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Mediterranean Journal of Chemistry **2018**, 6(6), 276-282

Functional properties of a new spread based on olive oil and honeybees

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Abstract: A new alimentary concept has been developed since the 80's. This one is called "functional food". In this context, the olive oil and honey are traditionally used in their initial state as a basic food. They are considered as a potential source of new bioactive products from which we can formulate several functional foods. This work will focus on the elaboration of a new spread of honey and olive oil using beeswax as an emulsifier. Physical-chemical characterization, antioxidant and antibacterial activity were evaluated. As for the phenols content, spreads prepared from thyme honey has the highest content (337 mg GAE/kg) compared to other spreads. The antioxidant activity was evaluated by three different methods namely: DPPH test, ABTS + test and the iron reduction method (FRAP) which proves that this last has a higher activity than the other spreads (EC_{50} of 70 mg /L using DPPH, EC_{50} of 20 mg /L using ABTS). An agar-well diffusion assay was used to assess the activity of honeys against seven bacteria strains. All prepared spreads honey samples showed highest antibacterial activity against all bacterial strains tested (diameter of ZI > 20mm). Hence, we note that our new spread proved by excellence to be a functional food due to the high content of phenols and the important antibacterial and antioxidant activities.

Keywords: honey; phenolic compounds; antioxidant activity; antibacterial activity; functional food

Introduction

Over the last few decades, consumer demands for food production have changed. Consumers believe more and more that foods have a direct impact on their health [1, 2]. Today foods are not intended to satisfy the only hunger for humans but also to prevent nutrition-related diseases and improve the physical and mental well-being of the human [3, 4].

Therefore, a new alimentary concept came to surface which is "Functional Food". This one is known for its benefits on health. These nutritional effects act as a stimulator for well being and decrease the risk of diseases. Any kind of food may be considered functional as soon as it provides benefits on one or more parts of the body organism [4, 5].

In addition to being used for therapeutic and medical purposes, honey is one of the most consumed foods in the world. The Codex Alimentarius defines honey as a natural sweet substance produced by the bees "*Apis mellifera*" from the nectar of the plants or from the secretions left on the parts of plants, which the bees forage, transform by combining them with specific substances Which they secrete, deposit, dehydrate, store and leave to refine and mature in the hive rays [6]. Besides, Honey has many medicinal effects such as antibacterial, hepatoprotective, hypoglycemic, reproductive, antihypertensive and antioxidant effects [7]. Beeswax (E901) is the natural wax produced by bees in the hive. It is used in cosmetics and skin care as a thickening agent, emulsifier, and a surfactant. Beeswax, however, is not valued in the food industry.

Olive oil is the main source of fat in the Mediterranean diet. It is much appreciated all over the world for its taste and aroma, as well as for its nutritional properties [8]. It's a functional food which besides of having a high level of monounsaturated fatty acid contains several minor components with bioactive properties such as the antioxidant phenolic compounds, squalene, and alpha-tocopherol. A large body of studies, either experimental or in animal models, have been performed to provide evidence that olive oil phenolic compounds contribute significantly to health benefits.

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DOI: http://dx.doi.org/10.13171/mjc66/01801182226-tekiki

Received December 4, 2017
Accepted, January 9, 2018
Published January 18, 2018

Olive oil and honey are traditionally used in their initial state as a basic food [9]. Indeed, thanks to their dietary, nutritional and functional interests [9], these products deserve to be better valued and introduced into our alimentary diet. So, a combination of honey and olive oil may be very beneficial as the impact on human health will be huge. However, due to their potential source of a bioactive molecule, they may be envisaged on the bargain as the origin of a new kind of functional food.

Low-fat spreads are available in the market but with limited range. Characterizations of selected spreads from peanut butter, pistachio butter [10], sesame, soy, and date [11] are available. All these facts suggest the possibility of producing a functional low-fat spread from olive oil and honey. The aim of this work is the characterization of a spread based on olive oil and honey using beeswax as an emulsifier. Beeswax is used as an emulsifier to substitute chemical additives in order to have spread rich in nutrients as antioxidants and retaining the bioactive properties of honey and olive oil. The main goal of this work is to examine bioactive properties of the spread. Antibacterial activity was tested against seven bacteria; an agar-well diffusion assay was used. Three assays were used to screen the antioxidant properties (DPPH, ABTS, FRAP).

Experimental

Samples

The study was carried out on monovarietal virgin olive oil from the Tunisian cultivars, namely *Chetoui*, planted in the north of Tunisia. The honey samples were derived from eucalyptus, thyme and polyfloral origins which came from the north of Tunisia. Finally, natural beeswax sample was provided by the Central Laboratory for Analysis and Testing.

Chemical reagents

Barbituric acid, p-toluidine, Trolox and Folin-Ciocalteau reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA); acetic acid, chloroform and methanol were purchased from Fluka Chemika (Buchs, Switzerland) of the highest purity available; 2.2'-azinobis (3-ethylenbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were purchased from Merck (Darmstadt, Germany).

Preparation of bread spreads

Our bread spreads are made from 20% of olive oil which is melted with 1% beeswax in a water bath at 80 °C. Once the emulsifier is liquefied 79% of honey is added. Homogenization of the mixture is carried out using a homogenizer (Ultraturax D-160 Homogenizer, Scilogex USA) by applying a speed of 15000 rpm for 7 minutes. After production, each sample was transmitted in a hermetically sealed jar and stored at room temperature.

Physicochemical analysis

Stability test

15 g (F_0) of each sample was transferred to test tubes (internal diameter 30 mm, height 500 mm) which were tightly sealed with plastic caps and then centrifuged for 30 min at 5000 rpm (Hettich, Roto silent/K, Germany). The weight of the precipitated fraction (F_1) was measured, and the emulsion stability was characterized as follows:

$$\textbf{Stability (\%)} = (F_1/F_0) * 100$$

Centrifugation was repeated three times to separate the oily fraction. Thus, the oily phase and the precipitate were recovered for the rest of analyses [12].

Peroxide value

The peroxide value was determined for the oily phase of the spread. This index was carried out in accordance with ISO 3960: 2001. It's expressed in meq O2 / Kg body fat.

Hydroxymethylfurfural (HMF) value

The HMF value was determined for the honey to separate from the spread. First, 10 g of each sample was dissolved in 20 mL water; next, 2 mL of the solution was mixed with 5.0 mL of p-toluidine solution and put into two different test tubes. Last, 1 mL of distilled water (reference solution) was added into one tube; while to the second tube, 1 mL of barbituric acid solution 0.5% (sample solution) was added.

The absorbance of the solutions at 550 nm was determined using a spectrophotometer (ChromoTek GmbH, Germany). The quantitative value of HMF was determined by using the proposed formula for the method [13].

Total phenolic content (TPC)

The content of total phenol compounds was determined according to the method described by A. Boussaid et al. [14]. 1 g of sample (honey and spread) diluted with 10 mL of ultrapure water and filtered through a 45 lm Minisart filter. 0.5 mL of this solution was then mixed with 2.5 mL of Folin-Ciocalteu reagent (0.2 N) and 2 mL of sodium carbonate (Na 2 CO 3) (0.7 M) for 7 min. After incubation in the dark at room temperature (≈ 25 °C.) for 2 h, the absorbance of the mixture was measured at 760 nm (spectrophotometer: ChromoTek GmbH, Germany). The total phenolic content was expressed in mg of gallic acid (equivalent mg GAE / Kg of honey).

Antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of honey and spreads were studied by evaluating the free radical scavenging test (DPPH) as described by S. Ž Gorjanović et al. [15]. 1g of each sample (honey, spreads) was dissolved in 1mL of distilled water. A serial of four dilutions was done.

Then, 200µl of each concentration was mixed with 1800µl of methanol solution DPPH (0.04 g /mL), incubation in dark for 30min. The absorbance of the reaction mixture was measured at 517nm (spectrophotometer: ChromoTek GmbH, Germany).

$$\boldsymbol{DPPH}\ (\%) = \left(\mathbf{1} - \frac{\boldsymbol{Ax}}{\boldsymbol{A0}}\right) \times \mathbf{100}$$

Where:

A_0: absorbance of the control (distilled water).

A_x: absorbance after reaction with honey or spread.

Results are expressed with EC50 using the serial dilution, which is the effective concentration of the sample at which 50% of initial amount of DPPH was scavenged. The Trolox was used as a standard.

ABTS radical-scavenging assay:

The ABTS assay was carried out according to the method of Re et al. (1999), partially improved by C. Cimpoiu et al. [16]. Radical scavenging activity was performed by mixing 100 µL of the aqueous honey solution at different concentrations with 3 mL of ABTS•+ solution. After 10 min of reaction in dark, the absorbance was read at 734 nm (spectrophotometer: ChromoTek GmbH, Germany). The blank used was 0.1mL of aqueous honey solution mixed with 3.0 mL of distilled water.

The result was expressed by EC50 (mg/L) values determined as described previously in DPPH• assay. The Trolox was used as a standard.

Ferric reducing power assay (FRAP)

The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe3+-TPTZ) to its ferrous colored form (Fe2+-TPTZ) in the presence of antioxidants [17]. The Fe3+ reducing power of honey was determined by the method of G.C Yen [18], with slight modifications by H. A. Alzahrani et al. [19]. Honey (2.5 mL) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated for 20 min at 50 °C. After incubation, 10% trichloroacetic acid (2.5 mL) was added to the mixtures, followed by centrifugation at 3000 rpm for 10 min (Hettich, Roto silent/K, Germany). The upper layer (1 mL) was mixed with distilled water (1 mL) and 0.1% ferric chloride (0.5 mL). The absorbance of the obtained solution was measured at 700 nm (spectrophotometer: ChromoTek GmbH, Germany).

Antibacterial activity

Agar well diffusion was used for detection of antagonistic activity of each sample of honey and spread against 7 bacteria strains: *E. coli* (DH5a, IPT), *P. aeruginosa* (9027), *Salmonella typhimurium* (ATCC 09040726) and *S. aureus* (ATCC 25923), *L. monocytogenes* (ATCC 070101121), *Klebsiella pneumonia* (CIP104727) and *Aeromonas hydrophila* (ATCC 7966). Preparation of the strain was done as described by C. Basualdo et al. [20]. The concentration of cultures was $1*10^7$ CFU/ mL.

Honey and spreads solutions were prepared immediately before testing by diluting honey or spreads in sterilized water 10% v/v. All samples were then incubated for 30 minutes at 37 °C in agitation. Fresh culture suspension of the test microorganisms (100 µL) was spread on nutrient agar. Well, about 9 mm diameter was drilled on the culture after solidification media and 100µl of each honey dilution were added to each hole.

Plates were incubated at 37 °C for 24 h, after which antagonistic activity was confirmed by the presence of a clear halo. Results were presented with ZDI (mm) the diameter of inhibition zone. ZDI is the mean of three replicates.

Statistical analysis

Statistical analysis and comparisons among means were carried out using the statistical package SPSS 17.0 (SPSS Inc., Chicago, IL.). Mean values of all data were obtained from the triplicate assay. The differences in mean values among samples were determined using one-way analysis of variance (ANOVA).

Results and discussion

Physical-chemical properties of the samples

Table 1 shows the physicochemical quality parameters of eight spreads studied. The most apparent problem of spreads is the tendency of the oil release. Using lipids with higher fluidness to produce spreads could affect the physical stability of the spreads; may significantly decrease the stability leading to oil release during storage [21]. This tendency to phase separation can be rapidly estimated by assessing the oil release from the spread upon centrifugation [22]. Our spreads samples showed a high stability. Values of stability are in the range 97% and 98.8% there is no significant difference ($p<0.05$) between samples. Moreover, the peroxide value of all the spreads was less than ≤ 20 mequiv (limit accepted by Regulation COI/T.15/NC 2015). The peroxide value showed no significant difference ($p<0.05$) between samples. The low index of peroxide value of prepared spreads proves the good fat quality of samples. For HMF (Hydroxymethylfurfural) value measured for all the samples are less than 40mg/kg (limit value). The lowest HMF value was 1.25 mg/kg and 1.23 mg/kg measured for one sample eucalyptus spread and a sample of thyme spreads.This low value indicates that the sample is freshly extracted honey. This result was in agreement with that of I.N. Pasias et al. [23], who confirmed that HMF content is below 10mg/Kg in freshly honey.

Table1. Physical-chemical property of parameters spreads.

Samples	Stability %	Peroxide Value meq O_2 / Kg	HMF value mg/kg
Polyfloral honey spread 1	97,73 ± 0,35	1,067 ± 0,02	25,6 ± 0,6
Polyfloral honey spread 2	97,267 ± 0,31	1,03 ± 0,02	30,97 ±0,21
Eucalyptus honey spread 1	97,83 ±0,35	0,95 ±0,08	12,53 ± 00,25
Eucalyptus honey spread 2	98,03 ± 0,6	0,97 ±0,1	19,36 ± 0,56
Eucalyptus honey spread 3	98,83 ± 0,29	1,46 ± 0,3	1,253 ± 0,258
Thyme honey spread 1	97,6 ± 0,5	1 ± 0,03	21,523 ± 0,509
Thyme honey spread 2	97,77 ± 0,33	1,01 ± 0,09	26,31 ± 0,580
Thyme honey spread 3	98,8 ± 0,46	1,42 ±0,26	1,23 ± 0,178

Total phenolic content

The results of the total phenolic content (TPC) of the eight Tunisian kinds of honey and eight spreads are shown in Table 2. TPC of honey ranged from 274 to 512 mg GAE/kg honey. It was observed that the TPC showed significant differences among the different samples. The highest concentration of polyphenols was 512.8 mg GAE/kg for sample honey thyme 3, while the lowest contents value was 201.27 mg GAE/kg for polyfloral honey 2. The TPC of our Tunisian honey samples is in the same range of Algerian honeys [24], Sundarban hneys [25] and Portuguese honey [26]. In addition, the TPC of the eight samples analyzed is higher than some Malaysian honey samples [27] and Turkish honey samples [28]. For our eight prepared spreads, TPC values are between 183 and 337 mg GAE/kg honey. Moreover, results showed that TPC values decreased significantly ($p < 0.05$) while preparing the spread for all the eight samples of honey. Because spreads are composed only 79% of honey, it was observed the same ranking for spreads as for honey; the richest honey in phenol give the richest spread in phenol. Despite, this diminution of phenol in our spread, the TPC values remains important and higher than some Malaysian honey samples [27] and Turkish honey samples [28].

Table 2. Total phenol content (TPC) and antioxidants activities of honeys and spreads.

Samples	TPC (mg GAE/	EC50 DPPH value	EC50 ABTS value	EC50 FRAP
Polyfloral honey 1	274,3 ± 12,2b	167 ± 0, 4f	67 ± 0, 4c	87 ± 0, 4d
Polyfloral honey 2	201,27 ±7,7a	153 ± 0, 4^e	83 ± 0, 4d	103 ± 0, 4^e
Eucalyptus honey 1	402,8 ±6,6c	90 ± 0, 1d	33 ± 0, 4b	65 ± 0, 7b
Eucalyptus honey 2	390,5 ± 9,33c	80 ± 0, 1c	37 ± 0, 9b	73 ± 0, 4c
Eucalyptus honey 3	410,1 ± 7,33c	77 ± 0, 4c	33 ± 0, 4b	57 ± 0, 4b
Thyme honey 1	456,6 ± 13,33d	40 ± 0, 1b	13 ± 0, 4a	57 ± 0, 9a
Thyme honey 2	479,5 ± 9,33d	30 ± 0, 1a	13 ± 0,44a	43 ± 0, 4a
Thyme honey 3	512,8 ± 8e	23 ± 0, 4a	12 ± 0, 4a	37 ± 0, 4a
Polyfloral honey spread 1	206,7 ± 3,67	199 ± 0, 7	110 ± 0, 4	92 ± 0, 8
Polyfloral honey spread 2	183,6 ± 3,2	180 ± 0, 4	110 ± 0, 7	113 ± 0, 4
Eucalyptus honey spread 1	261,8 ± 2,94	130 ± 0, 7	46 ± 0, 3	86 ± 0, 8
Eucalyptus honey spread 2	224,9 ± 2,933	107 ± 0, 5	50 ± 0, 5	90 ± 0, 5
Eucalyptus honey spread 3	229,5 ± 4,27	110 ± 0, 7	60 ± 0, 7	86 ± 0, 3
Thyme honey spread 1	325 ± 5,13	82 ± 0, 5	20 ± 0, 5	45 ± 0, 4
Thyme honey spread 2	330,2 ± 3,9	75 ± 0, 40	20 ± 0, 2	47 ± 0, 4
Thyme honey spread 3	337,5 ± 3,8	70 ± 0, 3	22 ± 0, 5	42 ± 0, 5

Different letters in the same row indicate significantly different mean ± standard deviation of triplicates ($p < 0.05$).

Antioxidant activity

The antioxidant activities were measured using three different spectrophotometric tests by scavenging radical DPPH·, ABTS•+ and reducing power FRAP. These are summarized in (Table2) and the results were expressed as EC_{50} values (milligram weight of sample per milliliter).

DPPH• radical scavenging assay

DPPH radicals measure the decrease in DPPH radical absorption after exposure to radical scavengers. While studying the radical-scavenging potential of honeys, the DPPH assay was frequently used because the antioxidant potential of honey has been correlated with its phenolic contents [24, 25]. The DPPH radical scavenging activities of the eight honey samples (expressed by EC_{50}) are shown in Table 2. Results showed that thyme honey samples were significantly ($p < 0.05$) more active (EC_{50} from 23 mg/mL to 40mg/mL) than other honey samples (EC_{50} from 77 mg/mL to 167mg/mL). Phenolic extracted from thyme honeys exhibited the higher content of phenols than those extracted from eucalyptus and polyfloral honeys (Table 2). Therefore, we can conclude that phenolic fraction could influence the

antioxidant potential of each honey. Several studies showed that phenols present in honey affect the antioxidant capacity and free radical scavenging activity. The EC_{50} values measured in this study were similar and in the same range of some Algerian, Indian and Malaysian honeys [29, 30]. The DPPH radical scavenging effect of honeys spreads prepared samples is also presented in Table 2. The EC_{50} DPPH scavenging activity ranged from 70 mg/mL to 199 mg/mL. Besides, results showed that EC_{50} DPPH scavenging values decreased significantly ($p < 0.05$) while preparing the spread samples only for thyme honey. Spreads from eucalyptus and polyfloral honeys kept the same EC_{50} DPPH scavenging values than honeys. So, spreads present a good DPPH scavenging activity. Yet, the result showed that the antioxidant activity of honey and prepared spreads vary greatly depending on the honey's floral source.

$ABTS^{\bullet+}$ radical scavenging assay

ABTS activity was also quantified in terms of percentage inhibition (EC_{50}) of the ABTS•+ radical cation of antioxidants in each honey sample. These two tests treat two different mechanisms of action using two different radicals (ABTS•+ and $DPPH^{\cdot}$) [31]. It is for this reason that the two tests are not always well correlated and don't give the same results. Results in Table 2 showed a significant variation in the percentage inhibition of the honey samples, thyme honey was the most efficient scavenger of the radical (EC50 13mg/mL) followed by eucalyptus honeys, while polyfloral honey had the lowest scavenger inhibition effect (EC50 67mg/mL to 83mg/mL). This classification is similar to DPPH scavenging activity. As well, honeys have been shown to be a more effective scavenger of ABTS radical (Table 2).

Table 3. Diameter of inhibition zones (mm) produced by honeys and spreads on tested bacteria.

Samples	*S. arizonae*	*E. coli*	*S. aureus*	*P. aeruginosa*	*A. hydrophila*	*L. monocytogens*	*K. pneumonie*
Polyfloral honey 1	28,33±0,44	27 ± 0,67	30,33 ± 0,44	25,33 ± 0,44	23,33 ± 0,44	23,33 ± 0,66	25,33 ± 0,44
Polyfloral honey 2	22,67±8,89	30 ± 0,44	24,33 ± 0,44	27,33 ± 0,44	22,33 ± 0,44	20,33 ± 0,44	24,33 ± 0,44
Eucalyptus honey 1	30,3 ± 0,44	33,44 ±0,44	29 ± 0,67	35,33 ± 0,44	34,33 ± 0,44	30,33 ± 1,33	30,33 ± 0,44
Eucalyptus honey 2	23 ± 13,3	30,33 ±0,44	31,33 ± 0,44	31,33 ± 0,44	29,33 ± 0,44	30,33 ± 0,44	31,33 ± 0,44
Eucalyptus honey 3	30,3 ± 0,44	36,33 ±0,44	30,66 ± 0,44	34,33 ± 0,44	33,33 ± 0,44	30,33 ± 0	29,33 ± 0,44
Thyme honey 1	22,67±13,1	31 ± 0,67	32,33 ± 0,44	30,33 ± 0,44	28,33 ± 0,44	29,33 ± 0,44	28 ± 0
Thyme honey 2	31,67±0,44	30,33 ±0,44	28,33 ± 0,44	30,33 ± 0,44	32,33 ± 0,44	3167 ± 0,89	27,33 ± 0,44
Thyme honey 3	38,67±0,44	35,33 ±0,44	29,33 ± 0,44	28,33 ± 0,44	31,33 ± 0,44	29 ± 0,67	31 ± 1,33
Polyfloral honey spread 1	25 ± 0,67	29,33 ±0,44	28,67 ± 0,44	28,67 ± 0,44	21,67 ± 0,44	24,67 ± 0,44	24 ± 0
Polyfloral honey spread 2	25,33±0,44	27,33 ±0,44	26,33 ± 0,44	26,67 ± 0,4	23,33 ± 0,44	21,33 ± 0,44	24,33 ± 0,89
Eucalyptus honey spread 1	29,33±0,44	31,67 ±0,44	32,67 ± 0,44	30,67 ± 0,44	31 ± 0,67	27,33 ± 0,44	30,33 ± 0,44
Eucalyptus honey spread 2	30,67±0,44	31,33 ±0,44	29,33 ± 0,44	26,67 ± 0,44	32,67 ± 0,44	29,67 ± 0,89	32 ± 0,67
Eucalyptus honey spread 3	28,33±0,44	33,33 ±0,44	30,33 ±0,44	27,67 ± 0,44	30,33 ± 0,44	30 ± 0,667	30,33 ± 0,44
Thyme honey spread 1	30 ± 0	29,67 ±0,44	32 ± 0	29,33 ± 0,44	29,33 ± 0,44	28,33 ± 0,889	28,33 ± 0,44
Thyme honey spread 2	29,33±0,44	30,67 ±0,44	28 ± 0,67	27,67 ± 0,88	30,67 ± 0,44	27 ± 0,667	26,67 ± 0,89
Thyme honey spread 3	32,33±0,44	32,67 ±0,44	29,33 ± 0,44	27,67 ± 0,44	30,33 ± 0,44	29,67 ± 0,89	30 ± 0,67

These results could be explained by the nature of phenolic compounds present in honeys which can react with ABTS•+ better than DPPH•. The EC_{50} values of spread prepared range from 12mg/mL to 110mg/mL, showing significant differences ($p < 0.05$) and keeping the same classification as honeys samples. Using ABTS activity, thyme honeys and prepared thyme honeys don't show significant differences ($p < 0.05$) conversely of the result showed with DPPH radical.

FRAP assay

Reducing power is another important parameter for the evaluation of antioxidant activity. The FRAP assay measured the ability of honey samples to reduce Fe (III) to Fe (II) in an acidic medium [31]. Based on the results of the FRAP assay in Table 2, we detected a significant difference ($p < 0.05$) between the honey samples. As shown, thyme honey samples were roughly three times more active than eucalyptus and polyfloral honey samples. The eight prepared spreads showed 50% reduction at 37mg/mL to 113mg/mL with significant differences ($p < 0.05$) between samples. In FRAP assay, only prepared eucalyptus spread showed significant differences ($p < 0.05$) with eucalyptus honey samples.

To summarize, the eight tested honey samples and the spreads prepared showed high antioxidant activity. In addition, we can say that spreads kept the same antioxidant levels as honeys.

Antibacterial activity

The results of the inhibition tests ran with honey samples and honey spreads on the bacterial strains used in this study are shown in Table 3 (Diameter ZI mm). The antibacterial activity was classified as: no sensitive, for diameters lower than 8 mm; sensitive, for diameters from 8 to 14 mm; very sensitive, for diameters from 15 to 19 mm; extremely sensitive, for diameters higher than 20 mm [32]. The results revealed that the eight samples of honey and their prepared spreads showed an excellent antibacterial activity, all diameter of ZI is more than 20mm. *Salmonella arizonae* and *Staphylococcus aureus* was the most sensitive strains against honeys samples and spreads. No single honey exhibited exceptional inhibitory activity than other honeys or spreads. The antibacterial activity of honey is related to several factors acting alone or in synergy. The most prominent of them are hydrogen peroxide, phenolic compounds, the pH of honey and the osmotic pressure exerted by honey [32, 33]. Hydrogen peroxide is the major inhibitor substance of honey. Concentration values of this compound in different honeys result in their varying antimicrobial effects [33, 34].

Conclusion

From the above results, we can conclude the possibility to formulate a functional spread based on olive oil and honeybee emulsified by beeswax. The physicochemical parameters (Stability, IP, HMF) give us information about the quality of the prepared spreads and their aptitude of conservation due to the low obtained values of peroxide and HMF. The high content of phenol and the strong bacterial, as well as antioxidant activities, makes from our new spread an important and interesting product for consumers who are currently looking for foods rich in bioactive properties.

Acknowledgments

This work was supported by grants from The Institution for Research and Higher Education in Agriculture (Ministry of Agriculture, Tunisia) and Central Laboratory of Analysis and Testing of Tunis.

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