F.A.S. and C. Azevedo, A.V. (2009). Principal Components Analysis in the Software Assistat-Statistical Assistance. In: 7th World Congress on Computers in Agriculture Conference Proceedings, 22-24 June 2009, Reno, Nevada (electronic only) 711P0409e. (doi:10.13031/2013.29066).

Singh, B. (2020). Vegetables:Source of Adequate Health Benefit. Annals of Horticulture 13 (2):124-130 DOI:10.5958/0976-4623.2020.00023.7

Singh, T., Pandey, V.K., Dash, K., Zanwar, S. and Singh, R. (2023). Natural bio-colorant and pigments: Sources and applications in food processing. Journal of Agriculture and Food Research, 12: 100628

Sule, S., and Abu, J.O. (2017). Consumption of Extruded Foods among Under–Fives: Vitamin A Awareness and Fortificant Preferences of their Caregivers in Makurdi, Nigeria. Nigerian Journal of Nutritional Sciences, 38(1):1–9.

Varga, E., Birtalan, N., Abushita, A. and Fülöp, I. (2024). The Effects of Different Solvents on Phenolic, Flavonoid, Anthocyanin Contents and Free Radical Scavenging Activity on Pomegranate Juice and Seeds. Periodica Polytechnica Chemical Engineering, 68(1):85–92.

Wallace, T.C., Bailey, R.L., Blumberg, J.B., Freeman, B. B., Oliver Chen, C-y., Crowe-White, K. M., Drewnowski, A., Hooshmand, S., Johnson, E., Lewis, R., Murray, R., Shapses, S. A., and Wang, D.D. (2020). Fruits, vegetables, and health: A comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. Critical Reviews in Food Science and Nutrition, 60 (13): 2174–2211. https://doi.org/10.1080/10408398.2019.1632258

Zaki, H.M. Elshawaf, A.M. El.Makhzangy, A. And Hussein, A. M. S. (2018). Chemical, Rheological and Sensory Properties of Wheat- Oat Flour Composite Cakes and Biscuits. J. Product. & Dev., 23(2):287- 306.