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Research Article

Functional Properties, Antioxidant Activity, and Organoleptic Quality of Novel Biscuit Produced by Moroccan Cladode Flour "*Opuntia ficus-indica*"

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This study aimed to develop a novel biscuit by supplementing cladode flour (CF) into whole-wheat flour (WWF) at different proportions 0, 25, 50, 75, and 100%. Proximate analysis revealed that CF had a high amount of ash (11.9%) and dietary fiber (41.04%). Major minerals determined by ICP-MS were calcium (4.47 g/100 g); potassium (1.25 g/100 g); magnesium (1.46 g/100 g); and trace elements such as zinc (1.77 mg/100 g), copper (0.95 mg/100 g), and selenium (148.5 μg/100 g). The analysis of total phenolics, total flavonoids, and antioxidant activity showed high values (649.88 mg gallic acid equivalents (GAE)/100 g; 399.16 mg catechin equivalent (CE)/100 g; and 72.37%, respectively). HPLC was used to identify four phenolic acids (gallic, ferulic, syringic, and caffeic acids) and only one flavonoid (rutin) in cladode flour. Biscuit hardness, $L*$, and $a*$ color values decreased corresponding to the incorporation level of CF. Sensory evaluation showed that the substitution level (up to 25%) is ideal to prepare an acceptable bio-biscuit. Cladode flour could be very useful for the food industry as a source of bioactive compounds with technological potential and nutritional and antioxidant properties.

1. Introduction

Opuntia ficus-indica (L.) Mill., commonly called prickly pear or nopal cactus, belongs to the dicotyledonous angiosperm Cactaceae family and includes about 1500 species of cactus. *O. ficus-indica* is a tropical and subtropical plant which grows in arid and semiarid climates with a geographical distribution encompassing Mexico, Latin America, South Africa, and Mediterranean countries [1]. All over the Mediterranean countries and especially in Morocco, prickly pear cultivation has an important role both in sustainable agriculture and fruit production. In fact, this plant grows spontaneously and is consumed exclusively as fresh fruit, cladodes, or animal feed, but only small quantities are used for processing.

Nowadays, the light is focused on functional food rich in natural antioxidants, dietary fibers, minerals, vitamins, low calories, low fat, and natural colorants and free of synthetic additives. Furthermore, some research studies have proved that the cladode of *Opuntia ficus-indica* showed high values of some nutrients like minerals and vitamins [2], as well as bioactive compounds which prove their efficiency in the treatment of several diseases, such as anti-inflammatory [3], antidiabetic [4, 5], antioxidant [6], antiatherogenic [7], and anticancer properties [8]. Hence, various compounds identified in the cladode may increase the health potential of this novel functional food (biscuits).

Functional and technological properties of cladodes remain underdeveloped, and few reports have published the industrial uses of this vegetative part as a source of bioactive compounds or supplement ingredients [2, 9]. Furthermore, in the literature, the available data have especially focused on physicochemical composition and processing of pulp, fruit, and seeds [10]. The various functional and nutritional properties make the cladode as a great substrate for fermentation [11] and food supplementation. In fact, the inclusion of 4% of nopal powder increases the calcium and fiber content of the commercial nixtamalized corn flour compared with the traditional products [12].

This study was undertaken for the first time to investigate the possibility of using cladode flour as a novel baking product. There were two specific objectives of this research; the first was to develop a biscuit by studying the nutritional, antioxidant, functional, and physicochemical properties of cladode flour and biscuits. The second was to evaluate the sensory attributes most critical for the formulation development. Results generated are expected to provide information for commercial and nutritional applications of cladode flour.

2. Materials and Methods

2.1. Preparation of Flour Samples. Two samples were used in this study: cladode flour (CF) of *Opuntia ficus-indica* and whole-wheat flour (WWF). One-year-old cladodes (spineless and undamaged) were collected from the Oulad Dlim region of Marrakesh (Morocco). Fresh cladodes were washed with distilled water and dried in an air oven (Ecocell standard, Germany) at 60°C for 48 h. Then, the dried cladodes were ground using a professional hammer mill (Model Monbroy/2000 W, China) and screened through a mesh sieve of 500 μm. The whole-wheat flour was purchased from the local market and also screened through a mesh sieve of 500 μm. All samples were kept in the refrigerator at 4°C prior use. Besides, the composite flours were prepared by blending CF with whole-wheat flour in ratio of 100 : 0 (F 100), 75 : 25 (F 75), 50 : 50 (F 50), and 25 : 75 (F 25), respectively. The whole-wheat flour was considered as control (F 0).

2.2. Functional Properties of Flour Samples. The functionality of flours contributes to the formulation and properties of the final product. Therefore, flours were analyzed for their physicochemical and functional properties. In this section, functional properties were tested for CF, WWF, and composite flours (F 25, F 50, and F 75) by methods described as follows:

The water-holding capacity (WHC) and oil-holding capacity (OHC) were based on the method outlined by Chandra et al. [13] with slight modifications. Flour samples (5 g) were mixed separately with 50 mL of distilled water for WHC or sunflower oil for OHC and centrifuged at 3000 g for 30 min. The volume of the supernatant was measured. The WHC and OHC were expressed as percent water or oil per gram flour. The method of Cheng and Bhat [14] was used for the determination of bulk density (BD). The result was calculated as weight of the sample per unit volume of the sample (g/cm^3). Swelling power (SP) was measured according to Baljeet et al.'s method [15]. SP values were expressed in mL. The water solubility index (WSI) was calculated using the method of Ammar et al. [16] with some modifications. Briefly, 2.5 g of sample flours was added to 30 mL of distilled water and heated at 90°C for 15 min in a water bath. The cooked paste was cooled at room temperature and centrifuged at 3000 ×g for 10 min. The supernatant was decanted and oven-dried overnight at 110°C. The final residue presents the amount of sample flour solubilized in water, and results were expressed as g per 100 g of sample flour on dry weight basis. The least gelation concentration (LGC) was investigated, employing the method of Adebowale et al. [17]. Gelatinization temperature (GT) was determined using the method reported by Chandra et al. [13]. All determinations were analyzed in triplicates.

2.3. Biscuit Preparation. The composite biscuits were prepared from various combinations of CF and whole-wheat flour in the ratio of 100 : 0 (B 100), 75 : 25 (B 75), 50 : 50 (B 50), and 25 : 75 (B 25), respectively. The whole-wheat biscuit is considered as control (B 0). The formulation of biscuit had ingredients such as 500 g flour, 40 mL of sunflower oil, and 250 mL of water. The cohesive dough obtained was kneaded for two minutes and then rolled to a thickness of 2 mm with help of a rolling pin and cut with a square plastic frame. All biscuits were transferred to an aluminum baking pan covered with sulfurized paper. Biscuits were baked at 160°C for 20 min in a baking oven (Polin rotary oven, Verona, Italy). The biscuits were cooled at room temperature and stored in airtight plastic containers for further analysis.

2.4. Proximate Composition. Moisture, ash, fat, protein, and dietary fiber contents were determined for flour and biscuit samples according to the AOAC standard method. The total solid soluble (°Brix), pH, and titratable acidity were also determined as recommended by AOAC [18]. Total carbohydrates and energetic value were approximately estimated using the following formulas:

Total carbohydrates (g/100 g) = 100 − (m fat + m ash + m proteins)

Energy (Kcal/100 g) = 4 ∗ (m proteins + m carbohydrates) + 9 ∗ (m fat) [19].

Mineral content analysis and hydrolysis of the samples were performed by ICP-MS (inductively coupled plasma mass spectrometry) according to the normalized method in REMINEX laboratory (Marrakesh). Briefly, samples (0.1 g) are hydrolyzed using a HNO3 : HCl (1 : 3) mixture, on a hot plate at 250°C for 5 min, diluted with ultrapure water, and filtered through 0.45 mm paper. Samples were analyzed in ICP-MS (PerkinElmer, USA).

2.5. Chlorophylls and Total Carotenoid Determination. The chlorophyll a, chlorophyll b, and carotenoids were carried out for flour and biscuit samples in a whole pigment extract acetone (Sigma–Aldrich, St. Louis, MO, USA) by UV-VIS spectroscopy (UV-2550, Shimadzu Corporation, Kyoto, Japan). Chlorophyll determination was based on the method described by Hynstova et al. [20]. Chlorophyll a, chlorophyll b, and total carotenoids were calculated using the following equations:

Chlorophyll $a = 11.24 * A662 - 2.04 * A645$ g/mL; chlorophyll $b = 20.13 * A645 - 4.19 * A662$ g/mL; total carotenoids = $(1000 * A470 - 1.90 * \text{Chl } a - 63.14 * \text{Chl } b)/214$ g/mL.

A662, A645, and A470 were the absorbance lengths of the studied pigments.

All results were reported by mg/g dry weight (DW).

2.6. Determination of Total Phenolics, Flavonoid Content, and Antioxidant Activity. Flour and biscuit samples (2 g) were extracted twice with 20 mL of 80% methanol for 2 h at room temperature. The two filtrates were combined, concentrated under vacuum using a rotary evaporator, and finally reconstituted in 10 mL of pure methanol and stored at 20°C until analysis. Total phenolic content (TPC) was determined according to Chougui et al. [21]. The methanol extract (0.5 mL) was mixed with 1.5 mL Folin-Ciocalteu reagent (Sigma–Aldrich, St. Louis, MO, USA), diluted 10 times. After 5 min, 1.5 mL of sodium carbonate (6%) was added. The mixture was incubated in darkness for 1 h, and then, the absorbance was measured at 760 nm against a blank. TPC was calculated by linear fitting using gallic acid as standard ($R^2 = 0.96$). Results are expressed in mg gallic acid equivalent (GAE) per 100 g of dry weight (DW). The total flavonoid content (TFC) was measured according to Chougui et al.'s [21] method. An aliquot of methanol extracts (1.5 mL) was added to 1.5 mL of $AlCl_3$ reagent (2%) and allowed to stand 30 min at room temperature in the dark. The absorbance was then recorded at 430 nm against a blank. The control was prepared by mixing 1.5 mL of methanol to 1.5 mL of $AlCl_3$ reagent (Sigma–Aldrich, St. Louis, MO, USA). TFC was calculated by linear regression using catechin as standard ($R^2 = 0.97$). The results were expressed in mg catechin equivalent (CE) per 100 g of dry weight (DW). The method of Abdel-Hameed et al. [22] was used for antioxidant potential determination (DPPH assay). DPPH (100 mM, Sigma–Aldrich, St. Louis, MO, USA) solution freshly prepared in methanol (1 mL) was added to 1 mL sample extract. For the control, 1 mL DPPH methanolic solution was mixed with 1 mL methanol. The reaction mixture was incubated in the dark for 30 min, and the optical density was recorded at 517 nm against the blank. The activity of scavenging (%) was calculated using the following formula: DPPH radical scavenging % = (OD control − OD sample)/OD control ∗ 100.

2.7. HPLC Analysis. The identification and quantification of phenolic compounds of flour and biscuit samples were carried out by high-performance liquid chromatography (HPLC) (Knauer, Berlin, Germany) equipped with a UV-Vis diode array detector (DAD) and C18 column (Eurospher II 100-5, 250 mm × 4.6 mm) maintained at 25°C. The flow rate was 1 ml/min, and the injected volume was 10 μl. The gradient mixture consists of eluent A (acidified water) and eluent B (acetonitrile) as a mobile phase for a total running time of 60 min. DAD detection is set at an acquisition range of 200–700 nm as preferred wavelengths. The phenolic compounds were characterized according to their UV spectra and retention times and compared with authentic standards (gallic acid, ferulic acid, vanillic acid, syringic acid, p-coumaric acid, vanillin, and caffeic acid) purchased from Solvachim laboratory (Casablanca, Morocco). For quantitative analysis, calibration curves were prepared from different standard compounds using the mass peak areas obtained from the chromatograms at a concentration between 0.5 and 1 g·L^{-1}. The results were expressed in mg per 100 g DW.

2.8. Physical Characteristics. Physical characteristics of biscuits (weight, length, width, diameter, thickness, and spread ratio) were determined based on the method described by Baljeet et al. [15]. Hardness was measured with the help of Hardness Tester (model PTB301, USA) ,and the value of hardness was expressed in Newton (N). Color measurement was determined using a Handheld Chroma Meter (model CR200, Minolta, Tokyo, Japan) on the basis of $L*$, $a*$, and $b*$ values, where $L*$ value indicates the lightness and its value ranges from 0 to 100, $a*$ value gives the degree of the red-green color, with a higher positive a value indicating more red ($_{+}a$), and the $b*$ value indicates the degree of the yellow-blue color, with a higher positive b value indicating more yellow ($_{+}b$). The colorimeter was calibrated using a standard white and black plate.

2.9. Sensory Analysis. Biscuit samples were analyzed for their organoleptic characteristics by a panel of 30 non-trained members (university students) using a 5-point hedonic scale. The panelists scored for different parameters with a maximum score: 5: like extremely, 4: like good, 3: neither like nor dislike, 2: dislike, and 1: dislike very much. The biscuits were evaluated for their taste, aroma, hardness, color, texture, appearance, after taste, and overall acceptability. The samples were placed on white plates and were identified with random three-digit numbers. The panelists evaluated all samples in a testing area with a good light condition. Panelists were instructed to rinse their mouth with water between samples to minimize any residual effects [23].

2.10. Statistical Analysis. Results generated in this work were expressed as mean ± standard deviation of three independent replications. The statistical significance of the generated data was analyzed by employing one-way analysis of variance (ANOVA) along with Tukey's test with a significance level of 5%.

3. Results and Discussion

3.1. Physicochemical Properties of Cladode Flour. The chemical composition of CF compared to WWF is shown in Table 1. The CF had the lowest moisture content and the highest ash content due to the mineral content. Dietary fiber content in CF is in accordance with those obtained for small cladodes [24]. Besides, CF presents a large decrease in humidity compared to the fresh cladode (88–95%), due to the drying process, which makes flour edible and prevents

Table 1: Proximate composition of cladode flour and whole-wheat flour.

Parameters	CF	WWF
Moisture (%)	$9.55^{b} \pm 0.52$	$14.03^{a} \pm 0.68$
Ash (g/100 g)	$11.90^{a} \pm 0.23$	$1.25^{b} \pm 0.32$
ph	$4.20^{b} \pm 0.02$	$6.05^{a} \pm 0.03$
Titratable acidity (% citric acid)	$1.17^{a} \pm 0.10$	$0.23^{b} \pm 0.04$
°Brix	$4.07^{a} \pm 0.12$	$0.67^{b} \pm 0.12$
Fat (g/100 g)	$2.30^{a} \pm 0.54$	$2.86^{a} \pm 0.36$
Protein (g/100 g)	$8.76^{b} \pm 0.11$	$13.22^{a} \pm 1.47$
Dietary fiber (g/100 g)	$41.04^{a} \pm 0.7$	$9.47^{b} \pm 1.07$
Carbohydrate (g/100 g)	$74.27^{b} \pm 1.44$	$82.67^{a} \pm 1.70$
Energy (Kcal/100 g)	$377.72^{b} \pm 6.92$	$409.28^{a} \pm 1.39$
Ca (g/100 g)	$5.52^{a} \pm 0.23$	$0.06^{b} \pm 0.04$
K (g/100 g)	$1.24^{a} \pm 0.21$	$0.41^{b} \pm 0.05$
Mg (g/100 g)	$1.54^{a} \pm 0.30$	$0.06^{b} \pm 0.02$
Fe (mg/100 g)	$16.38^{a} \pm 0.96$	$2.01^{b} \pm 0.66$
Cu (mg/100 g)	$0.83^{a} \pm 0.12$	$0.44^{a} \pm 0.17$
P (mg/100 g)	$342.02^{a} \pm 33.35$	$178.69^{b} \pm 13.10$
Mn (mg/100 g)	$3.87^{a} \pm 0.77$	$2.30^{b} \pm 0.68$
Zn (mg/100 g)	$1.12^{a} \pm 0.58$	$1.35^{a} \pm 0.13$
Se (μg/100 g)	$137.84^{a} \pm 12.77$	$0.03^{b} \pm 0.02$

Values are means ± standard deviation ($n = 3$). Mean values in the same line followed by different letters were statistically different (Tukey's test: $p < 0.05$). CF = cladode flour; WWF = whole-wheat flour.

the proliferation of microorganisms [25]. It is worth mentioning that the drying process and maturity stage of cladode had an effect on nutrient contents such as fiber, ash, and protein [26]. The same author confirmed that soluble fiber amount decreased with the age of cladode while insoluble fibers and ash content showed a reverse trend. The WWF had similar values for moisture, ash (1.25 ± 0.32), fat (2.86 ± 0.36), and protein (13.22 ± 1.47) as found by Chandra et al. [13], Liu et al. [27] and Boita et al. [28], respectively. The WWF contained a high amount of fiber (9.47 ± 1.07) than refined wheat flour due to the presence of bran [15]. However, fiber contents of WWF were lower than that of CF (41.04 vs 9.47%) and those of Boita et al. [28]. The high titratable acidity observed in CF is due to the presence of many organic acids as malic, citric, and oxalic acids [29], while soluble sugar contents (glucose) are responsible for the high values of °Brix in CF [30]. Differences in proximate composition might be due to differences in varieties, soils, climatic environment, and physical conditions. CF had low levels of carbohydrate and calorific value, contrary to WWF. The high calorific value of WWF was due to its high protein and high carbohydrate content. Similar results in WWF were reported by Cardoso et al. [19]. Furthermore, the same findings were reported by Chiteva and Wairagu [31] for the fruit pulp of *Opuntia ficus-indica*. The study indicated that cladode is not a good source of energy, but it could be advantageous for weight loss diets. The determination of mineral and trace elements has taken considerable importance in fruits and vegetables for both nutritional and technological processing [22]. The results as shown in Table 1 revealed that Ca was the mineral with the highest concentration (5.52% and 2.86%) followed by magnesium (1.54% and 1.42%) and potassium (1.24% and 1.23%) for CF and biscuit samples, respectively. These findings were in agreement with those of Méndez and Batista [32, 33] who reported that Ca values were clearly higher than 1.5-3.5% DW. Our data for iron and zinc fell well within the range described in the same literature but higher than those for prickly pear fruit, pulp, and juice [29, 32, 34]. The mineral quantities detected in CF could make it a good supplement for commercial foods. Hence, the consumption of cladode (flour or biscuit) contributes to the daily intake of some nutrients. Thus, one serving (10 g) of CF contributes 14% to 16% of WHO-recommended intake (DRI) for fiber. However, the same serving provides 59% of the DRI for Ca, 51% for Mg, 19% for Fe, and 12% for Cu. The contribution to selenium's daily intake is considerably higher and accounts for about 60% of the DRI. Furthermore, moderate contributions of K, Mn, P, and Zn were observed with values of the DRI 9.49, 7.7, 6.0, and 2.86%, respectively. These findings show that cladode could greatly contribute to human food as described by the WHO. Besides, only young cladodes with a very high nutritional potential are recommended for the fortification.

3.2. Functional Properties. Functional properties investigate the intrinsic physicochemical properties and the interaction between the composition, structure, and physicochemical properties of food compounds. The functional properties of CF and WWF are given in Table 2. Figure 1 presents the results of the supplementation effect. Statistically significant differences ($p < 0.05$) were noticed for most properties. CF showed the highest WHC (4.87% vs 2.47% for WWF) explained by the ability of CF to interact with free polar groups of the hydrophilic constituents (polysaccharides and fiber) [35]. Protein has both hydrophilic and hydrophobic nature, and therefore, they can interact with water in foods. Even if WWF has more proteins, CF has a high amount of fiber (41.04% vs. 9.47%) responsible for the water-holding capacity. The maturity stage of cladode could be responsible for differences of WHC and other properties as confirmed by Nuñez-López et al. for small, medium, and large cladodes [24]. The OHC of WWF was 3.47% and 2.6% for CF. These findings indicate that there are more hydrophobic interaction sites in WWF compared to the CF. The holding of liquid in flours can be a good index to determine the capacity of protein and dietary fiber to absorb and retain water or oil. Thus, WHC and OHC are important parameters affecting the texture, mouthfeel, and consistency of food products. These results indicated that CF possesses high potential encouraging their applications in various food products. By increasing the level of CF incorporation, WHC, SP, BD, and WSI were increased while OHC and GT were decreased, whereas LGC remains constant up to 25%. Our current results are in agreement with those of Chandra et al. [13]. For flour blends, the WHC ranged from 2.47 to 5.13% and OHC ranged from 3.87% to 2.6%; the highest value was shown for 25% supplementation level. Boukid et al. [36] suggest that OHC was correlated with temperature contrary to water-holding capacity, whereas Lobato et al. [37] found an opposite trend during drying at high temperatures. The BD of CF and WWF was recorded to be 0.95 and 0.66 g/cm^3,

Table 2: Functional properties of cladode flour and whole-wheat flour.

Functional properties	CF	WWF
Water-holding capacity (%)	$4.87^a \pm 0.10$	$2.47^b \pm 0.10$
Oil-holding capacity (%)	$2.60^b \pm 0.20$	$3.47^a \pm 0.12$
Swelling power (mL)	$30.17^a \pm 1.89$	$6.5^b \pm 0.50$
Bulk density (g/cm^3)	$0.95^a \pm 0.01$	$0.66^b \pm 0.00$
Least gelation concentration (%)	$18.00^b \pm 0.00$	$20.00^a \pm 0.00$
Water solubility index (g/100 g)	$10.75^a \pm 1.74$	$2.14^b \pm 0.66$
Gelatinization temperature (°C)	$59.00^b \pm 0.82$	$82.67^a \pm 2.05$

Values are means ± standard deviation ($n = 3$). Means with the same letter in a row are not statistically different from each other (Tukey's test: $p < 0.05$).

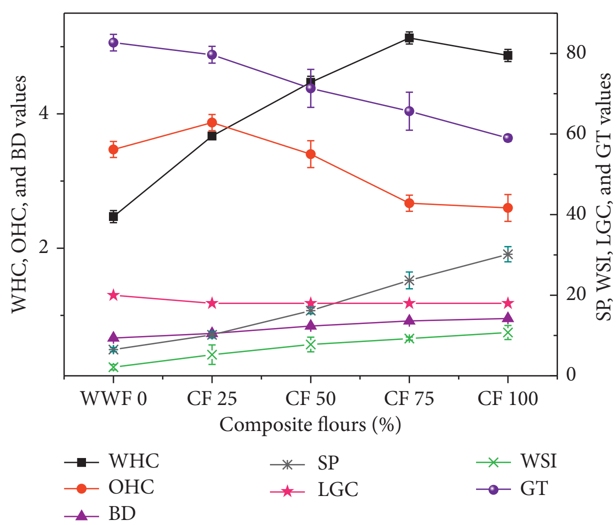

Figure 1: Effect of cladode flour incorporation on functional properties. CF = cladode flour; WWF = whole-wheat flour; WHC = water-holding capacity (%); OHC = oil-holding capacity (%); SP = swelling power (mL); BD = bulk density (g/cm^3); LGC = least gelation concentration (%); WSI = water solubility index (g/100 g); GT = gelatinization temperature (°C).

respectively. These values were greater than values observed by Ayadi et al. [38] in spineless cladode powder. The BD of composite flours is related to the supplementation level; it ranged from 0.66 g/cm^3 for WWF to 0.95 g/cm^3 for CF. The highest BD was observed for composite flour F 75 (0.92 g/cm^3), followed by F 50 (0.84 g/cm^3) and F 25 (0.73 g/cm^3). The high BD could depend on the particle size and initial moisture content of flours. The hydration properties described by SP and WSI showed significant difference ($p < 0.05$). WSI for CF was five times more than WWF (10.75% vs 2.14%) due to soluble molecule contents in CF (glucose and galactose) [16, 38]. However, other molecules (organic acids, proteins, and low molecular weight compounds) were eliminated by heating at 90°C and filtration as confirmed by the study of Majdoub et al. [35]. The SP of composite flour was strongly affected by the addition level ($r = 0.73$ at 100%), whereas the WSI was negatively correlated because of the hydration properties ($r = -0.74$ at 0% level). The LGC and the GT of WWF was 20% and ≈83°C, and 18% and 59°C for CF, respectively. These variations may be ascribed to the ratios of different compounds (protein, carbohydrates, and lipids) and also to their chemical interactions [39]. This study revealed that the high amount of dietary fiber of CF is the main cause of the drop-in temperature and time gelatinization. Hence, CF could be added as the gel-forming material for food products that require thickening and gelling.

3.3. Physicochemical and Physical Analysis of Biscuits

3.3.1. Physicochemical Properties. Physicochemical properties of flours and biscuits are given in Table 3. The results show a significant difference ($p < 0.05$) between all samples upon the blending ratio of CF. Moisture and pH of flours decreased from 14.26% to 9.55% for WWF and from 6.05% to 4.20% for CF, while °Brix and titratable acidity follow the reverse trend (0.67 to 4.07 and 0.24 to 1.83%, respectively). The same observations were noted for composite biscuits (Table 3). The moisture content was highly affected by blending and baking conditions, and it was assessed to determine the storability and enhance shelf-life of final products. On the other hand, pH, titratable acidity, and °Brix were not affected by the baking conditions unlike the blending ratio showing significant differences. In addition, color changes were observed for composite biscuits (green darker) due to the release of organic acids from the cell vacuole that causes pH-dependent reactions as the conversion of chlorophyll to pheophytin [30].

3.3.2. Chlorophyll and Carotenoid Determination. Chlorophyll a and b content is illustrated in Figure 2. The results was higher than the total chlorophyll found by Silva-Ortega et al. [40] who found 1 μg/g DW in cladode of *O. streptacantha* plant. However, data were lower than results reported by Ayadi et al. [38] for spiny and spineless cladode of *O. ficus-indica*. Total carotenoid content in the CF and biscuit have moderate changes from 0 to 100% level of incorporation. The values ranged between 1.85 to 6.11 mg/100 g DW and 2.33 to 4.40 mg/100 g DW for flour and biscuit, respectively. Data were very lower than findings of Jaramillo-Flores et al. [41] who reported that fresh cladode contains 23.18 mg/100 g DW with three identified carotenoids (β-carotene, α-cryptoxanthin, and lutein). Hence, the concentration of carotenoids showed special interest because of their antioxidant activity.

3.3.3. Total Phenolic Contents, Total Flavonoid Contents, and DPPH Scavenging Activity. Large interest was awarded to secondary metabolites for their antioxidant properties. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (% inhibition) of CFs and biscuits are displayed in Figure 3. The total phenolic content increased by supplementing CF. Furthermore, TPC ranged from 52.96 to 649.88 mg GAE/100 g DW for composite flour and from 47.18 to 513.75 mg GAE/100 g DW for composite biscuits. The cladode biscuit at 100% had the highest amount of total phenolic compounds followed by 75, 50, and 25%. Ashraf and Ahmed [42, 43] also reported an increase in total

Table 3: Physicochemical characteristics in flour and biscuit of composite samples.

Parameters		Moisture	°Brix	pH	Titratable acidity (% citric acid)
WWF 0%	Flour	$14.26^{abc} \pm 0.78$	$0.67^{f} \pm 0.12$	$6.05^{b} \pm 0.03$	$0.24^{abc} \pm 0.04$
	Biscuit	$10.65^{bc} \pm 1.32$	$0.67^{f} \pm 0.12$	$6.33^{a} \pm 0.02$	$0.19^{bc} \pm 0.06$
25%	Flour	$9.81^{cd} \pm 1.09$	$1.73^{e} \pm 0.12$	$5.35^{c} \pm 0.01$	$0.41^{cd} \pm 0.1$
	Biscuit	$7.96^{cd} \pm 1.48$	$1.47^{e} \pm 0.12$	$5.06^{d} \pm 0.02$	$0.43^{cd} \pm 0.04$
50%	Flour	$16.62^{a} \pm 1.09$	$2.37^{d} \pm 0.15$	$4.89^{e} \pm 0.06$	$0.77^{a} \pm 0.19$
	Biscuit	$14.23^{abc} \pm 1.45$	$2.37^{d} \pm 0.06$	$4.74^{f} \pm 0.02$	$0.70^{abc} \pm 0.06$
75%	Flour	$8.47^{cd} \pm 0.88$	$2.80^{c} \pm 0.20$	$4.79^{f} \pm 0.03$	$0.96^{cd} \pm 0.13$
	Biscuit	$8.47^{cd} \pm 0.80$	$3.33^{b} \pm 0.12$	$4.59^{g} \pm 0.01$	$0.89^{cd} \pm 0.02$
CF 100%	Flour	$9.55^{cd} \pm 1.49$	$4.07^{a} \pm 0.12$	$4.20^{h} \pm 0.02$	$1.83^{cd} \pm 0.15$
	Biscuit	$6.28^{d} \pm 0.95$	$4.27^{a} \pm 0.12$	$4.52^{g} \pm 0.01$	$0.99^{d} \pm 0.16$

Values are means ± standard deviation ($n = 3$). Mean values in the same column followed by different letters were statistically different ($p < 0.05$).

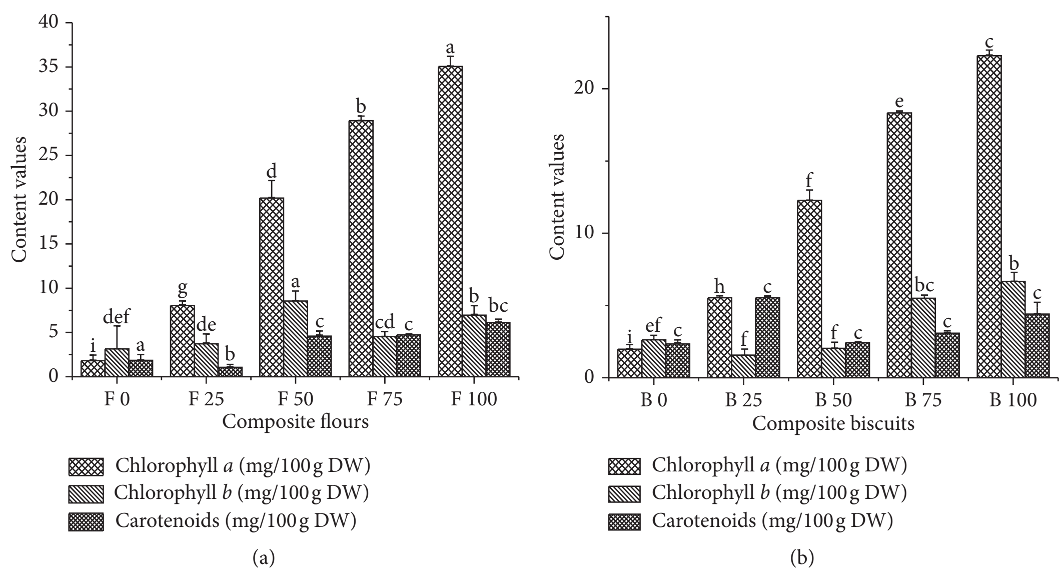

Figure 2: Chlorophyll *a* and *b* and carotenoids of flour and biscuit samples. Values indicated by different lowercase letters are significantly different ($p < 0.05$) according to Tukey's test.

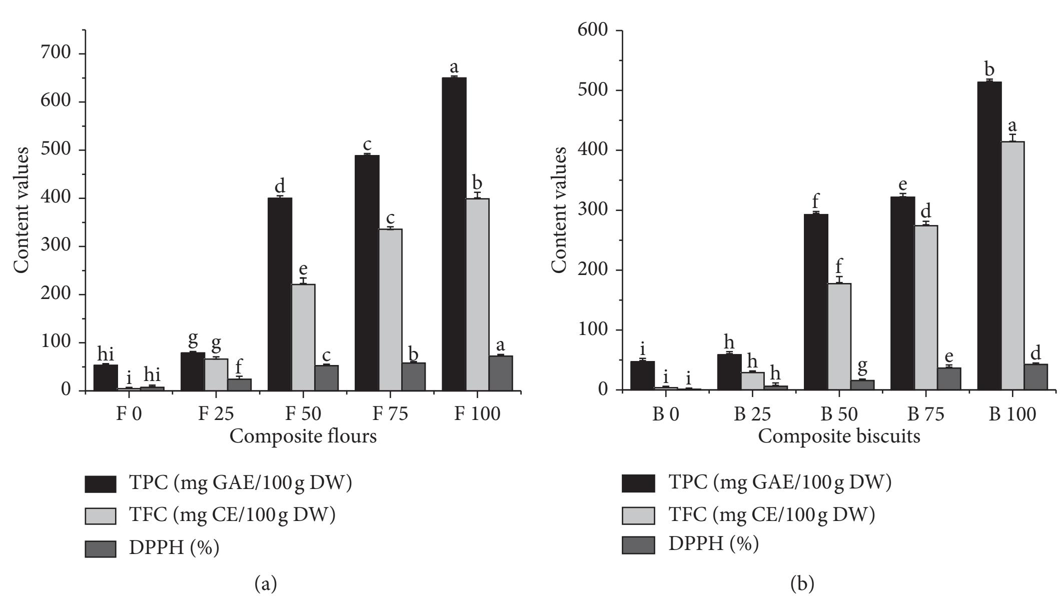

Figure 3: Total phenolics, total flavonoids, and antioxidant activity of flour and biscuit samples. Values indicated by different lowercase letters are significantly different ($p < 0.05$) according to Tukey's test.

phenolic content in biscuits fortified with apricot powder and *Hibiscus sabdariffa*, respectively. The TFC was found in smaller amount than phenolic acids and followed the same trend; it ranged from 4.71 to 399.16 mg CE/100 g DW for flour samples and from 3.62 to 414.16 CE/100 g DW for biscuit samples. These results were in agreement with those of Guevara-Figueroa et al. [44] for commercial varieties (*Blanco* and *Manso)* with 5.25 and 11.7 mg GAE/g. Besides, current findings were higher than *Tapon* varieties and commercial powders containing less than 2 mg GAE/g. Furthermore, the phenolic content of CF was higher than those found for raw and boiled cladode with 458 mg/100 g and 283 mg/100 g, respectively [45]. These differences might have arisen from different methodologies used, age of cladode, environment, soil type, and climate [46, 47]. Furthermore, these differences in TPC and TFC could be related to species biochemical characteristics because the secondary metabolite accumulation depends on biotic and abiotic factors. In fact, Astello-García et al. [46] highlighted a relationship between phenolic compound content and domestication gradient of *Opuntia* species. The present study showed that total phenolic content decreased by the baking process with a little effect on the antioxidant activity. The same observation was reported by Jaramillo-Flores [41] who found that total phenolics of nopalitos extract decreased to low values with simultaneous increase in the antioxidant activity after the thermal treatment at 93°C, which can be explained by the presence of other compounds such as carotenoids, tocopherols, and ascorbic acid. Nevertheless, Medina-Torres et al. [6] reported that dehydration process can preserve the bioactive compounds of nopal, and the best convective drying conditions were a temperature of 45°C and an air flow rate of $3\,m{\cdot}s^{-1}$. The antioxidant activity of sample extracts measured by the DPPH method showed that supplemented samples had an important free-radical scavenging ability. Significant variations in the antiradical power were observed between flour and biscuit samples. The % inhibition values of flours and biscuits ranged from 7.18 to 72.37% and from 1.10 to 42.54%, respectively. The antioxidant activity value of CF (72.37%) was in line with those reported by Jun et al. [48] and Jaramillo-Flores et al. [41] for Korean cladode (*Opuntia humifusa*) and nopalitos thermally treated.

3.3.4. Individual Phenolic Compounds. The contents and concentrations of phenolic compounds were identified by HPLC analysis and are presented in Table 4. Seven phenolic acids (gallic acid, vanillic acid, caffeic acid, syringic acid, ferulic acid, p-coumaric acid, and vanillin) and one flavonoid (rutin) were detected. The results revealed that gallic acid was the most abundant compound followed by syringic acid. It was observed that gallic acid, syringic acid, and caffeic acid were presented in all flour and biscuit samples. The concentration of gallic acid was noted to be highest in both CF and biscuit but lowest in WWF (918.63 and 216.73 mg/100 DW; 623.10 and 161.79 mg/100 g, respectively). However, syringic acid and caffeic acid presented an opposite trend. Ferulic acid was presented in flour samples (F 25, F 50, F 75, and F 100) and was absent in composite biscuits, while vanillic acid was detected only in cladode biscuit (B 100). However, vanillin was identified in all composite biscuits. These results could be due to the thermal degradation of ferulic acid during the cooking at 160°C/20 min, showing the small amount of the ferulic acid content in the composite flours (4 and 22 mg/100 g). This absence may be due to water-soluble phenols leaching into the cooking water and the structural changes of phenolics that occurred during the heat process. Further, ferulic acid could be transformed into useful phenolic compounds by the conversion of ferulic acid to vanillin or by the demethylation to caffeic acid [47, 49]. This statement was also supported by the study of Zhao et al. [50] who explained that the extent of polyphenol degradation depended on the processing conditions and also on the type of matrix being processed. F 75, F 100, and B 100 samples contained a low level of *p*-coumaric acid (1.87, 9.09 and 7.22 mg/100 g DW). However, vanillic acid was absent for all samples except for cladode biscuit with 2.41 mg/100 g DW. The increase of some phenolic acids such as vanillic acid could be explained by the degradation of gallic acid as a radiolytic product by baking. Boles et al. [51] mentioned that gallic acid decomposed rapidly at temperatures between 105° and 150°C by decarboxylation and carbanion transition generating vanillic and ellagic acids. Breaking down of gallic acid was also proved under nonthermal conditions. In fact, Melo et al. [52] and Madureira et al. [53] reported that gallic acid is a radiolytic product of syringic, vanillic, and protocatechuic acids by irradiation treatment. Hence, the increasing content of syringic and vanillic acids may be justified. Some flavonoids as catechin, kaempferol, and isorhamnetin were not identified in all investigated samples of this work despite being identified in several studies [54–56] as well as chlorogenic acid [46]. However, rutin was the only flavonoid detected and quantified in flour and biscuit (16.74–92.49 mg/100 g DW). Gallic acid and syringic contents indicated in our study were higher than those found by Jun et al. [47] for both flour and composite biscuit. However, the same author reported that p-coumaric acid and ferulic acid contents were higher than our data (61 and 222 mg/100 g, respectively). In addition, rutin content was in agreement with this study (61 mg/100 g) for flour and biscuit samples. These differences noticed in the phenolic acid content could be explained by various factors such as a difference in species, geographical conditions, and other environmental factors [57].

3.3.5. Physical Analysis of Biscuits. The physical properties of biscuits are given in Table 5. No significant differences were observed for weight, length, width, and diameter. However, thickness, hardness, and spread ratio were more affected. The changes in diameter and thickness were reflected in spread ratio. Thus, thickness is the main factor that influences the ratio due to steady diameter. The highest spread ratio was 10.93 at 100% supplementation level vs 5.71 for control. These findings are in opposition with those of Cheng and Bhat [14] for cookies prepared by supplementing jeering seed flour with wheat flour, and they are in

Table 4: Content of individual polyphenol compounds of 17 flour samples and composite biscuits determined by HPLC-DAD (mg/100 g DW).

	Gallic acid	Tyrosol	Vanillic acid	Rutin	Vanillin
F 0	$216.73^{f} \pm 1.84$	$863.48^{a} \pm 1.07$	ND	ND	ND
F 25	$348.26^{e} \pm 4.21$	ND	ND	$46.32^{e} \pm 2.10$	ND
F 50	$702.13^{bc} \pm 8.98$	ND	ND	$82.20^{b} \pm 0.99$	$8.93^{b} \pm 0.19$
F 75	$722.85^{b} \pm 2.46$	ND	ND	$74.20^{c} \pm 0.12$	ND
F 100	$918.63^{a} \pm 5.2$	ND	ND	$72.56^{c} \pm 0.51$	ND
B 0	$161.79^{f} \pm 6.35$	ND	ND	$16.74^{g} \pm 0.36$	$3.10^{f} \pm 0.06$
B 25	$337.97^{d} \pm 8.18$	ND	ND	$34.47^{f} \pm 0.90$	$5.36^{e} \pm 0.05$
B 50	$933.58^{a} \pm 3.17$	ND	ND	$55.84^{d} \pm 0.98$	$8.63^{c} \pm 0.10$
B 75	$896.02^{a} \pm 8.37$	ND	ND	$81.91^{b} \pm 0.24$	$6.22^{d} \pm 0.03$
B 100	$623.10^{cd} \pm 5.51$	ND	$2.41^{a} \pm 0.06$	$92.49^{a} \pm 2.53$	$9.40^{a} \pm 0.02$
	Syringic acid	Ferulic acid	*p*-Coumaric acid	Caffeic acid	Phenolic sum
F 0	$595.48^{a} \pm 3.07$	ND	ND	$251.35^{a} \pm 1.57$	1927.04 ± 7.63
F 25	$268.48^{d} \pm 4.26$	$4.19^{c} \pm 0.01$	ND	$30.54^{d} \pm 0.10$	697.79 ± 10.85
F 50	$357.27^{c} \pm 3.14$	$8.44^{b} \pm 0.11$	ND	$57.51^{bc} \pm 0.77$	1216.49 ± 14.47
F 75	$338.42^{c} \pm 1.72$	$3.33^{c} \pm 0.10$	$1.87^{c} \pm 0.06$	$49.31^{c} \pm 0.94$	1189.98 ± 5.46
F 100	$455.49^{b} \pm 3.13$	$22.55^{a} \pm 1.05$	$9.09^{a} \pm 0.07$	$64.48^{b} \pm 0.69$	1279.01 ± 10.65
B 0	$254.24^{d} \pm 1.65$	ND	ND	$19.19^{de} \pm 0.26$	455.06 ± 8.62
B 25	$143.11^{f} \pm 4.20$	ND	ND	$7.72^{e} \pm 0.08$	528.63 ± 13.41
B 50	$239.92^{de} \pm 3.18$	ND	ND	$26.93^{d} \pm 0.10$	1264.91 ± 7.53
B 75	$370.46^{c} \pm 0.94$	ND	ND	$46.05^{c} \pm 0.07$	1400.66 ± 9.65
B 100	$186.81^{ef} \pm 3.05$	ND	$7.22^{b} \pm 0.25$	$51.39^{bc} \pm 0.07$	971.82 ± 11.49

Values are presented as means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other according to |Tukey's test. ND: nondetected; F: flours at different incorporation levels; B: biscuit at different incorporation levels.

Table 5: Physical properties of composite biscuits.

Parameters	WWF 0%	25%	50%	75%	CF 100%
Weight (g)	$3.36^{ab} \pm 0.29$	$3.97^{a} \pm 0.58$	$3.32^{ab} \pm 0.30$	$2.99^{b} \pm 0.24$	$3.10^{ab} \pm 0.49$
Length (cm)	$3.53^{c} \pm 0.05$	$3.68^{bc} \pm 0.05$	$3.76^{b} \pm 0.10$	$3.75^{b} \pm 0.13$	$3.94^{a} \pm 0.05$
Width (cm)	$3.68^{b} \pm 0.13$	$3.73^{ab} \pm 0.1$	$3.85^{ab} \pm 0.10$	$3.83^{ab} \pm 0.05$	$3.91^{a} \pm 0.03$
Thickness (cm)	$0.46^{a} \pm 0.04$	$0.35^{b} \pm 0.02$	$0.31^{bc} \pm 0.03$	$0.31^{bc} \pm 0.03$	$0.25^{c} \pm 0.02$
Diameter (cm)	$2.59^{c} \pm 0.01$	$2.61^{c} \pm 0.03$	$2.67^{b} \pm 0.02$	$2.70^{ab} \pm 0.02$	$2.72^{a} \pm 0.01$
Spread ratio	$5.71^{c} \pm 0.57$	$7.47^{b} \pm 0.51$	$8.57^{b} \pm 0.60$	$8.71^{b} \pm 0.86$	$10.93^{a} \pm 0.88$
Hardness (*N*)	$118.50^{a} \pm 63.96$	$117.50^{a} \pm 52.98$	$78.25^{a} \pm 16.37$	$60.7^{a} \pm 15.90$	$73.25^{a} \pm 4.72$
Color					
$L*$	$66.74^{a} \pm 3.26$	$55.40^{b} \pm 2.23$	$55.09^{b} \pm 2.78$	$54.05^{b} \pm 2.64$	$53.22^{b} \pm 3.67$
$a*$	$-5.95^{a} \pm 0.72$	$-8.00^{b} \pm 0.44$	$-8.37^{bc} \pm 0.31$	$-8.81^{c} \pm 0.39$	$-9.00^{c} \pm 0.39$
$+b*$	$29.55^{a} \pm 0.82$	$28.93^{a} \pm 0.63$	$28.86^{a} \pm 0.89$	$28.02^{a} \pm 1.01$	$27.97^{a} \pm 0.95$

Values are means ± standard deviation ($n = 4$). Mean values in the same line followed by different letters were statistically different ($p < 0.05$). WWF: whole-wheat flour; CF: cladode flour.

agreement with those of Baljeet et al. [15] for the biscuits with buckwheat flour. Moreover, spread ratio serves as a parameter to evaluate the rising ability of bakery product, because it is related to the protein and fat content. In fact, high protein content limits the spread of biscuits as opposed to high-fat content. Fat, protein, and fiber content could be the parameters responsible for hardness observed (118.5 to 73.25 N) due to their interactions during dough development [14, 23]. Color measurement of food products is a critical parameter that affects the acceptance of consumers. Significant differences ($p < 0.05$) were observed for composite biscuits and control. $L*$ values were 66.74 for WWF and 53.22 for CF. The same pattern was found by Galla et al. [23]. In terms of $a*$ (redness) and $b*$ (yellowness), no red color was observed and the final product becomes green (darker) (−5.95 to −9 and 29.55 to 27.97, respectively). These results could be explained by the nonenzymatic reaction (Maillard reaction) that takes place between reducing sugars and amino acids causing a brown effect during cooking. Further, the biscuit's darkness color could be due to the presence of high content of chlorophyll a (22.29 mg/100 g DW) compared to other composite biscuits.

3.4. Sensory Evaluation of Biscuits. The effect of cladode powder incorporation on the organoleptic characteristics of biscuits is presented in Figure 4. Photographs of biscuits, spider plot according to the sensory hedonic test, and panelist suggestions improving biscuit formulation are given. The organoleptic evaluation indicated that CF addition had a significant effect on biscuit quality ($p < 0.05$) except for hardness, texture, and appearance. It was noted

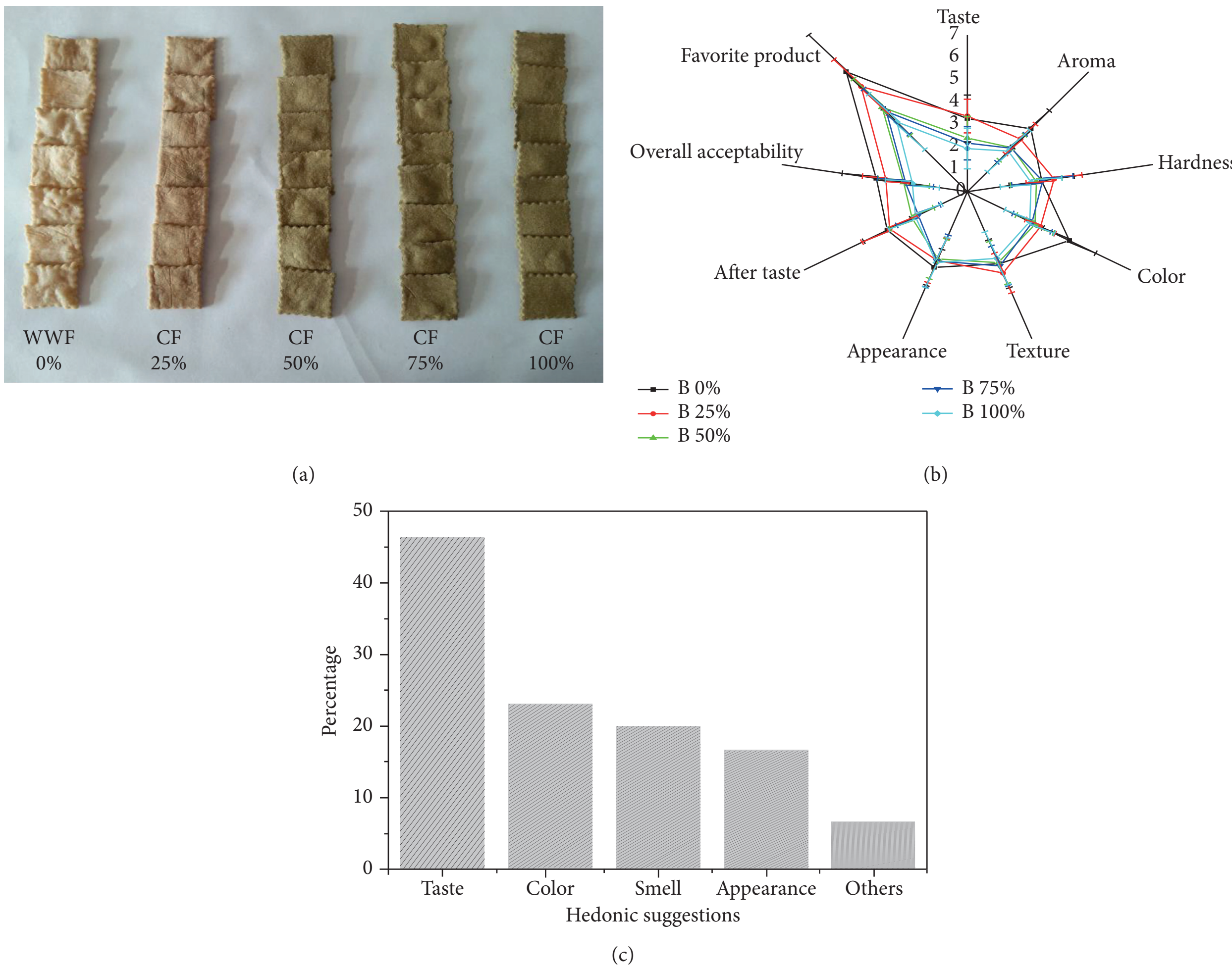

FIGURE 4: (a) Photographs of biscuit with levels of supplemental CF. (b) Sensory profile of composite biscuits. (c) Rate of suggestions improving the acceptability of biscuits.

that the mean score for taste (3.27 to 2.17), aroma (3.67 to 2.53), color (4.37 to 2.77), and after taste (3.43 to 2.1) decreased significantly with addition level. Furthermore, biscuits with CF had a natural color of pistachio (natural additive), which avoids the addition of other synthetic green colors. The overall acceptability on a five-point hedonic scale was 2.7 for biscuits at 25% level and did not differ from the control. However, for B 75 and B 100, the overall acceptability was rated as poor. This is due to the strong taste and aroma of CF. According to Ayadi et al. [38] and Kim et al. [58], CF could not be added at more than 5% and 9% levels, respectively. However, Boukid et al. [36] recommended 30% as a good level for rolled cake. The overall organoleptic assessment showed that up to 25% of CF did not influence consumer acceptance and had the highest scores among all the biscuits evaluated. Therefore, some improvements are required for biscuit formulation. Panelist's recommendations were analyzed and the results are presented in Figure 4(c). The taste (46.65%) and color (23.43%) were the most criteria affecting customer's acceptability. Thus, natural sweetener (stevia) could be an excellent additive improving the taste of biscuits without affecting their nutritional potential [59]. For that reason, studying the effect of stevia supplementation and other additives on biscuits properties and sensory evaluation is highly required.

4. Conclusion

For the first time in Morocco, we developed novel nutraceutical biscuits supplemented by local CF. The above results confirmed that CF can be a promising source of dietary fiber, minerals, and bioactive compounds (phenolic compounds and carotenoids), which is recommended for improving bioactivity and nutritional value of food products, cosmetics, and pharmaceutical preparations. The present study showed that the addition of CF in the proportion of 25% produced acceptable biscuits with a score of 6.13. Besides, taste and color are considered the major factors enhancing the overall acceptability of the final product. To summarize, CF can be considered a value-added ingredient paving way for consumption in Morocco as bakery products, serving as a nutrient supplementing vehicle in Moroccan diet and solving malnutrition problems in some areas. Furthermore, the nutritional potential of CF might be very attractive to the growing industry of "nutraceutical foods."

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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