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Development of Functional Biscuits Made of Wheat Flour and *Diospyros mespiliformis* Pulp

Djuikwo Nkonga Ruth Viviane[1], Ngueke Merveille[2], Ngatchic Metsagang Therese Josiane[3], Yadang Germaine[4], Youdom Patrick[5], Njintang Yanou Nicolas[6]

[1, 2]Laboratory for Food Science and Metabolism, Department of Biochemistry, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon

[3, 4, 5, 6]Department of Food Science and Nutrition, National School of Agro-industrial Science, Ngaoundere, P.O. Box 455, University of Ngaoundere, Cameroon

Abstract: ***'Functional foods' refers to traditional processed foods containing one or more ingredients that, in addition to their normal functions, promote specific physiological functions compared to the unmodified products from which they are derived'. Among the foods increasingly used for this purpose are biscuits. The objective of the present study was to design a functional biscuit enriched with the fruit pulp of Diospyrosmespiliformis and to evaluate its nutritional and anti-oxidant potential. The proximate composition of the fruit and biscuits was assessed. Antioxidant activity and sensory analysis characteristics were determined. Results revealed that Diospyros mespiliformis pulp is a potential source of carbohydrates, crude fibre and iron. An interesting antioxidant activity was noted, certainly caused by the presence of total polyphenols and β-carotene. The use of D. mespiliformis as a composite considerably enhances the nutritional composition of the biscuits. The significant increase of about 50% in the iron content suggests that Diospyros mespiliformis could contribute to the fight against anaemia. From the sensory analysis, it appears that the most appreciated biscuit after the control (BST) was BS 8 with 12.5 % wheat flour, 18.75 % sugar and 68.75 % D. mespiliformis powder.***

Keywords: *Diospyros mespiliformis*, functional biscuit formulation general acceptability, nutritional potential

1. Introduction [2]

Biscuits are pastry products made of cereals, fat, sweeteners and any other authorised ingredients, which, after baking, retain their organoleptic quality (**Cheblaoui *et al.*, 2016) [1].** These occupy an important place in the diet because of their affordability, their ready-to-eat state, and their appreciation by all age groups (**Antonios *et al.*, 20192].** Refined wheat flour is the main ingredient in their manufacture (**Divyasree *et al.*, 2020) [3].** As a result, biscuits are poor in important health-preventing components like dietary fibre, minerals, vitamins, bioactive compounds (**Sozer *et al.*, 2014) [4].**

The production of functional biscuits based on wheat flour and other locally available materials is increasingly being observed. This is the case with fruits. In India some authors made biscuits from wheat flour and ripe mango peels **(Ajila *et al* 2008) [5].** It was found that the fortified biscuits had higher dietary. Fibre content and antioxidant activity compared to the control biscuits. In South Africa people prepared biscuits made from wheat flour, prickly pear and banana peel **(Mahloko *et al.*, 2019) [6].** They noted a clear improvement in dietary. Fibre, bioactive compounds of the fortified biscuits compared to the control. It is therefore true that fruit improves the nutritional value of biscuits.

Cameroon benefits from a great diversity of fruit trees (**Mapongmetsem *et al.*, 2012) [7],** a significant part of which is still unexploited (**Tchiégang *et al.*, 2001) [8].** This is the case of *Diospyros mespiliformis*.

D. mespiliformis Hochst. Ex. of the *Ebenaceae* family, is a medicinal plant found in the Far North of Cameroon. It is known as Jackal's bay or *Poupoui* among the fufuldé (**Gautier *et al.*, 2002) [9].** Its sweet and sour fruits are sometimes eaten fresh, as jelly or dried in some countries (**Janick *et al.*, 2008) [10].** In Nigeria, **Suleiman, (2019) [11]** showed that the fruit pulp of *D. mespiliformis* has good nutritional value and a powerful antioxidant capacity. It was shown in Cameroon that the powdery fractions of its fruits had an antihyperlipidemic effect in rats (**Mai-Mbé *et al.*, 2019**) [12]. Thus, there is need to investigate whether the use of *D. mespiliformis* fruits as a composite in the production of functional biscuits could be an asset.

2. Material and Methods

2.1 Sampling

The mature and dried fruits of *D. mespiliformis were* collected at Kaélé in the Far North region of Cameroon. The fruits were sorted and cleaned, crushed manually and sieved to separate the pulp from the pits. This pulp was ground to a powder (PDm) using a *Retsch* electric grinder (*grindomix*) at 800 rpm and sieved using a 200 µm sieve. The wheat flour, granulated sugar, eggs and margarine used in this study were purchased from the local market in Ngaoundéré.

2.2 Production of biscuits

The recipe used to produce the composite biscuit is shown in Table 1. This was based on the recipe of **Miller and Mathew (1985) [13]** for the production of 500g of shortbread type biscuit dough. The *Mintab* software, using a Centroid Simplex mix design with constraints on the proportions of *D. mespiliformis* powder, wheat flour and sugar, were used to produce twelve mixtures. The biscuits were prepared as follows:

The eggs were beaten and added to the margarine/sugar mixture until a snow-white dough was obtained. The yeast and flour/powder were homogenised and added to the dough (fat, sugar and eggs) while continuing to mix by hand until a homogeneous dough was obtained. The dough was then rolled out with a wooden rolling pin on a flat surface and cut out with star-shaped cookie cutters. The biscuits were placed on finely greased baking trays and baked in a *Mat index* oven at 190°C for 15 minutes. Once baked, the biscuits were cooled at room temperature for 15 minutes and packed in plastic wrappers. They were then placed in large lidded jars for later use.

Table 1: The different ingredients and their proportions (%) / quantities (g) in each biscuit preparation

	PDm		Wheat flour		Sugar		Margarine	Yeast	Eggs
Codes	%	(g)	%	(g)	%	(g)	(g)	(g)	
TSB	0	0	66.67	250	33.33	125	125	10	3
BS 1	25	112.17	50	224.34	25	112.17	125	10	3
BS 2	33.33	198.69	66.67	250	0	0	1 25	10	3
BS 3	43.75	196.30	37.5	168.25	18.75	84.12	125	10	3
BS 4	50	224.34	50	224.34	0	0	125	10	3
BS 5	56.25	252.38	37.50	168.25	6.25	28.04	125	10	3
BS 6	62.50	250	0	0	33.33	125	125	10	3
BS 7	66.67	280.43	25	112.17	12.25	56.08	125	10	3
BS 8	68.75	308.47	12.50	56.08	18.75	84.12	125	10	3
BS 9	75	336.51	0	0	25	112.17	125	10	3
BS 10	81.25	364.56	12.50	56.08	6.25	28.04	125	10	3
BS 11	100	448.69	0	0	0	0	125	10	3

2.3 Determination of the nutritional potential of *D. mespiliformis* powder and biscuits

The water, ash and fat contents were determined using the methods described by **AOAC (1984) [14],** while the nitrogen content was determined by the Kjeldahl method (**AFNOR, 1984) [15]** and the colorimetric method of **Devani** et ***al.*, (1989 [16]).** The nitrogen content was converted to protein using the factor 6.25. Carbohydrates were assessed by the differentiation method. The energy value was calculated according to the formula: $_{EV} = (9 \times \%$ Fat) + (4 × % Protein) + (4 × % Carbohydrate) (**Livesey *et al.*, 1995) [17].** The β-carotene content was determined by the method described by **Wolff (1968 [18]).** The analysis was done in triplicate. Minerals such as iron, zinc, manganese and copper were determined by the atomic absorption spectrophotometry method described by **Benton and Vernon (1990) [19].**

2.4. Phenolic and tannin contents

The spectrophotometric method using Folin-Ciocalteu reagent described by **Wafa*et al.*, (2014) [20]** was employed to quantify total polyphenols. In alkaline medium, polyphenols reduce the Folin-Ciocalteu reagent to blue coloured tungsten-molybdenum oxide. The intensity of this blue colour provides information on the content of total polyphenols in the mixture (**Dewanto*et al.*, 2002) [21].** The latter show an absorption maximum at 760 nm, the intensity of which is proportional to the quantity of polyphenols present in each sample.

The tannins were determined by the spectrophotometric method using catechin as a standard (**Bainbridge *et al.*, 1996) [22].** In the presence of tannins, catechin develops a red coloration that absorbs with a maximum at 500 nm.

2.5 Assessment of *in vitro* antioxidant activity by free radicals

There are several methods for the determination of the *in vitro* anti-free radical activity of free radical activity of food products. In the context of this study, three methods were chosen. In this study, three methods were chosen: the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical test, the ABTS (2, 2'azion-bis (3-ethylbenzothizoline-6-sulphonic acid) stable radical test, and the FRAP (Ferric Reducing Antioxidant Power) test.

Preparation of the extract

The extract was obtained by mixing a mass of 0.1 g of each sample in 10 ml of 70% methanol. It was then stirred for two hours on a magnetic stirrer to homogenise. The resulting mixture was filtered through wattman paper No. 1 and the filtrate was collected for evaluation of the *in vitro* antioxidant activity by the following methods.

2.5.1. DPPH anti-free radical activity

The DPPH assay is used to measure the free radical scavenging capacity of the extracts. The DPPH radical scavenging activity was measured according to the protocol described by ***Zhang et al. (*2004) [23].** Indeed, this process is based on the reduction of the synthetic radical 2, 2diphenyl-1-picrylhydrazyl which is a stable radical with a violet colouration. The maximum of its absorption in the visible range is around 515-517 nm caused by the antioxidants. In the presence of the free radical scavengers, the purple DPPH (2, 2-diphenyl-1-picryl hydrazyl) is reduced to the yellow 2, 2 diphenyl-1-picrylhydrazine. Then the IC50 (50% inhibitory concentration) were obtained by graphical determination from the percentages of inhibition as a function of the concentrations obtained by extrapolation from the curve of the percentages of inhibition of the different extracts at different concentrations.

2.5.2. Method using the stable radical ABTS

The method used is the one described by **Re *et al, (*1999) [24].** The ABTS radical method is one of the most widely used tests for the determination of free radical concentration. It is based on the neutralisation of a cation radical resulting from the mono-electronic oxidation of the synthetic chromophore ABTS (2, 2' azino-bis (3-ethylbenzothiazoline -6-sulphonic acid).

2.5.3. Method using the FRAP test

Antioxidant activity was assessed by reducing antioxidant power (FRAP). This determined their ability to reduce iron (III) to iron (II) **Oyaizu (1986) [25].** The presence of reducing compounds is indicated by the ability of these extracts to reduce ferric ions (Fe3+) from the ferrocyanide complex to ferrous ions (fe2+)$^{.}$ This causes the solution to change colour from yellow to green. This colour change is a function of the reducing power of the sample and its intensity is examined at 700 nm.

2.6 Functional properties of *D. mespiliformis* powder and biscuits

2.6.1. Determination of the water absorption capacity of biscuits

It was determined by the method of **Phillips *et al. (*1988) [26]** which consist of weighing about 1 g of *D. mespiliformis* powder and biscuits in 15 ml centrifuge tubes. Approximately 10 ml of distilled water was added to each tube and mixed thoroughly for 2 min and left at room temperature for 30 min, then centrifuged for 20 min at a centrifugal force of 3000rpm. The pellet was weighed ($_{M2}$) and oven dried at 105 ± 2°C for 24 hours. The mass of the dry pellet (M1) was then determined. The absorbed water was calculated as water absorption capacity (WAC) according to the following formula: CAE = (M2 M0)/M1×100.

2.6.2. Determination of mass, thickness, density and friability of biscuits

Mass was measured by weighing and thickness by calliper. The quality of dry biscuits is generally assessed by their density, a technological parameter considered to be the best index of the textural properties of biscuits (**Divyasree*et al.*, 2020) [3].** A low density corresponds to a light biscuit and therefore a high specific volume (V). The density is expressed by the following formula: D = M /V Where D = density of the biscuit (g/cm3); M = mass of the biscuit (g).

2.6.3. Determination of the cracking resistance of biscuits

It was determined by the penetrometric test (**Gregson *et al.*, 1999) [27]** which consisted of inserting a probe into the biscuit, the force required to reach a certain penetration depth was measured. The following formula was used to convert this into N: 1g = 0.102N.

2.7 Sensory evaluation of the biscuits

The different biscuits were subjected to a sensory evaluation. A total of thirty untrained panellists from the University of Yaoundé 1, due to their constant consumption of biscuits, participated in the evaluation. The parameters assessed included colour, texture, flavour, taste and general acceptability. A five-level hedonic scale where 1 = not liked and 5 = very liked (**Larmond, 1977) [28]** was used for the evaluation.

2.8 Statistical analysis

Statistical analyses were performed using the *Statistical Package for Social Science* (SPSS) version 20.0 for Windows. The significance level was set at 5% based on an Analysis of Variance (ANOVA).

3. Results and Discussion

3.1. Nutritional potential and antioxidant activity of *D. mespiliformis* powder

3.1.1. Proximal composition and bioactive compounds of *D. mespiliformis* powder

The results of the proximal analysis of *D. mespiliformis* powder presented in Table 2 show that the water content of 7.87% is below 10%. Indeed, **Sidibé (2017) [29]** showed that water contents of food flours below 10% favour good preservation. The protein content of *D. mespiliformis* powder is 6.81%. Also, **Ahmad *et al.*, *(*2019) [30]** showed that *D. mespiliformis* contains seven essential amino acids for the body namely threonine, lysine, leucine, isoleucine, valine, phenylalanine and histidine. This makes it a good source of protein. The fat content is 3.73%. This value is similar to the one found by **Ahmad *et al. (*2019) [30]** on the same fruit which was 4.48%. **Sidibé (2017) [29]** showed that flours containing less than 5% lipids have a longer shelf life. As for the total carbohydrate content, the powder of *D. mespiliformis*is 67.02%, higher than that found by **Jacob *et al. (*2016) [31]** which was 60.47%. This would be due to the difference in soils, maturity stage or environmental conditions (**Mhakalao *et al.*, 2015) [32].**

D. mespiliformis would be a good source of carbohydrates. The energy value of the pulp of this fruit is 328.84 Kcal/100g DM. The ash content gives an idea of the amount of minerals in the food. Generally, the ash content of feed is less than 5% (**Marshall, 2010**) **[33].** In this study, the ash content of *D. mespiliformis* powder is 3.01%. Among the trace elements analysed in this study, the iron content (8.37mg/100g DM), was found to be much higher than others, followed by Zinc (1.183), Manganese (0.546) and finally Copper (0.473). All these values are more or less low than those found on the same fruit in Nigeria by **Jacob, (2016) [31]** and by **Olaleke *et al.*, *(*2019) [34].** The observed differences could be explained by environmental variations, nature of soil, maturity status of the fruits (**Makalao *et al.*, 2015**) **[32].** The content of (β-carotene) which is quite high. Indeed, a value of 7638.89 ± 154.32 µg/100g DM was found in the pulp of *D. Mespiliformis* fruits, compared to that of orange carrot which is 8290 µg/100g (**APRIFEL, 2017) [35].**

The results show that the fruit of *D. Mespiliformis* has an interesting nutritional potential and can be used as an ingredient in preparations to improve their nutritional quality. Similarly, its phenolic compounds content is not negligible as the results showed a content of 1204.59 ± 67.07 mg EAG/100g DM. **Ahmad *et al.*, *(*2019) [30]** on the same fruits obtained a content of 2069.00 mg EAG/100g DM. These results suggest that the pulp is a good source of polyphenols. Numerous studies have shown that polyphenols effectively reduce the risk of cardiovascular disease, are considered powerful antioxidants and fight against free radical phenomena (**Adou *et al.*, 2012) [42*)*.** The tannin content of *D. Mespiliformis* pulp which is 129.53 mg Eq cat/100g MS, is lower than that found by **Ahmad *et al.*, *(*2019) [30]** in the pulp of the same fruit (384 mg/100gMS). This could be due to the difference in soils, the condition of the fruit (fresh or dry) (**Makalao *et al.*,**

2015) [32] and the method used which differs from that used in this study (gravimetric method). Tannins are compounds that can form insoluble complexes with divalent ions; they have the ability to inhibit non-heme iron absorption, reduce protein digestibility (**Ogbadoyi *et al*, 2011) [36].** However, according to **Chung *et al.*, (1998) [37],** tannins, at a certain concentration, should not be considered as anti-nutritional as they possess anti-carcinogenic and antimutagenic potential related to their antioxidant property important to protect against cellular oxidative damage. The tannin content of the studied pulp is below the safe dose which is between 150 and 200 mg/100g (**Schiavone *et al.*, 2008) [38].**

Table 2: Proximal composition and bioactive compounds of *Diospyros mespiliformis* powder

Elements analysed	Contents
Water content (g/100g DM)	7.87 ± 0.20
Total fat (g/100g DM)	3.73 ± 0.06
Total protein (g/100g DM)	6.81 ± 2.37
Crude fibre (g/100g DM)	11.55 ± 0.01
Total ash (g/100g DM)	3.01 ± 0.02
Total carbohydrates (g/100g DM)	67.02 ± 0.21
Calorific energy (Kcal/100g)	328.84 ± 0.80
Iron (mg/100gMS)	8.372 ± 0.01
Zinc (mg/100gMS)	1.161 ± 0.01
Manganese (mg/100gMS)	0.546 ± 0.01
Copper (mg/100gMS)	0.473 ± 0.01
Total polyphenols (mg EAG/100g DM)	1204.59 ± 67.07
β-carotene (µg/100g DM)	7767.49 ± 154.32
Tannins (mg Eq cat/100g DM)	129.47 ± 0.69

GAE: gallic acid equivalent; Cat; Catechin

3.1.2. Antioxidant activity of *D. mespiliformis* powder

The IC50 expresses the amount of antioxidant required to decrease the concentration of the free radical by 50%, and is inversely related to the antioxidant capacity of the compound, i.e., the lower the IC50 value, the greater the antioxidant activity of the compound. The results obtained in this study correspond to this logic (Table 3; Figures 1 and 2). The IC50 by DPPH test gave a value of 0.37 ± 0.01 µg/ml. **Ahmad *et al* (2019) [30]** showed that of all unexploited wild fruits studied in Egypt, this fruit had the highest antioxidant activity. The ABTS test gave an IC50 value of 2.32 ± 0.03 µg/ml. This value is higher than the one found by **Fidrianny *et al*, (2017) [39]** on *Hylocereuscostaricensis* fruits which was 1.55 µg/ml.

In view of these results, the fruit pulp of *D. mespiliformis* would be a good raw material for the manufacture of a functional biscuit.

Table 3: Antioxidant activity of *Diospyros mespiliformis* fruit powder

	DPPH anti-radical activity (µg/ml)		Anti-radical activity ABTS (µg/ml)		FRAP (mgEAA/gMS)
	PDm	Vitamin C	PDm	Vitamin C	
IC50	0.37 ± 0.01^b	0.01 ± 0.01^a	2.32 ± 0.05^b	0.03 ± 0.01^a	101.09 ± 2.34

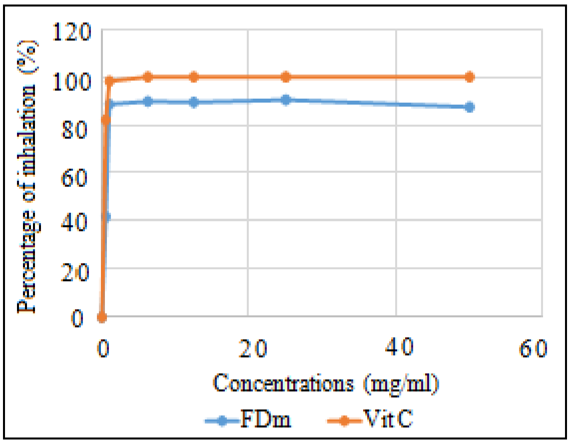

Figure 1: Percentage inhibition of the DPPH radical of PDm

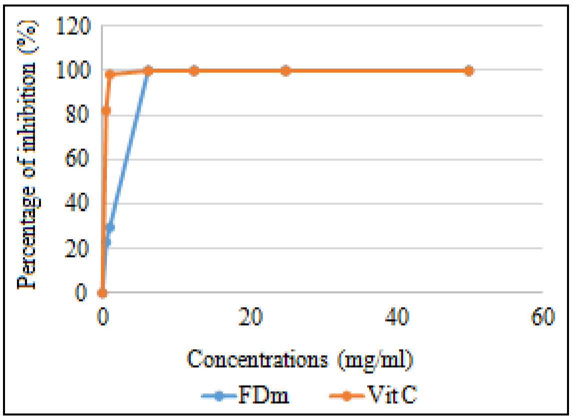

Figure 2: Percentage inhibition of the ABTS radical of PDm

3.2 Proximal composition of the biscuits

The results for the water content of the biscuits ranged from 6.55 to 11.11%. The water contents increase with the amount of *D. mespiliformis* powder. This would be due to the water content of the *D. mespiliformis* powder added in the preparation of the biscuits. According to **FAO** and **WHO (2007) [40],** the water content of certain fried foods should not be higher than 10%; therefore, all biscuits except BS 9, BS10 and BS 11 meet the standard and could be stored for a relatively long time.

The addition of *D. mespiliformis* powder increased ash, carbohydrate and lipid contents. For example, in the BST control biscuits (without *D.* mespiliformis powder) an ash content of 1.56 ± 0.65 g /100g DM was obtained and that of 3.04 ± 0.95 g /100g DM for the BS 11 biscuits with 100% *D. mespiliformis* powder. **Okoye *et al (*2008) [44] obtained** similar results for wheat and soybean flour biscuits. The protein content did not follow the same trend. Indeed, the highest protein content was observed in the control biscuits (46.16 ± 0.01 g/100g DM) and the lowest values were found respectively in the BS11, BS10, BS9 biscuits (26.18 ± 0.24; 25.76 ± 0.01; 25.45 ± 0.05). This could be explained by the nature of the flours used, which have low protein contents (6.81 ± 2.37), whereas wheat flour has an average protein content that varies between 9 and 15 g/100 g DM (**Pomeranz, 1983) [42].**

From these results, we can say that the use of *D. mespiliformis* powder as a composite significantly enhances the nutritional composition of biscuits. Similar observations were made on wheat and African breadfruit biscuits. **Agu *et al (*2007) [43]** The calorific energy evaluated in the biscuits showed no significant difference ($p < 0.05$).

Table 4: Proximal composition of the different biscuits

	Water content (%)	Lipids (%)	Protein (%)	Carbohydrates (%)	Ashes (%)	Energy (Kcal/100gDM)
TSB	6.55 ± 0.19^{a}	23.22 ± 0.13^{a}	46.16 ± 0.01^{e}	21.76 ± 0.16^{a}	1.56 ± 0.65^{a}	480.56 ± 0.93^{c}
BS 1	7.93 ± 2.81^{abc}	24.28 ± 0.98^{abc}	39.33 ± 8.77^{de}	21.94 ± 1.86^{a}	1.90 ± 0.08^{b}	460.27 ± 5.53^{a}
BS 2	7.44 ± 0.38^{ab}	23.65 ± 0.38^{ab}	36.70 ± 0.01^{bcd}	27.04 ± 0.36^{e}	2.12 ± 0.02^{c}	467.81 ± 3.11^{bc}
BS 3	7.11 ± 0.19^{a}	24.35 ± 1.14^{abc}	32.46 ± 0.10^{bcd}	26.39 ± 1.17^{bc}	2.30 ± 0.03^{cd}	467.67 ± 1.08^{bc}
BS 4	7.58 ± 0.52^{ab}	23.70 ± 0.48^{ab}	30.79 ± 0.02^{bcd}	30.99 ± 0.36^{e}	2.33 ± 0.01^{d}	460.46 ± 4.24^{bc}
BS 5	9.34 ± 0.32^{abc}	24.62 ± 0.37^{abc}	27.87 ± 0.02^{ab}	29.69 ± 0.30^{de}	2.46 ± 0.01^{de}	451.81 ± 3.27^{ab}
BS 6	8.41 ± 0.13^{abc}	25.00 ± 0.29^{bc}	37.48 ± 0.01^{cde}	22.26 ± 0.10^{cde}	2.34 ± 0.01^{d}	464.00 ± 2.23^{bc}
BS 7	8.50 ± 0.17^{abc}	25.60 ± 0.11^{c}	28.57 ± 0.02^{abc}	29.38 ± 0.21^{cde}	2.37 ± 0.14^{d}	462.18 ± 1.23^{bc}
BS 8	9.47 ± 0.55^{abc}	25.38 ± 0.46^{bc}	28.37 ± 0.01^{abc}	28.21 ± 0.76^{abc}	2.75 ± 0.01^{f}	454.13 ± 2.97^{abc}
BS 9	10.83 ± 0.83^{bc}	24.00 ± 0.54^{abc}	25.45 ± 0.05^{a}	30.55 ± 1.09^{e}	2.62 ± 0.55^{ef}	444.70 ± 4.30^{ab}
BS 10	10.68 ± 1.23^{bc}	25.02 ± 0.44^{bc}	25.76 ± 0.01^{a}	28.01 ± 1.26^{bcde}	3.00 ± 0.05^{g}	440.36 ± 5.57^{ab}
BS 11	11.11 ± 1.10^{c}	25.83 ± 0.04^{c}	26.18 ± 0.24^{a}	25.50 ± 2.72^{b}	3.04 ± 0.95^{g}	426.28 ± 3.61^{a}

Values are expressed as mean ± standard deviation of the mean (percentage change of the mean).

All the minerals studied (F, Zn, Mn and Cu) increased with an increasing of *D. mespiliformis* fruit in the preparation of biscuits.

As for iron, its content varied between 4.939 ± 0.01 mg/100g for the control biscuits without pulp (BST) and 9.431 ± 0.01 mg/100g for the biscuits with 100% pulp (BS11) (Table 5). The iron content of this fruit is 8 mg/100g DM. It is therefore obvious that the iron content increases with the proportion of fruit powder in the mixture. The same trend was found for Zn, Mn and Cu contents, Of all the minerals studied, Cu appears to have very low levels ranging from 0.12 to 0.54 mg/100g. Copper is an important trace element for the organism and plays a role as cofactor in several metabolic reactions (**FNB, 2001) [44].** The low concentration of Cu in biscuits is an advantage when considering the potential health implications once consumed in overdose.

The significant increase of about 50% in iron content suggests that *D. mespiliformis* could contribute to the fight against anaemia, which is a real public health problem in Cameroon and even in the world. A study of the bioavailability of this iron would help to reassure us.

Table 5: Iron, zinc, manganese and copper content (mg/100 g DM) of biscuits

Samples	Iron	Zinc	Manganese	Copper
TSB	4.94 ± 0.01^{a}	0.43 ± 0.01^{a}	0.22 ± 0.01^{a}	0.12 ± 0.01^{a}
BS 1	5.61 ± 0.01^{c}	0.83 ± 0.01^{e}	0.22 ± 0.01^{b}	0.16 ± 0.01^{b}
BS 2	5.22 ± 0.01^{b}	0.76 ± 0.01^{d}	0.30 ± 0.01^{c}	0.20 ± 0.01^{d}
BS 3	6.96 ± 0.01^{e}	0.53 ± 0.01^{b}	0.39 ± 0.01^{e}	0.17 ± 0.01^{c}
BS 4	7.07 ± 0.01^{f}	0.91 ± 0.01^{f}	0.40 ± 0.01^{f}	0.22 ± 0.01^{e}
BS 5	6.86 ± 0.01^{d}	0.66 ± 0.01^{c}	0.42 ± 0.01^{h}	0.22 ± 0.02^{e}
BS 6	7.89 ± 0.01^{g}	0.98 ± 0.01^{h}	0.31 ± 0.01^{d}	0.25 ± 0.01^{f}
BS 7	8.15 ± 0.01^{h}	0.92 ± 0.01^{g}	0.41 ± 0.01^{g}	0.31 ± 0.01^{g}
BS 8	8.41 ± 0.01^{i}	$1 .00 \pm 0.01^{i}$	0.43 ± 0.01^{i}	0.32 ± 0.01^{h}
BS 9	9.04 ± 0.01^{j}	1.06 ± 0.01^{j}	0.47 ± 0.01^{j}	0.33 ± 0.01^{i}
BS 10	9.13 ± 0.01^{k}	1.09 ± 0.01^{k}	0.48 ± 0.01^{k}	0.45 ± 0.03^{j}
BS 11	9.43 ± 0.01^{l}	1.18 ± 0.01^{l}	0.64 ± 0.04^{l}	0.54 ± 0.01^{k}

Values are expressed as mean ± standard deviation of the mean (percentage change of the mean).

3.3. Composition of secondary metabolites

Secondary metabolites

The values of total phenolic compounds obtained ranged from 208.92 ± 2.89 mgEAG/100g DM for the control biscuits without pulp (BST) to 1170.40 ± 52.26 mg EAG/100g DM for the biscuits with 100% pulp (BS11). In general, the increase in the proportion of pulp in the preparation leads to an improvement in the content of phenolic compounds. This finding corroborates that of some authors (**Ajila *et al.* 2008) [5]** on soft biscuits with the incorporation of mango peel powder.

The same is true for the tannin contents which range from 7.88 ± 0.67 mg Eqcat/100g DM for the control biscuits without pulp (BST) to 76.87 ± 1.92 mg Eqcat/100g DM for the control biscuits with 100% pulp (BS11). The safe value for tannin content in food was reported**)** 150 to 200 mg/100g (**Schiavone *et al (*2008) [38].** All tannin values obtained for the different biscuits are below this range.

3.4 Antioxidant activity of biscuits

The different β-carotene contents range from 120.88 ± 8.91 to 4912.55 ± 89.10 µg/100g DM (Table 6). It can be observed that the incorporation of *D. mespiliformis* significantly improves the β-carotene content of the biscuits compared to the control biscuits without BST pulp. This remark corroborates with those of **Mridula, (2011) [45]** on defatted soy enriched biscuits.

The results of the different contents of total phenolic compounds (TPC) as presented in Table 6 indicate values ranging from 208.92 ± 2.89 to 1170.40 ± 52.26 mgEAG/100g DM. The increasing addition of *D. mespiliformis* powder in the preparation of the biscuits clearly improves the TPC content. The biscuits with 100% of D.*mespiliformis* powder have the highest value (1170.40 ± 52.26 mgEAG/100g DM). This finding corroborates with that of **Ajila *et al.*, *(*2008) [5]** on soft biscuits with mango peel powder incorporation.

Tannins (Ta) are sometimes considered as anti-nutritional but their antioxidant activity at a certain threshold has been proven (**Schiavone *et al*., 2008) [38].** The presented tannin contents found for the biscuits are between 7.88 ± 0.67 mg and 76.87 ± 1.92 mg Eqcat/100g DM. However, the safe value of tannin content in food was reported is 150 - 200 mg/100g **(Schiavone *et al.* *(*2008) [38]** all tannin values obtained for the biscuits are below this range.

The anti-free radical activity of the different preparations was evaluated by the DPPH, ABTS tests by expressing the percentages of inhibition at different concentrations (figures 3 and 4). These different inhibition profiles were used to obtain their 50% Inhibitory Concentrations, abbreviated as IC50 (Table 6), indicating the concentration of antioxidant required to inhibit the DPPH, ABTS radical by 50%. The lower the IC50, the higher the antioxidant activity of the bioactive molecules, the reducing activity was assessed by the FRAP test (Table 6). The statistical analysis reveals that there are some significant differences between the extracts:

The IC50 values ranged from 0.35 ± 0.01 to 3.81 ± 0.03 for the DPPH method, 2.49 µg/ml and 17.84 ± 0.08 µg/ml for the ABTS method. After vitamin C, used as a reference antioxidant, the anti-oxidant activity is increasingly higher when the proportion of the fruit increases in the biscuit preparation in contrast to the control (BST). This increase in free radical scavenging activity is thought to be due to the nature of *D. mespiliformis* possessing bioactive compounds capable of scavenging and neutralizing free radicals by surrendering their protons or electrons through their hydroxyl group-rich structure (**Perron *et al.*, 2011) [46].**

The results of the FRAP test for the biscuits were ranged from 2.52 ± 0.01 to 20.41 ± 1.41 EAA/g DM. The preparations with the highest reducing power are those with the highest proportions of the fruit on the other hand, the control biscuits (BST) without pulps of the fruit have the lowest values. This is because the pulp would contain biologically active compounds with metal ion reducing activity unlike the refined wheat flour used.

Overall, the results show that the increasing addition of *D. mespiliformis* in the preparation of biscuits leads to their increase in the antioxidant activity. These results corroborate with those of **Mahloko *et al.*, (2019) [6]** on Barbary powder and banana biscuits.

Table 6: Secondary metabolite content and antioxidant activity of biscuits

	β-Carotenes (µg/100g DM)	CPT (mg EAG/ 100g DM)	Ta (mg Eq cat/ 100g DM)	IC50 DHHP (µg/ml)	IC50 ABTS (µg/ml)	FRAP (mg EAA/ g DM)
TSB	120.88 ± 8.91^{a}	208.92 ± 2.89^{a}	7.88 ± 0.67^{a}	3.81 ± 0.03^{i}	17.84 ± 0.08^{m}	2.52 ± 0.01^{a}
BS 1	208.66 ± 0.33^{b}	426.56 ± 2.20^{b}	21.04 ± 0.67^{bc}	0.49 ± 0.02^{g}	8.44 ± 0.01^{l}	6.80 ± 0.14^{c}
BS 2	329.22 ± 31.18^{cd}	476.45 ± 16.99^{b}	16.95 ± 0.21^{b}	0.53 ± 0.02^{h}	7.12 ± 0.55^{k}	7.18 ± 0.72^{c}
BS 3	321.50 ± 4.45^{b}	572.84 ± 29.17^{c}	23.65 ± 1.32^{c}	0.44 ± 0.01^{f}	5.95 ± 0.83^{j}	7.69 ± 0.51^{d}
BS 4	352.37 ± 27.10^{cd}	630.11 ± 7.87^{d}	25.02 ± 0.80^{c}	0.44 ± 0.01^{e}	5.44 ± 0.17i	8.52 ± 0.13f
BS 5	$354.94 \pm 7.72b^{c}$	626.19 ± 1.65^{cd}	44.24 ± 3.45^{d}	0.47 ± 0.01^{e}	4.33 ± 0.01^{h}	8.87 ± 2.32^{h}
BS 6	408.95 ± 7.71^{c}	682.58 ± 0.84^{d}	44.98 ± 1.23^{d}	0.42 ± 0.01^{d}	4.01 ± 0.01^{f}	8.62 ± 0.18^{e}
BS 7	412.81 ± 3.86^{de}	793.10 ± 6.08^{e}	59.03 ± 0.65^{e}	0.44 ± 0.01^{e}	4.22 ± 1.01^{g}	8.47 ± 0.09^{e}
BS 8	443.67 ± 27.01e	843.89 ± 19.11^{e}	57.96 ± 1.16e	0.39 ± 0.02^{c}	3.75 ± 0.01^{d}	9.47 ± 0.69^{i}
BS 9	582.56 ± 3.86^{f}	903.80 ± 8.50^{f}	64.71 ± 0.58^{e}	0.39 ± 0.01^{c}	3.83 ± 0.22^{e}	14.59 ± 0.72^{j}
BS 10	1620.55 ± 0.18^{g}	916.83 ± 4.20^{f}	78.03 ± 1.70^{g}	0.35 ± 0.01^{b}	2.77 ± 0.10^{c}	16.19 ± 2.59^{k}
TSB 11	$4912.55 \pm 89.^{10h}$	1170.40 ± 52.26^{g}	76.87 ± 1.92g	0.35 ± 0.01^{b}	2.49 ± 0.08^{b}	20.41 ± 1.41^{l}
Vit C	/	/	/	0.01 ± 0.01^{a}	0.03 ± 0.01^{a}	/

Values are expressed as mean ± standard deviation of the mean (percentage change of the mean). GAE: Gallic Acid Equivalent; cat: catechin. BST: Shortbread biscuits.

The values assigned to different letters in the same column are significantly different ($p < 0.05$).

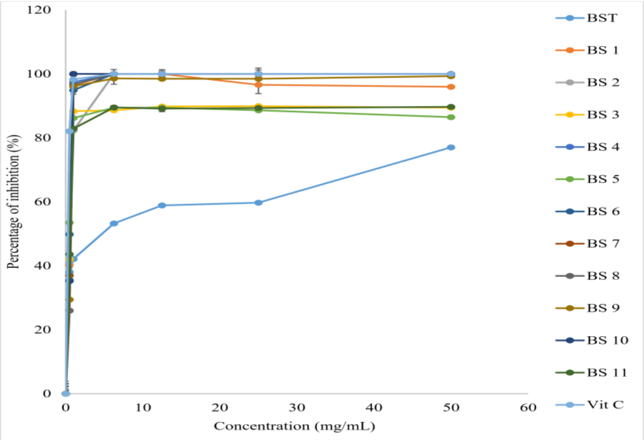

Figure 3: Percentage of DPPH radical inhibition of biscuits

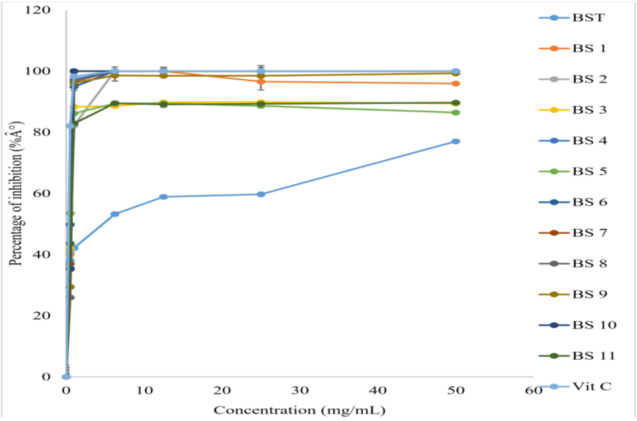

Figure 4: Percentage inhibition of the ABTS radical in biscuits

3.5. Textural and physical characteristics of biscuits

The physical measurements (hardness, mass, thickness, density, volume, reliability, water absorption capacity) of the formulated biscuits are reported in Table 8.

The hardness of the biscuits increases with the addition of *D. mespiliformis* powder. The values obtained range from 170.47 ± 16.13 N for the control biscuits (BST) to 517.11 ± 46.44 N for the biscuits with 100% *D. mespiliformis* (BS 11). This could be explained by the protein content of *D. mespiliformis (*6.81 g/100g DM) compared to 10 to 13 g/100g DM for wheat flour. Indeed, the structured gluten network of wheat gives a compact texture to the biscuits, whereas in the case of *D. mespiliformis*, the absence of such a network weakens the dough, making it more resistant to breakage (**Divyasree *et al.*, 2020) [3] These** results are in agreement with those of some authors on ripe mango peel biscuits (**Ajila *et al. (*2008) [5].**

Different biscuits were weighed and the values obtained ranged from 8.05 ± 1.07g (BS 11 with 100% *D. mespiliformis* to 10.86 ± 1.48g (BST with 0% *D. mespiliformis*. The masses of the biscuits are a function of the proportion of *D. mespiliformis.* The more the fruit increases the more the mass decreases. This could be due to the joint effect of the dough rising phenomenon thanks to the gluten network and the protein nature of the *D. mespiliformis*. These results are in agreement with those found for wheat and *Treculiaafricana* biscuits **(Agu *et al (*2007) [43].**

The values of the thickness of the biscuits were ranged from 0.70 to 1.26 cm. It was observed through the values obtained that the thickness of the biscuits decreases with the addition of *D. mespiliformis*. This may be due to the dilution of gluten (**Divyasree *et al.*, 2020) [3]** with increasing incorporation of *D. mespiliformis*in the preparation. Some authors made a similar observationon biscuits. (**Ajila *et al.* (2008) [5]**

The quality of a biscuit is often judged by its low density (**Divyasree *et al.*, 2020) [3].** In this case, the low density of the biscuits indicates that they would have good textural properties. The density results presented in Table 8 show no significant difference. The granulometry, a factor influencing the density, would be at the origin of these results; indeed, a finer flour would allow to obtain a dense biscuit (**Mancebo *et al.*, 2015) [47].** In contrast to the fine size of biscuit flours which oscillates between 50 and 130µm, that of the incorporated *D. mespiliformis* is larger (≥ 200µm).

The friability values obtained are presented in Table 8. The smaller the value, the higher the friability. The friability of the biscuits was ranged from 55.67 ± 3.48% to 92.93 ± 3.21% and increased with the proportion of sugar and gluten in the preparation. This would be due to their low proportions of wheat flour. Indeed, the structured network of gluten formed in biscuit doughs leads to biscuits with a high friability (**Gallagher *et al.*, 2003) [48].**

The water absorption capacity is the capacity of the food to absorb water. The values obtained for this purpose were ranged from 109.89 ± 1.92 g to 159.05 ± 1.02 g. Overall, the water absorption capacity increases with the incorporation of *D. mespiliformis*. This could be explained by the absence of a higher protein content in *D. mespiliformis* in contrast to wheat flour. Indeed, protein-rich flours absorb much more water due to their positive and negative charge that can bind water (**Adebowale *et al.*, 2003) [49].** Thus, the control biscuits (BST) and the biscuits with the highest proportions of wheat flour have the highest water absorption capacities.

Table 7: Physical characteristics of biscuits

Codes	Hardness (N)	Mass (g)	Thickness (cm)	Density (g/cm^3)	Friability (%)	CAE (g)
TSB	155.55 ± 3.77^{a}	10.21 ± 1.30^{cd}	1.26 ± 0.06^{e}	0.04 ± 0.01^{ab}	55.67 ± 3.48^{a}	159.05 ± 1.02^{e}
BS 1	183.12 ± 13.69^{ab}	10.06 ± 1.48^{d}	0.98 ± 0.09^{d}	0.04 ± 0.01^{ab}	78.41 ± 064^{b}	146.02 ± 2.46^{cde}
BS 2	219.83 ± 16.60^{bc}	9.85 ± 1.57^{bcd}	0.89 ± 0.01^{bcd}	0.05 ± 0.01^{ab}	86.93 ± 0.51^{cde}	140.63 ± 10.33^{bcde}
BS 3	201.18 ± 15.13^{ab}	9.72 ± 1.10^{bcd}	0.93 ± 0.03^{cd}	0.04 ± 0.01^{ab}	83.79 ± 2.08^{bc}	148.80 ± 9.77^{de}
BS 4	210.55 ± 5.02^{b}	9.75 ± 0.94^{bcd}	0.86 ± 0.05^{abcd}	0.04 ± 0.01^{ab}	86.61 ± 1.55^{cde}	117.60 ± 5.19^{ab}
BS 5	264.92 ± 4.20^{c}	9.63 ± 1.16^{bcd}	0.91 ± 0.04^{cd}	0.04 ± 0.01^{ab}	90.45 ± 1.78^{def}	128.69 ± 7.20^{abcd}
BS 6	437.69 ± 32.51^{e}	8.91 ± 0.87^{abc}	0.77 ± 0.06^{abc}	0.04 ± 0.01^{ab}	85.79 ± 0.70^{cd}	111.33 ± 6.42^{a}
BS 7	322.80 ± 29.40^{d}	9.34 ± 1.35^{abc}	0.80 ± 0.03^{abc}	0.05 ± 0.01^{b}	89.09 ± 0.93^{cde}	125.34 ± 6.78^{abc}
BS 8	515.35 ± 33.81^{f}	9.07 ± 0.83^{abc}	0.79 ± 0.04^{abc}	0.04 ± 0.01^{ab}	89.60 ± 2.14^{def}	126.67 ± 0.50^{abcd}
BS 9	543.30 ± 1.03^{f}	8.91 ± 0.92^{abc}	0.74 ± 0.11^{ab}	0.04 ± 0.01^{ab}	92.61 ± 1.08^{f}	146.54 ± 1.90^{cde}
BS 10	498.80 ± 0.58^{f}	8.68 ± 1.12^{ab}	0.70 ± 0.03^{a}	0.04 ± 0.01^{a}	92.93 ± 3.21^{f}	139.24 ± 5.37^{bcde}
BST 11	543.89 ± 46.44^{f}	8.05 ± 1.07^{a}	0.70 ± 0.04^{a}	0.04 ± 0.01^{ab}	91.57 ± 0.79^{ef}	109.89 ± 1.92^{a}

3.6 Sensory characteristics of the biscuits

The scores obtained from the sensory analysis of the biscuits are presented in Table 8. The scores of the different sensory criteria to be evaluated are the lowest for the biscuits with high proportions of *D. mespiliformis* in contrast to the control biscuits without *D. mespiliformis* (BST) except for the texture. Wheat flour is known to develop more flavour, good texture and pleasant colour than any other food flour (**Gallagher *et al.*, 2003) [48].** Similar results were found by **Okoye *et al.*, *(*2008) [41].** Substitution of wheat flour and sugar by *D. mespiliformis* in the preparation of biscuits gave good results regarding the overall acceptability for BS 1 (25: 50: 25), BS 3 (43.75: 37.5: 18.75), BS 6 (25: 12.25: 62.5), BS 2 (33.33: 66.67: 0).

Table 8: Sensory characteristics of the biscuits

Biscuits	Colour	Texture	Flavour	Taste
TSB	4.13 ± 1.19^{b}	1.53 ± 0.83^{a}	3.73 ± 0.96^{b}	4.27 ± 0.80^{c}
BS 1	3.13 ± 1.06^{ab}	1.93 ± 1.10^{ab}	2.67 ± 1.11^{ab}	3.13 ± 1.19^{abc}
BS 2	3.07 ± 0.96^{ab}	2.00 ± 0.85^{abc}	2.67 ± 1.29^{ab}	2.60 ± 0.91^{bc}
BS 3	3.13 ± 0.92^{ab}	2.40 ± 0.99^{abcd}	2.60 ± 1.35^{ab}	3.13 ± 1.30^{abc}
BS 4	2.53 ± 1.13^{a}	2.33 ± 0.98^{abcd}	2.07 ± 0.80^{a}	2.33 ± 0.90^{ab}
BS 5	3.07 ± 0.88^{ab}	2.40 ± 1.24^{abcd}	2.00 ± 1.07^{a}	2.00 ± 0.93^{ab}
BS 6	2.27 ± 0.88^{a}	2.40 ± 1.40^{abcd}	2.33 ± 1.29^{ab}	2.60 ± 1.24^{ab}
BS 7	2.53 ± 1.06^{a}	3.00 ± 1.20^{bcd}	2.00 ± 1.20^{a}	2.00 ± 1.13^{ab}
BS 8	2.60 ± 0.99^{a}	3.00 ± 0.63^{e}	3.07 ± 1.39^{ab}	3.27 ± 1.22^{bc}
BS 9	2.53 ± 1.25^{a}	3.40 ± 1.12^{de}	2.33 ± 1.23^{ab}	2.00 ± 1.13^{ab}
BS 10	2.53 ± 0.83^{a}	3.27 ± 1.22^{cde}	2.13 ± 1.13^{a}	2.27 ± 0.88^{ab}
BS 11	2.67 ± 1.35^{a}	3.53 ± 1.25^{de}	2.27 ± 1.44^{a}	1.87 ± 1.13^{a}

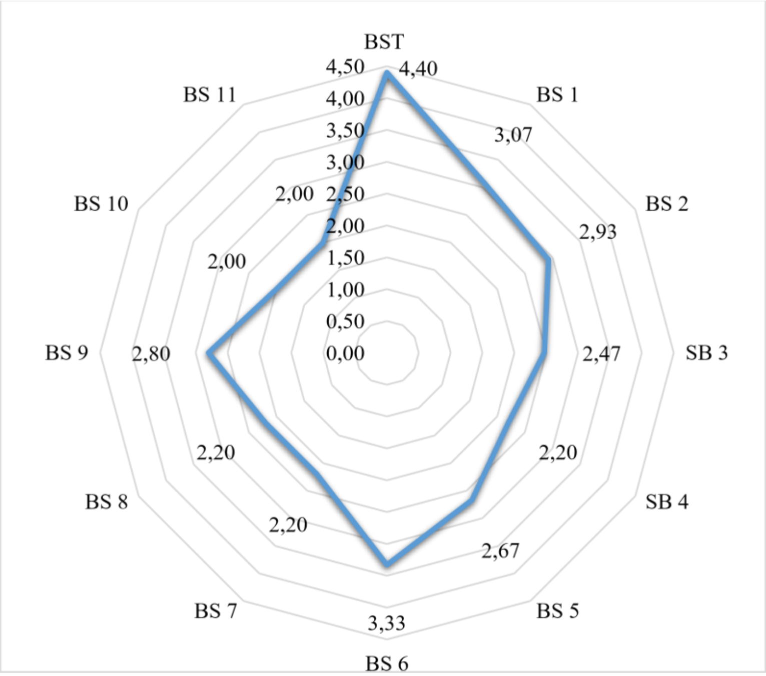

Figure 5: General acceptability of biscuits

4. Conclusion

This study showed that the incorporation of *D. mespiliformis* powder in the preparation of biscuits improves their nutritional profile and can therefore contribute to the fight against malnutrition. These biscuits are harder, less bulky and crumbly. The sensory analysis showed that the most appreciated biscuit was BS6. A study of the bioavailability of the nutrients in these biscuits would be interesting to promote their use in the fight against malnutrition in Cameroon.

The biscuits obtained from the mixtures between *D. mespiliformis*, wheat flour and sugar have different physical characteristics from the controls, the addition of *D. mespiliformis* in increasing proportions makes the biscuits harder and thinner, less voluminous; the acceptability of these biscuits depends on the proportion of substitution of *D. mespiliformis* with wheat flour and sugar, in fact the most appreciated biscuits were obtained for the mixtures: BS 2 (50 :25 :25), BS 5 (37.5 :18.75 :43.75), BS 6 (25 :12.25 :62.5), BS 9 (66.67 :0 :33.33).

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