

Effect of addition of Tunisian *Zizyphus lotus* L. Fruits on nutritional and sensory qualities of cookies

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Received: 26 June 2021; Accepted: 19 October 2021; Published: 7 December 2021

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PAPER

Abstract

Zizyphus lotus, which belongs to Rhamnaceae family, has been widely used to formulate many healthy food products. The aim of this work was to formulate new functional cookies enriched with different amounts of *Zizyphus lotus* powder (ZLP; 15%, 30%, 45% and 100%). The chemical properties of ZLP were also determined. The formulated cookies were evaluated for their physicochemical, textural and sensory characteristics. Results revealed that ZLP contained various bioactive components, fatty acids, and antioxidants. ZLP-added cookies demonstrated higher phytochemical and antioxidant activities than control cookies prepared without ZLP. The activity of ZLP cookies was enhanced with increase of ZLP level. Hardness and fracturability (**brittleness**) of cookies increased with increasing amount of ZLP. Results of Fourier-transform infrared spectroscopy and thermogravimetric analysis also revealed the presence of many bioactive compounds in formulated cookies. All cookie samples were generally accepted, but the panelists indicated a higher preference for cookies containing 15% ZLP.

Keywords: antioxidants; cookies; phytochemical compounds; sensory characteristics; texture; *Zizyphus lotus*.

Introduction

Bakery products such as cookies are widely consumed around the world. This is mainly due to their higher energy, low cost, ready-to-eat nature, availability in different tastes and extended shelf life (Ajila *et al.*, 2008). In addition, cookies may represent an excellent model product for enhancement of nutritional value and fortification. The main ingredients used for production of cookies are wheat flour, fat and sugar. To obtain homogeneous dough, water is also added. Nowadays, the enrichment of these cookies with vitamins, minerals, polyphenols and fibers is considered as a good alternative to produce high nutritional value foods to improve human health. To achieve this goal, many fruits and vegetable powders are used

for the formulation of cookies such as papaya pulp flour, choke-berry extract, grape marc extract, sour cherry pomace extract and Japanese quince (Antoniewska *et al.*, 2019; Molnar *et al.*, 2015; Pasqualone *et al.*, 2014; Šaponjac *et al.*, 2016; Varastegani *et al.*, 2015). In addition, many fruits being used are gaining attention because of attractive flavor as well as diverse antioxidant, anticarcinogenic and antimutagenic substances included in them (Antoniewska *et al.*, 2019). Besides, the consumption of these products has health benefits against several chronic diseases, including cardiovascular disease and certain types of cancers (Ludwig *et al.*, 2018). Genus *Zizyphus* belonging to Rhamnaceae family is widespread in tropical and subtropical regions: Asia, Africa, North America, South America, Oceania and Europe, with the center of diversity in Asia

(Richardson *et al.*, 2004). Besides, previous studies have described the secondary metabolites and pharmacology of genus *Zizyphus*. Traditionally, *Zizyphus* plant has been used in medicines to treat different diseases such as diarrhea, cholera, bronchitis, diabetes, hypertension, inflammation and intestinal spasms (El Maaïden, 2020; Le-Floc'h, 1983). In Tunisia, the commonly known species of *Zizyphus* shrub is *Zizyphus lotus*, which is named as 'Sedra' and its edible fruit is called 'Nbeg' (Mkadmini *et al.*, 2015). Various studies have described the nutritional and beneficial effects of *Zizyphus lotus* fruits (Yamada *et al.*, 1985). This small and round fruits are a rich source of sugar (Ghedira, 2013), minerals (calcium, magnesium, iron, sodium, potassium and phosphorus), carbohydrates, fatty acids and proteins (Abdeddaim *et al.*, 2014). Authors have also highlighted the presence of large amounts of vitamins A, C and D (Chouaibi *et al.*, 2011; Ghedira, 2013). Therefore, Owing to the presence of high healthy functional components, such as polyphenols, fibers, bioactive compounds, including micronutrients and phytochemicals (Da Silva *et al.*, 2007), *Zizyphus lotus* fruits are widely consumed in many countries and used to formulate many food products such as pastes, purees, syrups and confections. Moreover, *Zizyphus* fruits are usually used as food additive in many bakery products to improve their technological quality and nutritional value. In this context, the aim of the present work was to characterize *Zizyphus lotus* fruit powder and to evaluate its effect on physico-chemical properties and sensory evaluation of cookies.

Materials and methods

Preparation of *Zizyphus lotus* powder

Zizyphus lotus fruits were collected in July 2020 from Goubollat region of Beja in the northwest of Tunisia. The edible part of the fruits were manually isolated from seeds, sun-dried for 120 h, and milled. The obtained samples were then sieved through a 75- μ m sieve to get fine powder of *Zizyphus lotus* fruits (ZLP) and finally stored in plastic containers at 4°C until further use.

Chemical composition

Moisture, protein, fat and ash contents of ZLP and cookie samples were determined according to the method described by Association of Official Analytical Chemists (AOAC, 2005). Total carbohydrate content was calculated by difference (100 – sum of protein, fat, ash and moisture). The results were expressed as g per 100 g of dry weight. Energy was calculated as follows:

$$\text{Energy (kcal/100 g)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat}).$$

The water activity (A_w) was determined using Aqua Lab (Meter, AquaLab Series 3TE, USA) at 25°C. Each assay was carried out in triplicate.

Techno-functional properties of ZLP

Water absorption capacity (WAC) was determined according to the method described by Anderson *et al.* (1970). In a pre-weighed centrifuge tube, 1-g sample was mixed with 10-mL distilled water. The tube was stirred for 2 min and then centrifuged at 3,000 rpm for 15 min. The supernatant was decanted into a tared evaporating dish for determining its solid content and the sediment was weighed. The WAC was calculated as weight of sediment (g) per weight of sample (g) on a dry basis. Oil absorption capacity (OAC) was determined according to the method described by Kaur and Singh. (2005). An amount of 0.5 g of sample was mixed with 6 mL of corn oil in pre-weighed centrifuge tubes, stirred for 1 min, left for 30 min and centrifuged at 3,000 g for 25 min. The separated oil volume was recorded and the tubes were inverted for 25 min to drain out the oil prior to reweighing. The OAC was expressed as gram of oil bound per gram of sample on a dry basis. Swelling index (SI) was determined by the procedure described by Rosell *et al.* (2009). An amount of 1 g of sample was transferred into a graduated cylinder, it was gently leveled and the volume was noted. Then 10 mL of distilled water was added to the sample; the cylinder was swirled and left to stand for 60 min while the swelling (change in volume) was recorded every 15 min. The swelling index of samples was determined as a multiple of original volume.

Fatty acid extraction and analysis

Zizyphus lotus powder (20 g) was extracted in a Soxhlet apparatus with 250 mL of ether of petroleum at 40°C. Between each step, the extract was filtered with a Whatman filter paper (N°4), concentrated under rotary vacuum evaporator (Rotovapor-EL, Labortechnik AG, Büchi, Switzerland) at 40 C and conserved at 4°C for analysis

Fatty acid composition of ZLP lipid fraction was determined as fatty acid methyl esters using gas chromatography (Agilent 19091S-433) equipped with a flame ionization detector and a polar phenylmethyl-siloxane capillary column (60 m \times 25 mm \times 0.25 μ m film thickness) as described by Zarroug *et al.* (2015).

Formulation of cookies enriched with ZLP

Cookies were formulated according to the method described of Tyagi *et al.* (2020) with minor modification.

The ingredients used for preparing cookies are as follows: wheat flour, ZLP, 25-g powdered sugar, 26-g margarine, 0.6-g baking powder, 12-mL water, 0.7-g salt and 18-g whole eggs. Control cookie (CC) samples were prepared with wheat flour (100%) and other ingredients whereas the enriched cookie samples were formulated using combinations of wheat flour and ZLP. The ZLP was incorporated into cookies at the following five levels: 0% (CC), 15% (C15), 30% (C30), 45% (C45) and 100% (C100) by replacing the equivalent amount of wheat flour from the formulation. For preparation of cookies, the process consisted of mixing wheat flour (or ZLP), margarine and sugar using a mixer (Kenwood, Poland) for 7 min. Then whole eggs and required amount of water were added to this mixture with the rest of ingredients to obtain homogeneous dough. Prepared dough was rolled out to 2-mm thick circular shapes and biscuit dough was cut with a circular cutter to obtain pieces of 5-cm diameter. Finally, cookies were baked in oven (Convection, Type De Dietrich) at 175°C for 15 min. The baked cookies were cooled for 30 min at ambient temperature and stored in air-tight bags for further analysis.

Physical, color and textural analysis of cookies

Physical parameters, including weight (g), thickness (T), diameter (D) and spread ratio (D/T) were determined for cookie samples according to the procedure described by Hussai *et al.* (2006). Color measurement of the surface of the studied cookie samples was determined by a Chromameter (Konika Minolta, Sensing INC, Japan) using the hunter L^* , a^* and b^* . The L^* values measure black (0) to white (100), a^* values measure redness (+100) and greenness (-100), and b^* values measure yellowness (+100) and blueness (-100). The hardness of formulated baked cookies was studied by three-point bending tests at room temperature. A texture analyzer TAXT2i (Perten, TVT 6700, UK) was used with 1,000-N load cell and a sharp blade cutting probe (HDP/BSK blade set with knife). The distance between support bars was 4 cm and the probe travelling speed was set at 1 mm/s. Texture analysis were carried out in triplicate for each cookie formulation.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis of cookie samples was carried out in TGA-4000 Perkin Elmer (CURIE GRANT) and analyzed by the Pyris Manager software. The analysis was carried out by standard protocol. Weight loss (weight%) was observed with respect to temperature, and graphs were obtained for each sample with the Pyris software.

Fourier-transform infrared spectroscopy (FTIR)

Functional groups present in cookie samples were characterized by the Perkin Elmer FTIR instrument equipped with the software Perkin Elmer Spectrum Version 10.4.3. The samples were grinded with potassium bromide pellets and measured at a wavelength range of 400–4,000 cm^{-1} .

Scanning Electron Microscopy (SEM)

Microstructure of baked cookies was evaluated using SEM (JEOL/JSM-5400) as described by Adebisi *et al.* (2016). Cookies were milled, sieved and then observed using two magnification levels of $\times 400$ and $\times 1,500$.

Phytochemical Analysis and Antioxidant Activities of ZLP and Formulated Cookies

Extract preparation

Zizyphus lotus powder was extracted by maceration using the following solvents: acetone (60%), ethanol (60%), methanol (60%) and water. This extraction was done according to the method described by Mau *et al.* (2001). Triplicate samples of 2.5 g of dry matter were extracted by mixing with 25 mL of solvent. The mixture was stirred for 30 min and kept in darkness for 24 h at 4°C. Finally, this mixture was filtered with a Whatman filter paper ($N^{\circ}4$) and concentrated under rotary vacuum evaporator at 40°C. The dry residues were stored at 4°C for further analysis. Each extraction was done in triplicate. For cookie samples, extracts were prepared using the method described by Blanco Canalis *et al.* (2020). Cookies were milled and defatted in 30-mL hexane at 70°C for 20 min. Cookie sample, 100 mg, was extracted with 1 mL of acetone:water (70:30 v/v) mixture, and agitation in vortex was applied for 5 min at room temperature. Then the extracts were centrifuged for 10 min at 800 \times g and supernatants were collected. The supernatants were filtered and stored for further analysis.

Determination of total polyphenols (TPC), total flavonoids (TFC) and condensed tannins content (CTC)

Content of TPC was determined using the Folin–Ciocalteu spectrophotometric method (UV–VIS) as described by Dewanto *et al.* (2002). TPC was expressed as milligram of gallic acid equivalent per gram of dry matter (mg EAG/g DW) through the calibration curve with gallic acid. For determining TFC, 250 μL of methanolic extract was combined with 75- μL NaNO_2 (5%) (Dewanto *et al.*, 2002). TFC levels were expressed in

milligram of quercetin equivalent per gram of dry matter (mg EC/g DW). The protocol followed in the extraction of CTC was that recommended by Sun *et al.* (1998). CTC was expressed in milligram of catechin equivalent per gram of extract (mg CE/g DW).

Antiradical activity

Methanol extract, 1,000 μL , was added to 500 μL of 2,2-diphenyl-1-picrylhydrazyl (DPPH, 0.2 mM; Hatano *et al.*, 1988). After vigorous stirring, the mixture was kept at room temperature for 30 min in the dark and the absorbance was measured at 517 nm. The antiradical activity was calculated as percentage of inhibition (PI) of DPPH using the following formula:

$$\text{Percentage of inhibition (PI)} = \frac{\text{Do}_{\text{control}} - \text{Do}_{\text{extract}}}{\text{Do}_{\text{control}}}$$

where $\text{Do}_{\text{control}}$ is the absorbance of control at 30 min and $\text{Do}_{\text{extract}}$ is the absorbance of extract. The antiradical activity was finally expressed as IC_{50} ($\mu\text{g mL}^{-1}$). A lower IC_{50} value corresponds to a higher antioxidant activity of the extracted sample (Patro *et al.*, 2005). All samples were analyzed in three replications.

Reducing power

This method consists of mixing 1,000 μL of fraction at different concentrations with 1,250 μL of phosphate buffer (0.2 mol/L, pH 6.6) and 1,250 μL of $\text{K}_3\text{Fe}(\text{CN})_6$ (1%) (Ferreira *et al.*, 2007). The solution was then kept in water bath for 20 min at 50°C. To stop reaction, 1,250 μL of trichloroacetic acid (TCA) (10%) was added to the solution followed by centrifugation at 650 g for 10 min at 25°C. Finally, at the intake of 1,250 μL of supernatant, 1,250 μL of H_2O and 250 μL of FeCl_3 (0.1%) were added to the solution and the absorbance was measured at 700 nm.

Sensory analysis of cookies

Sensory analysis of cookies was conducted by 15 semi-trained panelists (research students and laboratory staff) from the Field Crops Laboratory, INRAT, Tunisia. They were asked to evaluate cookie samples for flavor, color, texture, after taste and overall acceptability. After cooling, the cookies were coded and served to panelists in plastic containers with mineral water to cleanse the palate between each tasted sample. The scores were based on the following criteria: 9 = liked extremely; 8 = liked very much; 7 = liked moderately; 6 = liked slightly; 5 = neither liked nor disliked; 4 = disliked slightly; 3 = disliked moderately; 2 = disliked very much; 1 = disliked

extremely. Average of the scores was calculated and rounded off to the nearest whole number. Sensory analysis of cookie samples was carried out in triplicate for each sample.

Statistical analysis

Each analytical determination was performed at least in triplicate. Values of different parameters were expressed as mean \pm standard deviation ($\bar{X} \pm \text{SD}$). Statistical analyses were performed with the STATISTICA software. The Duncan's test was used to evaluate the significance of differences between mean values at $p < 0.05$.

Results and Discussion

ZLP characterization

Chemical composition and techno-functional properties of ZLP

Results of chemical composition and techno-functional properties of ZLP are presented in Table 1.

The ZLP had a moisture content of 9.55%, which was lower than those reported for ZL fruits from Algeria (Saadoudi *et al.*, 2017), but was within the recommended moisture contents (<14%) for safe storage, minimal microbial growth and chemical deterioration, leading to longer shelf life. Protein, fat and ash contents of ZLP were 2.64%, 5.37% and 3.01%, respectively. Fat and ash contents in Tunisian ZLP were higher than those found by Choi *et al.* (2016) for Korean jujubes. However, the protein content in ZLP was higher than those found by Ghalem *et al.* (2014) (2.10%) and Saadoudi *et al.* (2017) (1.43%) in *Zizyphus lotus* from Algeria. The carbohydrates content in Tunisian ZLP, about 79.43%, was lower than those found by Li *et al.* (2007) in five Chinese *Zizyphus lotus* cultivars (ranged from 80.86% to 85.63%). The observed difference in chemical composition of ZLP depended on the location and the used species. In comparison to other fruit powders, the protein and ash contents in ZLP were lower than those found in the *Tinospora cordifolia* stem powder (proteins: 5.21% and ash: 6.26%) by Tyagi *et al.* (2020).

Referring to the techno-functional properties of ZLP, the values of WAC, SI and OAC in ZLP were about 2.81 g/g, 5.5 mg/g and 2.96 g/g, respectively. The obtained WAC (about 1.32 g/g) and OAC (about 1.20 g/g) values were higher than those detected in pearl millet flour (Adebiyi *et al.*, 2016). The WAC of ZLP was lower than those found in some cereals, such as in rice bran (around 5.21 g/g) (Sangnark and Noomhorm, 2004) and fruit coproducts such as passion fruit (13.5 g/g) and pineapple

Table 1. Chemical composition and techno-functional properties of ZLP.

Parameters	ZLP
Moisture (%)	9.55 ± 0.07
Fat (%)	5.37 ± 0.42
Protein (%)	2.64 ± 0.07
Ash (%)	3.01 ± 0.02
Carbohydrates (%)	79.43 ± 1.6
Energy (kcal/100 g)	388.65 ± 2.92
SI (mg/g)	5.5 ± 0.02
WAC (g/g)	2.81 ± 0.07
OAC (g/g)	2.96 ± 0.22
Fatty acids (% total fatty acid)	
Palmitic acid (C16:0)	13.12 ± 0.41
Stearic acid (C18:0)	4.72 ± 0.22
Arachidic acid (C20:0)	1.00 ± 0.01
Behenic acid (C22:0)	0.92 ± 0.06
Tetracosanoic acid (C24:0)	0.60 ± 0.02
Oleic acid (C18:1)	55.73 ± 0.85
10-Octadecenoic acid methyl ester (C19:1)	0.96 ± 0.05
Gadoleic acid (C20:1)	2.64 ± 0.01
Linoleic acid (C18:2)	20.31 ± 0.24
∑ Saturated fatty acids (%)	20.36
∑ Monounsaturated fatty acids (%)	59.33
∑ Polyunsaturated fatty acids (%)	20.31
Ratio: Unsaturated:saturated fatty acids	3.91

Values are expressed as mean ± SD of three determinations.
SI: swelling index; WAC: water absorption capacity; OAC: oil absorption capacity.

(14.6 g/g) (Martínez *et al.*, 2012). As noted, the hydration properties of ZLP were related to the chemical structure of polysaccharides, proteins and the fruit source. However, OAC is the capability of vegetable apolar chain protein to physically bind lipids by capillary attraction. This interaction is essential in food applications, because oil is known as retainer of flavor and enhances mouth-feel of food-formulated products. In addition, a better SI improved functionality of flour, which would ultimately yield a good product (Adebiyi *et al.*, 2016).

Composition of fatty acids

In ZLP, saturated fatty acids represented 20.36% of total fatty acids, while monounsaturated fatty acids accounted for 79.64%. Nine fatty acids were identified, where oleic acid was the major one accounting for 55.73%, followed by linoleic acid with 20.31% of total fatty acids (Table 1). Since ZL oil was rich in both oleic and linoleic acids, it might be considered healthier for human consumption. It has long been recognized that plant oils containing relatively low concentrations of omega-6 and higher levels of

monounsaturated fatty acids (mainly oleic acid) may contribute to the lower rate of coronary heart disease (CHD) and a nutritional perspective (Ryan *et al.*, 2007). Other representative fatty acids were palmitic (13.12%), stearic (4.72%) and gadoleic (2.64%) acids. In addition, arachidic, behenic, tetracosanoic and 10-octadecenoic acid methyl ester acids were minor fatty acids with contents varying from 0.60% to 1%. These results are similar to those reported by Chouaibi *et al.* (2011) for ZL seed oil.

Phytochemical composition and antioxidant activities of ZLP

Total polyphenols, TFC and CTC of different extracts are summarized in Figure 1. The highest contents of TPC, TFC and CTC were obtained in the acetone (60%) extract with values of 3.12 mg GAE/g DW, 0.49 mg CE/g DW and 1.09 mg CE/g DW, respectively. The lowest contents were recorded in the methanol fraction. According to Wojdyło *et al.* (2016), ZL fruit is considered as a rich source of bio-active components, including polyphenols, triterpenic acids, polysaccharides, nucleosides and nucleobases. In general, phenolic compounds in ZL fruit contribute to the antioxidant potential of its powder extracts. Authors have reported correlation between phenolic compounds and antioxidant activity (Elfalleh *et al.*, 2011). Antioxidant activity of different extracts of ZLP was examined by its radical scavenging activity using the stable radical DPPH and by reducing powers (Table 2). Methanol (60%) and acetone (60%) fractions exhibited the uppermost anti-radical capacities with IC₅₀ reaching 70 µg/mL and 75 µg/mL, respectively. The obtained results are lower than those found in Moroccan ZL (IC₅₀ = 477.6 µg/mL) using methanol solvent (70%) (Bakhtaoui *et al.*, 2014). Previous study conducted on ZL fruit from another region of Tunisia revealed IC₅₀ = 289 µg/mL (Mkadmini *et al.*, 2015) and 310 µg/mL (Ghazghazi *et al.*, 2014). The *Zizyphus mauritiana* fruits from Nigeria exhibited IC₅₀ = 338.45 µg/mL (Okala *et al.*, 2014), which was higher than the value obtained in the present study. The consequences of solvent on the antioxidant ability were also analyzed by determining reducing powers (Figure 1). The highest antioxidant potential was observed in acetone fractions (330 µg/mL). The obtained result was similar to that found in southern Tunisian ZL fruits (289 µg/mL) (Mkadmini *et al.*, 2015). The observed variability of phytochemical contents and antioxidant activities of *Zizyphus* genus could be explained by various factors, such as the geographical provenance, grown conditions, types of species and the nature of solvent used for extraction (Ksouri *et al.*, 2008).

Cookies characterization

Nutritional composition of formulated cookies

Nutritional composition of enriched cookies with ZLP is presented in Table 2. Results established that the addition

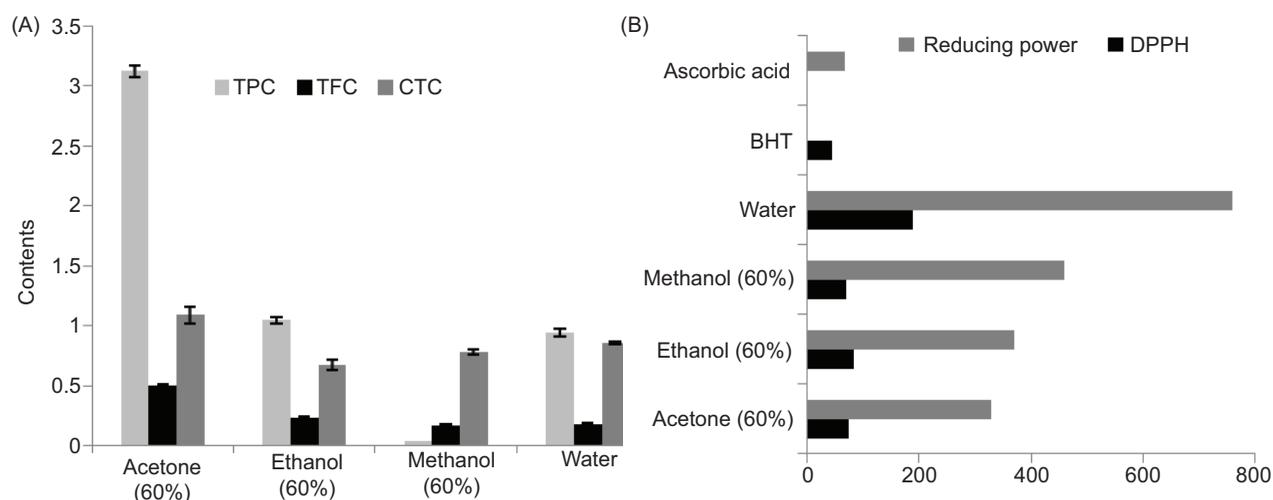


Figure 1. (A) TPC, TFC and CTC of different solvent extracts, and (B) antioxidant activities of ZLP. TPC: total polyphenols content (mg GAE/g MS); TFC: total flavonoids content (mg CE/g MS); CTC: condensed tannins content (mg CE/g MS); DPPH (IC₅₀, µg/mL); reducing power (EC₅₀, µg/mL); GAE: gallic acid equivalents; CE: catechin equivalents.

Table 2. Nutritional analysis of formulated cookies.

Cookies	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrates (CHO %)	Water activity (Aw)	Energy (kcal/100 g)
CC	5.14 ± 1.20 ^e	17.10 ± 0.01 ^a	7.10 ± 0.03 ^e	0.84 ± 0.02 ^a	69.82 ± 1.22 ^a	0.50 ± 0.01	461.58 ± 4.57 ^a
C15	6.55 ± 0.32 ^d	18.90 ± 0.01 ^b	6.60 ± 0.02 ^d	0.92 ± 0.03 ^b	67.03 ± 0.34 ^b	0.53 ± 0.02	464.62 ± 2.13 ^a
C30	7.29 ± 0.37 ^c	19.50 ± 0.02 ^c	6.35 ± 0.02 ^c	1.10 ± 0.03 ^c	65.76 ± 0.41 ^c	0.55 ± 0.13	463.94 ± 1.33 ^a
C45	7.62 ± 2.48 ^b	19.70 ± 0.01 ^d	6.06 ± 0.02 ^b	1.03 ± 0.01 ^d	65.59 ± 2.48 ^c	0.57 ± 0.01	463.90 ± 9.29 ^a
C100	7.64 ± 0.53 ^a	19.90 ± 0.05 ^e	4.94 ± 0.01 ^a	1.50 ± 0.01 ^e	66.02 ± 0.51 ^d	0.59 ± 0.01	462.94 ± 1.21 ^a

Mean values with different superscript alphabets in the same column differ significantly. CC: control cookies; C15, C30, C45, C100 are cookie samples containing wheat flour:ZLP in the ratio of 85:15, 70:30, 55:45 and 0:100, respectively.

of ZLP significantly increased ($p < 0.05$) the moisture, ash, fat and carbohydrates contents in cookies when compared to control cookies whereas protein values were decreased significantly.

In all formulated cookies, carbohydrates were the major component, followed by fat, protein, water and ash. Results demonstrated statistically significant differences ($p < 0.05$) between formulated cookies, especially for moisture, fat, protein and ash contents. Only for carbohydrates content, a nonsignificant difference was observed. Similar results revealed increase in ash and moisture contents in cookies enriched with spinach (5–15%) (Galla *et al.*, 2017) and *Tinospora cordifolia* stem (2–12%) powders (Tyagi *et al.*, 2020). In the same context, Bhat *et al.* (2020) reported consistent results in cookies containing tomato powder and crude lycopene. The energy values of enriched cookies ranged from 461.58 kcal in CC sample to 464.62 kcal in C15 sample. These results were in consistency with previous studies carried by Kaur *et al.* (2017) and Saadoudi *et al.* (2017) on cookies enriched with ZLP

from Algeria and raw flaxseed flour. About water activity, similar to that observed for moisture content, addition of ZLP in cookies provoked a significant increase in this parameter. The highest water activity value was observed in C100 sample (0.59), but it was still below than that allowed for growth of microorganisms ($Aw > 0.6$) (Chieh, 2006). These findings were higher than those reported by Milićević *et al.* (2020), who revealed water activity values ranging from 0.297 to 0.411 in cookies prepared with wheat bran gels.

Physical, textural and color characteristics of formulated cookies

According to Pareyt and Delcour (2008), quality of good cookies is related to large piece diameter, tender but snapping final product, and uniform surface-cracking pattern. The physical, textural and color characteristics of formulated cookies are depicted in Table 3. The weight of cookies enriched with ZLP varied from 12.5 g in C15 sample to 13.27 g in C100 sample. After addition of ZLP, the weight of cookies increased significantly ($p < 0.05$) in

Table 3. Physical, textural and color analysis of formulated cookies

Cookies	Diameter (D) (mm)	Thickness (T) (mm)	Weight (g)	Spread ratio (D/T)	L*	a*	b*	Hardness (N)	Fracturability (mm)
CC	57.60 ± 0.24 ^a	5.03 ± 0.02 ^a	13.13 ± 0.21 ^b	2.60 ± 0.24 ^a	66.72 ± 0.56 ^c	4.17 ± 0.12 ^a	47.83 ± 0.17 ^e	22.33 ± 1.53 ^a	0.58 ± 0.03 ^a
C15	56.70 ± 0.92 ^a	5.09 ± 0.09 ^a	12.50 ± 0.95 ^{ab}	1.70 ± 0.92 ^a	46.62 ± 0.02 ^d	10.06 ± 0.07 ^b	36.97 ± 0.014 ^d	40.00 ± 4.58 ^b	0.99 ± 0.06 ^{ab}
C30	57.10 ± 0.43 ^a	5.50 ± 0.32 ^b	12.70 ± 0.36 ^{ab}	2.10 ± 0.43 ^a	39.72 ± 0.25 ^c	11.58 ± 0.08 ^c	34.56 ± 0.17 ^c	51.33 ± 1.53 ^c	1.41 ± 0.05 ^b
C45	57.23 ± 0.66 ^a	5.55 ± 0.33 ^b	12.80 ± 0.21 ^b	2.23 ± 0.66 ^a	36.88 ± 0.82 ^b	11.43 ± 0.29 ^c	32.86 ± 0.14 ^b	65.00 ± 2.65 ^d	2.45 ± 0.06 ^c
C100	57.32 ± 0.46 ^a	6.15 ± 0.10 ^c	13.27 ± 0.38 ^b	2.32 ± 0.46 ^a	32.53 ± 0.31 ^a	12.33 ± 0.18 ^b	29.64 ± 0.05 ^a	74.33 ± 2.52 ^c	6.80 ± 0.66 ^d

Mean values with different superscripts on the same column differ significantly (Duncan's LSD test, $p < 0.05$). Where CC: control cookies, C15, C30, C45, C100 are cookies samples containing wheat flour: ZLP (85:15, 70:30, 55:45, 0:100).

comparison to CC sample. This increase in the weight of cookies was related to the presence of dietary fibers in ZLP, which enables to bind with water molecules and prevents loss of moisture during baking process. These results are in the trend of those observed by Saadoudi *et al.* (2017) on biscuits made with Algerian ZLP. In all formulated cookies, the diameter ranged from 56.70 mm to 57.60 mm. Maximum diameter was observed in CC sample, while minimum was in C15 cookie sample. These variations could be due to the dilution of gluten present in wheat flour. Results also demonstrated increase in the thickness of cookies from 5.03 mm (CC sample) to 6.15 mm (C100 sample). The obtained results of cookies enriched with ZLP are in accordance with the studies reported by Kaur *et al.* (2017) and Saadoudi *et al.* (2017) on some fortified cookies. A decrease in the spread ratio of cookies was observed with the inclusion of ZLP; this reduction could be due to the water absorption capacity of fibers present in ZLP. Similar results were obtained by Das Chagas *et al.* (2020), Ismail *et al.* (2014) and Toledo *et al.* (2017) when evaluating the effect of addition of by-products of pomegranate peels, pineapple, apple, melon, and camu-camu in cookies.

In order to evaluate the acceptability of prepared cookies by consumers, it is very important to determine their color parameters. The photographic images (Figure 2) of formulated cookies revealed that ZLP was the main ingredient that developed dark color, and C100 sample was the much darker cookies, looking like biscuits made with chocolate. The statistical analysis revealed a significant difference ($p < 0.05$) between all cookie samples regarding L* and b* values. In addition, as observed in Table 4, CC sample had the highest L* and b* values as compared to the cookies enriched with ZLP. Moreover, addition of ZLP decreased L* and b* values but increased a* values of formulated cookies. These effects could be explained by the dark color of cookies because of added ZLP, which is related to its richness in natural pigments such as polyphenols (Ajila *et al.*, 2008; Takeungwongtrakul and Benjakul. 2017). According to Masmoudi *et al.* (2021), the dark color of cookies could be also due to the more pronounced Maillard and caramelization reactions during the baking process, when wheat flour was substituted by ZLP containing higher content of sugar. These findings are in agreement with those of biscuits supplemented with Doum dietary fiber (Aboshora *et al.*, 2019) and bamboo shoot powder (Ajila *et al.*, 2008).

Regarding texture analysis, results revealed that the hardness and fracturability (**brittleness**) of cookies were increased significantly ($p < 0.05$) with the addition of ZLP. In all formulated cookies, C100 sample had the highest hardness and fracturability values (74.33 N and 6.8 mm) compared to CC sample (22.33 N and 0.58 mm). Similar results were also observed in previous findings in

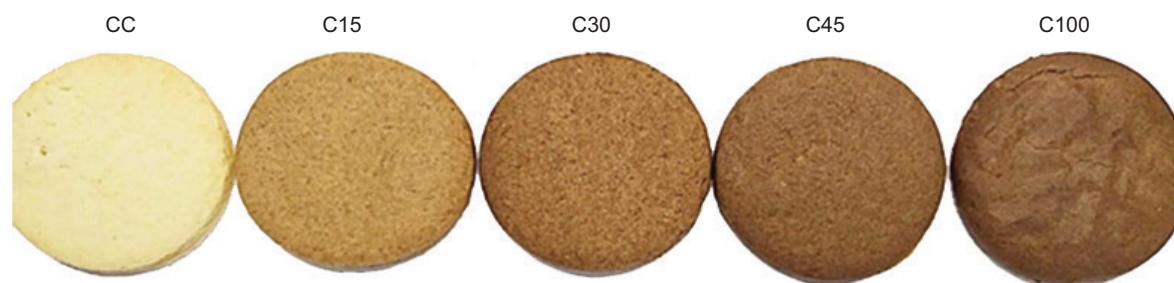


Figure 2. Photographic images of cookie samples. CC: control cookies, C15, C30, C45, C100 are cookie samples containing wheat flour:ZLP in the ratio of 85:15, 70:30, 55:45 and 0:100, respectively.

which cookies were enriched with bamboo shoot, spinach, *Tinospora cordifolia* stem and tomato powder (Ajila *et al.*, 2008; Bhat *et al.*, 2020; Galla *et al.*, 2017; Tyagi *et al.*, 2020). This increase in texture parameters are explained by the presence of ZLP, with high content of polysaccharides, which may lead to the dilution of gluten and extensive gluten structure. In addition, substituting ZLP for wheat flour in cookies formulation decreased its gluten content responsible for the viscoelastic character of dough. Das Chagas *et al.* (2020) affirmed in their study on cookies enriched with camu-camu coproduct powder that reduction of glutenin and gliadin proteins could have a remarkable effect on the formation of viscoelastic dough, which may possibly lead to increased hardness.

TPC, TFC and CTC of cookies

Table 4 reveals the TPC, TFC and CTC values of defatted methanolic extract of formulated cookie samples. Results revealed that the addition of ZLP increased significantly ($p < 0.05$) TPC, TFC and CTC in enriched cookie samples when compared to CC sample, and this increase was effectively due to the high content of phenolic

compounds such as protocatechuic acid, gallic acid, chlorogenic acid, caffeic acid and ascorbic acid present in ZLP (Koley *et al.*, 2016). In all formulated cookies, C100 sample contained the highest contents of TPC (0.11 mg GAE/g), TFC (0.05 mg QE/g) and CTC (1.07 mg QE/g). Our results were generally in agreement with the findings of other studies regarding the substitution of wheat flour in cookies by some fruit and vegetable powders such as apple, pineapple and melon coproducts (Toledo *et al.*, 2017), watermelon rind powder (Naknaen *et al.*, 2016) and camu-camu coproduct (Das Chagas *et al.*, 2020).

Antioxidant potential of cookies

Table 4 indicates that the addition of ZLP in cookies significantly ($p < 0.05$) increased the free radical scavenging activity and reducing power as compared to CC sample. This implies that the addition of higher level of ZLP improved the antioxidant activity of cookie products. In all formulated cookies, the highest free radical scavenging activity and reducing power were observed in C100 sample. This observation was in accordance with previous studies carried out with incorporation of

Table 4. Phytochemical analysis and antioxidant activities of formulated cookies.

Cookies	Phytochemical parameters			Antioxidant activities	
	TPC ^a	TFC ^b	CTC ^b	DPPH ^c	Reducing power ^d
CC	<0.01	0.01 ± 0.01 ^a	0.57 ± 0.14 ^e	454 ± 0.54 ^a	950 ± 3.42 ^a
C15	0.01 ± 0.02 ^a	0.02 ± 0.01 ^b	0.80 ± 0.15 ^d	355 ± 0.21 ^a	820 ± 2.45 ^d
C30	0.02 ± 0.01 ^b	0.03 ± 0.02 ^c	0.93 ± 0.1 ^b	280 ± 0.15 ^b	410 ± 1.34 ^c
C45	0.05 ± 0.01 ^c	0.04 ± 0.01 ^d	1 ± 0.05 ^a	165 ± 0.1 ^c	380 ± 3.5 ^b
C100	0.11 ± 0.04 ^d	0.05 ± 0.01 ^e	1.07 ± 0.01 ^c	95 ± 0.24 ^d	320 ± 2.45 ^a
Synthetic standards					
BHT (IC ₅₀ , mg/mL)	–	–	–	46.6 ± 0.08	–
Ascorbic acid (EC ₅₀ , mg/mL)	–	–	–	–	68 ± 0.06

^a(mg GAE/g), ^b(mg QE/g), ^c(IC₅₀, µg/mL), ^d(EC₅₀, µg/mL).

BHT: Butylated hydroxytoluene; TPC: total polyphenols content; TFC: total flavonoids content; CTC: Condensed tannins content; CC: control cookies; C15, C30, C45, and C100: cookie samples containing the wheat flour:ZLP in the ratio of 85:15, 70:30, 55:45, 0:100, respectively. BHT: butylated hydroxytoluene. an values with different superscripts in the same column differ significantly (Duncan's LSD test, $p < 0.05$).

freeze-dried Japanese quince fruits (Antoniewska *et al.*, 2019) and tomato powder in cookies (Bhat *et al.*, 2020). Several authors also demonstrated that the DPPH radical scavenging activity and reducing power increased in cookies with baking temperature, which could be due to the formation of melanoidins produced in the Maillard reaction (Bhat *et al.*, 2020). In addition, high concentration of bioactive compounds in ZLP lead to an increase in the antioxidant potential of formulated cookies. This could be of interest for human health and could provide an extended shelf life of cookies for food industry.

FTIR analysis

The FTIR spectra of cookie samples in the range of 400–4,000 cm^{-1} are revealed in Figure 3. Results revealed that cookie samples demonstrated common peaks with small variations in intensity. In all cookie samples, major peaks were observed at 3,500 cm^{-1} and 2,800 cm^{-1} , which indicated the stretch vibrations of O–H groups. In addition, the observed peak at 2,943 cm^{-1} resulted from the stretching vibration of C–H bond. However, the peaks found between 1,645 cm^{-1} and 1,742 cm^{-1} were attributed to the C–O stretch vibration of α,β -unsaturated compound. Similar results were reported by Tyagi *et al.* (2020) on cookies enriched with *Tinospora cordifolia* stem powder. The observed peaks at 1,485.71 cm^{-1} were essentially assigned to water molecules absorbed in the amorphous region. The established peaks at a range of 1,142–1,000 cm^{-1} could be attributed to the C–O bond and aliphatic C–N stretching; these peaks were with higher intensities in cookie samples enriched with ZLP compared to CC sample. These peaks are related to the presence of phenolic compounds, β -glycoside and glucoside (Tyagi *et al.*, 2020), which were essentially present in

ZLP. Besides, decrease in the intensity of peaks in cookie samples reflects their change in crystallinity.

Thermogravimetric analysis

In general, when materials are heated, they lose weight because of simple processes, such as drying, or because of chemical reactions that release gasses (Blanco Canalis *et al.*, 2018). The observed weight loss, which was related to reduction of water by evaporation can provide valuable information about the volatile and heat labile compounds present in cookie samples and their thermal stability. The TGA and differential TGA (Dr-TGA) curves of cookie samples are revealed in Figure 4(a) and Figure 4(b). The TGA of all studied cookie samples demonstrated four typical stages of weight loss. Figure 4(b) indicates that no significant differences in shape and intensity were observed between C45 and C100 samples. Comparison between the cookies enriched with ZLP and control samples indicated that the maximum rate of weight loss was in C45 sample (92.69%) followed by C100 (91.72%). The first stage of weight loss ranged from 190°C to 211°C, and was attributed to the loss of free and bound water with increasing temperature. It is suggested that inclusion of ZLP in cookies reduced the amount of water available for protein hydration, allowing faster water evaporation. In addition, the observed increase in weight loss suggested the formation of a weak gluten network in cookies dough. According to Blanco Canalis *et al.* (2018), water in cookies dough is shared between different components (such as starch, gluten and sucrose) responsible for trapping water until it is released because of heating. The second, third and fourth stages ranged from 229.54°C to 254.38°C, 311.85°C to 373.63°C and 426.29°C to 470.3°C, respectively. The second stage represented

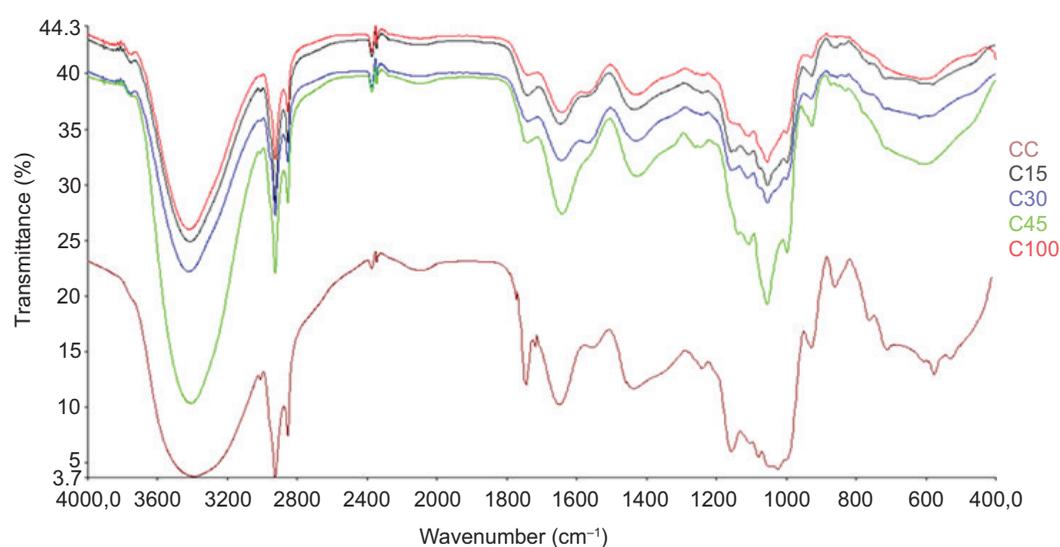


Figure 3. FTIR spectra of cookie samples; CC: control cookies; C15, C30, C45 and C100 are cookie samples containing wheat flour:ZLP in the ratio of 85:15, 70:30, 55:45 and 0:100, respectively.

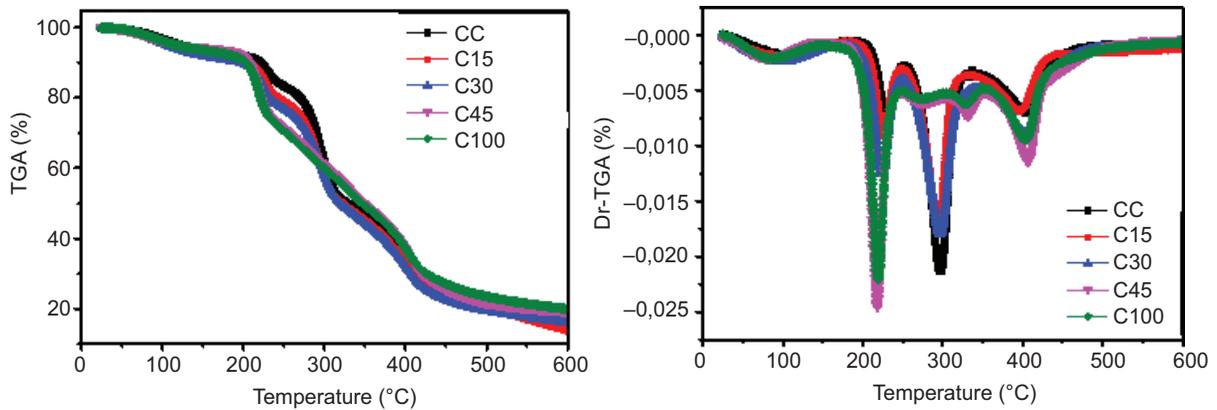


Figure 4. (A) Thermogravimetric, and (B) differential thermogravimetric curves of cookie samples; CC: control cookies; C15, C30, C45 and C100 are cookie samples containing wheat flour:ZLP in the ratio of 85:15, 70:30, 55:45, 0:100, respectively.

the depolymerization and degradation of organic matter (starch, gluten and polysaccharides). However, the third stage of weight loss represented the oxidation of organic matter that lead to the formation of ash residue. In the last range of temperature (close to 500°C), the total weight loss measured for cookie samples was not noticeably different.

Microstructure of cookie samples

Scanning electron microscopy was used to study the microstructure of baked cookie samples. The microstructure observation indicated that the protein network matrix was well developed and the starch granules were more intact in the case of control cookies (Figure 5). However, a change in the structure of cookies was observed with increase in ZLP content. This change was strongly observed in the case of C100 sample. Alteration in the microstructure of cookies could be due to the richness of ZLP in polysaccharides, which have good water retention capacity; this could also be due to the gelatinization of starch and denaturation of protein matrix. These results are in agreement with those found by Adebisi *et al.* (2016), who reported a similar microstructure of cookies enriched with millet flours.

Sensory evaluation of cookies

The flavor, color, texture, after-taste and overall acceptability scores recorded for the cookies enriched with ZL powder are depicted in Table 4. Results of sensory evaluation revealed that color, flavor and after-taste parameters increased with increase of ZLP in cookies, reaching the values of 7.87, 8.89 and 3.20, respectively, noted essentially in C100 sample. The sensory evaluation of cookies' color surface paralleled the colorimetric measurement (Table 3), with ZLP-enriched cookies being darker than the control. In addition, increase in ZLP concentration demonstrated a decrease in texture parameter in comparison to control cookies; these results were in agreement with the instrumental data of hardness and fracturability

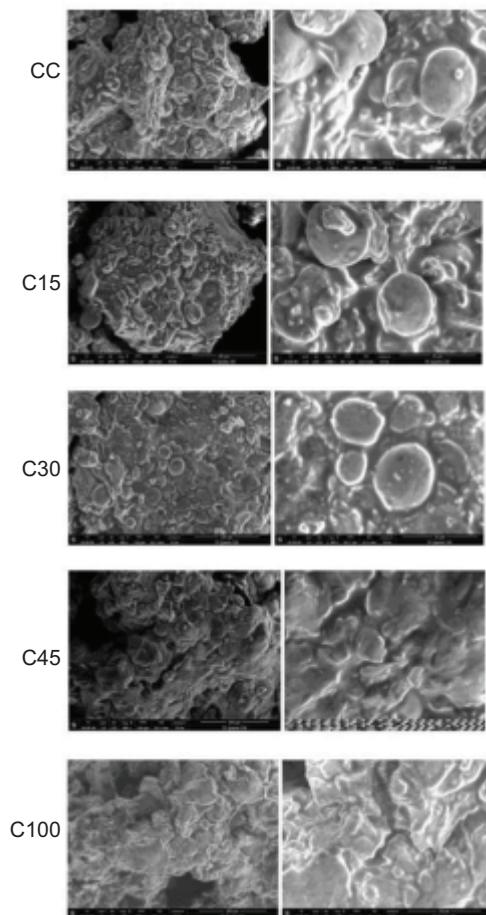


Figure 5. SEM of cookies samples. Magnification $\times 400$ and $\times 500$; CC: control cookies. C15, C30, C45, C100 are cookies samples containing wheat flour: ZLP (85:15, 70:30, 44:45, 0:100).

(Table 3). Similar effect was reported by Krystijan *et al.* (2015), Saoudi *et al.* (2017) and Šaponjac *et al.* (2016) with cookies enriched with bee pollen, jujube, and sour cherry pomace extract, respectively. In all enriched cookies, the C15 sample had a sensory score near to the CC

Table 5. Sensory acceptability of both control and formulated cookies.

Cookies	Color	Flavor	After-taste	Texture	Overall acceptability
CC	4.27 ± 1.12 ^a	6.54 ± 0.71 ^c	8.47 ± 1.30 ^a	6.60 ± 1.60 ^b	7.20 ± 2.04 ^a
C15	5.20 ± 1.00 ^b	7.33 ± 0.63 ^c	7.93 ± 0.96 ^a	5.66 ± 1.13 ^{a,b}	6.47 ± 1.30 ^b
C30	5.60 ± 0.64 ^b	7.41 ± 1.03 ^c	7.33 ± 1.05 ^a	5.06 ± 1.03 ^c	5.00 ± 1.31 ^{a,b}
C45	6.67 ± 0.63 ^c	8.27 ± 0.52 ^d	5.36 ± 1.39 ^b	4.87 ± 1.44 ^a	4.68 ± 1.25 ^a
C100	7.87 ± 0.63 ^c	8.89 ± 0.41 ^d	3.20 ± 2.01 ^b	4.07 ± 1.74 ^a	4.20 ± 2.61 ^a

Values are expressed as mean ± standard deviation of three determinations. Mean values with different superscripts in the same column differ significantly ($p < 0.05$). CC: control cookies; C15, C30, C45 and C100 are cookie samples containing wheat flour:ZLP in the ratio of 85:15, 70:30, 55:45, 0:100, respectively.

sample. For the overall acceptability of results, CC and C15 samples were most acceptable, while C100 sample was not preferred by panelists. Masmoudi *et al.* (2021) established that biscuits supplemented with jujube flour had as acceptable quality as the control for all its doses (5%, 10% and 15%). Reduction in acceptability of cookies at higher level of substitution with ZLP was due to more plant undertones contributed by ZLP. Similar effect was reported for cookies supplemented with flaxseed flour (Kaur *et al.*, 2017) and Japanese quince fruit (Antoniewska *et al.*, 2019).

Conclusion

The results of the present study reveal that ZLP contains several bioactive components, fatty acids, phenolics and antioxidants that are beneficial for human health. Results demonstrate that this powder has a great nutritional potential to be used in food industry and to substitute the conventional wheat flour in the formulation of new functional cookies. Addition of ZLP improves the phytochemical composition and the antioxidant potential of formulated cookies as revealed by the increase in DPPH and reducing power values in cookies. The FTIR and TGA results revealed the presence of many bioactive compounds in ZLP and formulated cookies. Besides improving the nutritional value, addition of ZLP affects the appearance, physicochemical, textural and sensorial properties of formulated cookies. Based on the sensory analysis, it is concluded that the acceptability of formulated cookies is directly dependent on the amount of added ZLP, and cookies enriched with 15% ZLP (C15) seems to have gathered the overall preference of panelists.

Acknowledgments

This work was supported by the Ministry of Higher Education and Scientific Research, Tunisia and the

Ministry of Agriculture, Water Resources and Maritime Fisheries, Tunisia. The authors wish to thank the technical staff, particularly Hanen Nasri and Nesrine Haj Yahya, for their valuable contribution in performing various analyses. We are also grateful to Tesnim Zarroug for her valuable efforts to check proofs of this paper.

Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the research reported in this paper.

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