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Studying the quality of local propolis and evaluation of its effect as antimicrobial food additive

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Abstract

The effect of using Propolis alone or mixed with chemical preservative on the shelf-life time of Luncheon was evaluated using local(Egyptian) propolis extract collected from farms in different governorates in Egypt. The Minimum Inhibitory Concentration of the extracted propolis was determined and was found to be 20 ppm. Raw minced meat was mixed with different ingredients according to Egyptian Standards with different formulae as follows: (T2) mixture + 50 ppm Sodium Nitrite, (STD) mixture + 125 ppm Sodium Nitrite, (T1) mixture + 20 ppm Propolis, (T3) mixture + 50 ppm Sodium Nitrite + 20 ppm Propolis and (T4) mixture + 125 ppm Sodium Nitrite + 20 ppm Propolis. Total Plate Count (TPC), Total Coliform Count (TCC), Faecal Coliform Count (FCC), Staphylococcal Count, *Bacillus cereus* count, *Salmonella* count, Total Yeast Count (TYC) and Total Fungal Count (TFC) were tested to estimate the effect of the used treatments on the microbial quality and subsequently the shelf life time of processed Luncheon. Panel test was performed on formulae under study to evaluate the effect of the used treatments on the sensory parameters of the products. The obtained results all over the study revealed that, adding proplis in a concentration of 20ppm together with Sodium Nitrite in a concentration of 125ppm increased the shelf life time of luncheon to be 10 days instead of 7days in case of luncheon contained Sodium Nitrite in a concentration of 125ppm. Also, using proplis as the only preservative had the same effect on shelf life time as that of Sodium Nitrite at 125 ppm, making it possible to use proplis as an alternative for chemical preservatives which have many documented disadvantages.

Keywords: propolis, luncheon, food preservatives, shelf life, microbial quality.

1. Introduction

Propolis is a naturally resinous composition made by honeybees from plant parts, buds, and exudates. Propolis is also known as bee glue. Bees need propolis in the formation and preservation of their hives because of its waxy nature and mechanical properties for sealing gaps, smoothing out the internal walls, and as a protective barrier against external strangers such as snakes, lizards, wind, and rain [1]. For its antibacterial and bactericidal qualities, propolis was tested as a food preservative [2]. According to the US Food and Drug Administration (FDA), contamination of meat and its products is expected to be caused through contamination of carcasses by both pathogenic and food spoiling bacteria which could survive the usual antiseptics used at the slaughterhouses [3]. The number of bacteria on/in food materials increases with the amount of exposed surface area. Hence, minced meat has the highest number of pathogenic/food spoilage bacteria [4]. Preservatives are compounds that interfere with the enzymatic and biochemical processes on or in food, enabling and encouraging the existing pathogenic bacterial community to multiply and use the nutritive elements present, making the food harmful to consumers [1].

Researchers have tried to find different sources of preservatives and tried to study its effect not only on food but also evaluated its risk and health hazards on consumers and on the environment. Salt is considered as one of the famous preservatives used in the meat industry but in fresh and partially processed products, salt concentration may reach 17% which renders the final product unpalatable [4]. Nitrite and Nitrite also

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were used as preservatives due to its antimicrobial effect and also due to its sensory effect as it can colour the final product with the good and nice red/pink colour, but the abuse of these chemical substances in larger amounts was confirmed to cause dangerous health hazards for the consumers [5]. So, researchers have started to find out natural preservatives to be used in food processing safely. Propolis is a resinous compound that is carried by the hind legs of honey bees during collection of flower syrup. It is used by serving bees as antibacterial and antifungal compounds inside the hives and also to cover and close all fishers and unwanted openings in the hives [6]. The chemical composition of Propolis varies according to its botanical source but mainly it is composed of 30% wax, 50% resin, 10% essential and aromatic oils, 5% pollen and 5% other substances including organic debris [5].

Propolis is used in the food industry as it has reliable antimicrobial and antioxidant effects which not only keep food healthy and safe but also extend the storage time of such food [6]. The antimicrobial and the antioxidant activity of Propolis were attributed to its richness in flavonoids and phenolic compounds which have a bactericidal effect against different types of food spoilage bacteria that causes deterioration of essential fatty acids and vitamins. Also these compounds have scavenging capacity which causes neutralization of free radicals that are formed during the oxidation process of fatty acids by lipolytic bacteria and which are known to have carcinogenic effects [7].Using ethanol extract of Propolis as a preservative was applied in some meat products like sausage and showed a positive effect as antimicrobial against some gram negative and gram positive bacteria causing extension of shelf life time without altering its sensory properties [7]. In this study, the quality of different sources of Propolis, the MIC of ethanol extract of Propolis against some food poisoning bacteria, the preservative effect of Propolis on Luncheon as a safe alternative to some chemical preservatives were investigated.

2. Experimental

Propolis sample collection:

Three samples of Egyptian produced propolis were collected from Kafr El Sheikh, Gharbeya, and Qualubeya Governorates, Egypt by using wire traps. The Propolis samples of Egyptian origin were mixed together, forming one sample of about 50 g in weight. Weighted sample of 20 g propolis, representing the four seasons (summer, autumn, winter and spring), which was then extracted with Ethanol according to [3] as follows:

Preparation of ethanolic extract of propolis (EEP): To investigate the antimicrobial activity of the Egyptian collected propolis, the samples were prepared as follows:

Extraction of propolis sample:

One flask was prepared with 200 ml of 70% ethanol solution. Ultrasound sonication (frequency: 40 kHz) was used to dissolve the propolis for 20 min. After that, the flask was shaken daily many times by using a shaker for 4-5 days. The sample was filtered and the ethanol was partially removed by the rotary evaporator "Hei-VAP-Advantage motor lift G3 Rotary Evaporator 1300 watt" [8].

Determination of chemical composition of Propolis extract by GC-MS/MS and LC-MS/MS:

Active ingredients of extracted propolis sample under study was determined using GC-MS/MS (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD Agilent 7000) and equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane), while we used LC-MS/MS (SCIEX 4000 QTRAP) according to [9].

Determination of antimicrobial effect of Propolis:

Different concentrations of ethanolic extract of propolis were prepared using dimethyl sulfoxide (DMSO) according to [10] to give (5,10, 20, 30, 40, and 50 ppm) concentrations. All prepared concentrations were kept in clean, dark bottles at room temperature for more practical work.

Preparation of bacterial suspension:

Bacterial strains used in this study (*Staphylococcus aureus*, *B. cereus*, and *Escherichia coli*) were kindly supplied by the Food Safety Laboratory, Regional Center for Food and Feed, Agricultural Research Center, Egypt. Each suspension of *S. aureus*, *B. cereus* and *E. coli* was mixed with about 50 ml of brain heart infusion (BHI) broth and incubated at 37 ºC for 24 hrs. according to [7, 11 and 12], respectively.

Determination of the minimum inhibitory concentrations:

The antibacterial activity of different concentrations of EEP was assessed using the well-diffusion assay (cup-plate method), in which 200 µl of the different concentrations of EEP were added into a well of 10 mm in diameter made in the plate containing nutrient agar medium inoculated with the test microorganisms *S. aureus, B. cereus,* and *E.* coli. The plates were incubated at 37°C for 24 hrs. Growth inhibition appeared as a measurable clear zone around the well [13 and 14].

Preparation of luncheon samples:

Table (1) Preparation of luncheon according to [15]:

Materials	STD	T1(1)	T2(2)	T3(3)	T4(4)
Meat %	80	80	80	80	80
Fat %	10	10	10	10	10
Salt %	3	2.2	3	2.7	2.9
$Na_2H_2P_2O_7$ %	0.15	0.15	0.15	0.15	0.15
$NaNO_2$ (ppm)	125	-	50	50	125
Spices %	1	1	1	1	1
Skim milk %	0.3	0.3	0.3	0.3	0.3
Starch %	3	3	3	3	3

Water %	2.5	2.5	2.5	2.5	2.5
Propolis (ppm)	-	20	-	20	20

Standard: Meat + Sodium nitrite (125 ppm)

(1) luncheon without sodium nitrite + Propolis

(2) Sodium nitrite (50 ppm) without Propolis

(3) Sodium nitrite (50 ppm) + propolis

(4) Sodium nitrite (125 ppm) + propolis

Using Propolis extract as preservative in Luncheon processing:

Processing was performed according to [15] as follows:

The mixture was packaged in a thermal transparent bag, then stretched well and warped with aluminum foil, and processed in boiling water for 30 minutes [16].

Determination of the microbiological quality of luncheon:

Samples of luncheon preparation Table 1 was performed according to the NMKL methods, after which the following microbial analysis: Total Bacterial Count, Total Coliform Count, Faecal coliform Count, Staphylococcal Count, *B. cereus* count, and Total Yeast and Mold count according to [6, 17, 7, 11, 12 and 5], respectively and Salmonella count according to [18]. All microbiological parameters were investigated after 0, 1, 3, 5, 7, 10 and 14 days of storage.

Panel test:

For subjective evaluation of luncheon quality, the panel test was carried out according to [16]. The luncheon was subsampled into seven groups and sliced into little pieces before being rated for color, smell, texture, and taste by 10 persons using the following scale: (-) dislike, (+) fair, (++) good, (+++) very good, and (++++) excellent.

Statistical analysis:

Data were analysed using the General Linear Model (GLM) and using the SAS 9.4 TS Software (2013). Means are compared using Duncan's Multiple Range Test. The mean differences are significant at P-value ($P < 0.05$) [19].

Results

Characterization of extracted propolis:

GC-MS/MS analysis:

From the microbiological tests of the extracted propolis sample, it is clear that it was free from all indicator parameters: total Bacterial count (TPC), total coliform count (TCC), faecal coliform count (FCC), and total fungal count (TFC). The microbial quality assessment is an indicator of a good and reliable extraction technique.

Table 2 illustrates the active ingredients determined in the Egyptian extracted propolis sample measured by GC-MS/MS. It is clear from the data that this tested sample was rich in many flavonoids and phenolic substances, with 7,4-Dimethoxy-3-hydroxy flavone found to have the highest amount of active ingredients together with 6,4-Dimethoxy-7-hydroxyisoflavone and prunetin, which was concluded by comparing the obtained peak area of all detected ingredients. Ethyl 7 and 3-6 Dimethoxy were found to be the 4th and 5th predominant active ingredients in the Egyptian propolis sample.[20]

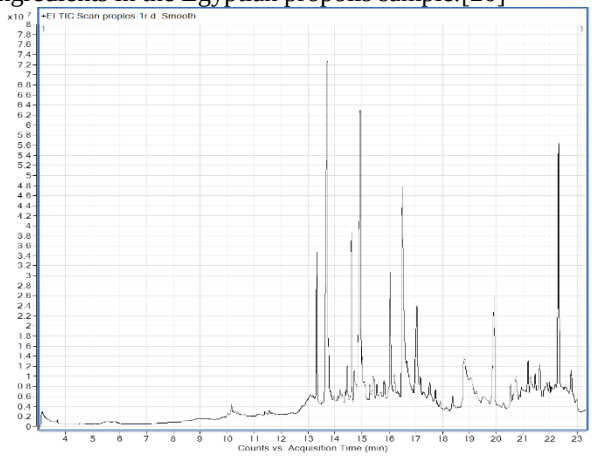

Fig. 1: Chromatograph of the scan of Egyptian extracted propolis sample by GC-MS/MS

LC-MS/MS analysis:

Data obtained in Table 3 and Figure 2 illustrated the active ingredients present in Egyptian extracted propolis sample analysed by LC-MS/MS. It is clear from the obtained data that the most predominant active ingredients were Chrysin parent ion 253m/z with predominant daughter of 143m/z, Galangin parent ion 271m/z with predominant daughter of 153m/z and Apigenin parent ion 269m/z with predominant daughter of 117m/z in an ascending manner.

The intensity of the obtained peaks for parent ions are illustrated in table 3 and figure 2

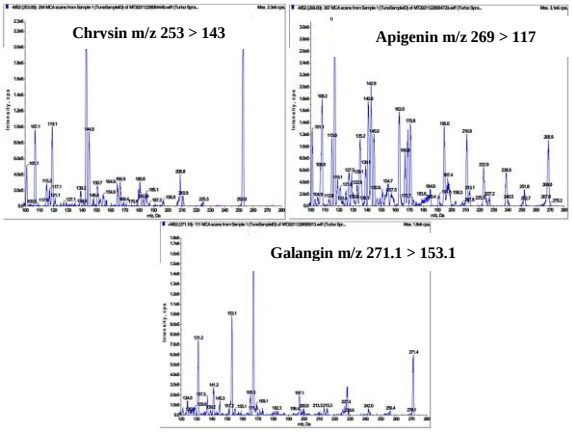

Fig. 2: Mass spectrometry in positive and negative ion mode monitoring for phenolic compounds in propolis

Table (2) Peak area of detected active ingredients in local Propolis samples under study:

	Detected compounds	Peak area
1	7,4'-Dimethoxy-3-hydroxyflavone	14.24
2	6,4'-Dimethoxy-7-hydroxyisoflavone	10.07
3	Prunetin	9.00
4	Ethyl 7-hydroxycoumarin-4-carboxylate	7.25
5	3,6'-Dimethoxy-2'-hydroxychalcone	4.53
6	Quercetin 3,5,7,3',4'-pentamethyl ether	3.21
7	5,7,3',4',5'-Pentahydroxyflavone	3.5
8	3,4'-Dimethoxy-5,7,3'-trihydroxyflavone	2.58
9	5,7-Dimethoxy-3-hydroxyflavone	2.23
10	(S)-(-)-Citronellic acid	2.17
11	Genistin	2.04
12	cis-Trismethoxyresveratrol	1.59
13	7,3',4',5'-Tetramethoxyflavanone	1.41
14	Quercetin-3,7,3',4'-tetramethyl ether	1.34
15	3-Hydroxy-7,8,2',3'-tetramethoxyflavone	1.24
16	3-Hydroxy-6,2',3'-trimethoxyflavone	1.22
17	7-Methoxy-8-methylisoflavone	1.13
18	4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	1.97
19	Formononetin	2.92
20	5,7,3',4',5'-Pentamethoxyflavone	4.89
21	4'-Benzyloxy-5,7-dimethoxyflavone	2.88
22	6-Methyl-3-(4-methoxyphenyl)-4-phenylcoumarin	5.85
23	5,7,2'-Trimethoxyflavone	5.76
24	3-(3,4-Dimethoxyphenyl)-7-hydroxy-4-phenylcoumarin	1.71
25	Ononin	-
26	6,7,3',4'-Tetramethoxyisoflavone	-
27	3-Hydroxy-3',4',5'-trimethoxyflavone	-
28	4-Hydroxy-2',4',6'-trimethoxychalcone	-
29	3,4,5-Trimethoxycinnamic acid	-

Table (3) List of the most dominant active ingredients of an extract of locally extracted propolis analyzed by LC-MS/MS expressed as estimated qualitatively through Peak area[**9**]**:**

Ethanol extract	**Chrysin**	**Galangin**	**Apigenin**
(Intensity, cps)	807	$3.18e^3$	$7.00e^3$

Antimicrobial activity of propolis [10]:

Table 4 shows that the Minimum Inhibitory Concentration (20 ppm) of local propolis extract was used in the manufacturing of luncheon with partial and complete replacement of the commonly used preservative sodium nitrite. The effect of different inclusion concentrations of preservatives under study on the microbial quality of luncheon before, during, and after processing was investigated. Evaluation of the antimicrobial effect of propolis samples under study against some pathogenic bacteria (Table 4) revealed that, at a concentration of <10 parts per million all tested organisms could survive the antimicrobial effect of propolis meanwhile at 10 ppm *E. coli* could survive.

Table (4) Diameter of inhibition zone (mm) caused by local extracted propolis sample against some pathogenic bacteria:

Propolis conc. (ppm)	***Staphylococcus spp.***	***B. cereus***	***E. coli***
5	R	R	R
10	18	15	R
20	22	22	20
30	31	23	25
40	39	26	29
50	38	32	30

Data obtained in Table 5 illustrated the total bacterial count (TPC) obtained from the analysis of raw minced meat. The obtained count $60x10^5$cfu/g was significantly decreased after mixing and addition of the ingredients as clear from the reduction of log10 by 1 log which indicates significant difference. These

results showed that ingredients represented by adding propolis only (20ppm) used in T1 could control TPC and keep the count within the limit till the 7th day and had a preservative effect on TPC as the effect of standard which is routinely used in luncheon manufacturing. It was clear that propolis made a marked reduction of bacterial count which was >10 till the 3rd day of storage and only 5x10 (cfu/g) on the 5th day.

During the 7 days of storage, Total Bacterial Count was significantly higher in T2 and T3 when compared with that obtained in standard (STD), but T1 and T4 were within the permissible level according to Egyptian standards (maximum10^4 cfu/g). On the other hand, after the 14 days of storage, the most effective treatments were T1 and T4 which gave the same value as that of STD still within the permissible limit according to Egyptian standards but the values obtained in T2 and T3 were higher than that obtained in STD and exceeded the permissible limit according to Egyptian standards.

Table 5: Comparison between the effect of local propolis extract and different concentrations of sodium nitrite on Total bacterial count in processed luncheon:

Sample No	Minced meat Before Mixing and Process cfu /g	Before Processing after Mixing cfu /g	After processing (cfu/g)						
			0d	1d	3d	5d	7d	10d	14d
STD		$14x10^4$	ND	$30x10^2$	$14x10^3$	$20x10^3$	$11x10^3$	$10x10^4$	$90x10^4$
T1		$90x10^4$	ND	ND	ND	5x10	$20x10^4$	$30x10^4$	$60x10^4$
T2	$60x10^5$	$14x10^4$	5x10	29x10	$50x10^3$	$26x10^3$	$23x10^5$	$37x10^5$	$>10^5$
T3		$90x10^4$	18x10	6x10	17x10	$10x10^3$	$68x10^5$	$34x10^5$	$>10^5$
T4		$90x10^4$	ND	$20x10^2$	$10x10^2$	$50x10^2$	$13x10^3$	$50x10^3$	$30x10^4$

In Table (6), it was mentioned that total coliform count (TCC) was 70x104cfu/g in minced meat before mixing. After mixing there was a significant reduction in TCC which occurred due to the effect of the ingredients such as salt, sodium Nitrite and used spices which was significantly declined after processing by the effect of the used ingredients and heat. In T2, the processing technique could inhibit all bacteria as indicated by the absence of total coliform on the 0 to 7th days, but at the 9th and 14th days of storage, injured bacteria could survive, giving a count of 15x10 and 24x10cfu/g, respectively. From the start, mixtures in T1, T3 and T4 showed complete inhibition of total coliform count from the start till the end of storage time.

In Table (7), the trend and the pathway of the growth and the inhibition of faecal coliform count were identical to those of total coliform count from the start of the experiment till the end of the storage period. All treatments under study showed an excellent inhibitory effect on total faecal coliform. Also, Table (6 and 7) it was clear that all treatments could keep the count of TCC and FCC within the permissible limit according to the Egyptian standards.

Table 6: Comparison between the effect of local propolis extract and different concentrations of sodium nitrite on Total coliform count in processed luncheon:

Sample No	Minced meat Before Mixing and Process cfu/g	Before Processing after Mixing cfu/g	After processing (cfu/g)						
			0d	1d	3d	5d	7d	10d	14d
STD		34x10	ND	ND	ND	ND	ND	6x10	3x10
T1		37x10	ND	ND	ND	ND	ND	ND	ND
T2	$70x10^4$	34x10	ND	ND	ND	ND	ND	15x10	24x10
T3		37x10	ND	ND	ND	ND	ND	ND	ND
T4		37x10	ND	ND	ND	ND	ND	ND	ND

Table 7: Comparison between the effect of local propolis extract and different concentrations of sodium nitrite on faecal coliform count in processed luncheon:

Sample No	**Minced meat Before Mixing and Process cfu/g**	**Before Processing after Mixing cfu/g**	**After processing (cfu/g)**						
			0d	**1d**	**3d**	**5d**	**7d**	**10d**	**14d**
STD	$12x10^3$	8x10	ND	ND	ND	ND	ND	20x10	20x10

T1	20x10	ND	ND	ND	ND	ND	ND	ND
T2	8x10	ND	ND	ND	ND	ND	3x10	5x10
T3	20x10	ND	ND	ND	ND	ND	ND	ND
T4	20x10	ND	ND	ND	ND	ND	ND	ND

The data in table (8) demonstrated the effect of the treatments used on staphylococcal count. It is clear from the mentioned values that the initial staphylococcal count obtained from raw minced meat ($5x10^3$cfu/g) was significantly increased after mixing in all treated groups. This increase was attributed to the staphylococcal counts present in the used ingredients and also to the bacterial load that was added during handling and processing. All groups showed complete inhibition of staphylococci just after cooking (0 time) except for T4, which had counts of 30x10 cfu/g. Injured bacteria started to revitalize in all treatments during the whole storage period, except in T3, where no growth was detected till the end of the experiment. Only treatmentsT1 and T2 could keep the staphylococcal count within the normal limit till the 7th day of storage and hence extend the shelf life of the product if compared to raw minced meat (STD) luncheon, which were made with the recommended standard procedure by codex alimentations [15].

On the other hand, *B. cereus* count, *Salmonella* spp. and total fungal count in raw minced meat were $20x10^2$, $80x10^2$ and $16x10^3$cfu/g, respectively. All groups showed complete inhibition of their pathogens from the start to the end of the experiment and so discovered that propolis had an antimicrobial effect on bacterial count and an antifungal effect on food-borne fungi.

In Table 9, the counted yeast in raw minced meat ($16x10^3$cfu/g) was significantly increased after mixing and the addition of spices, salt, chemical preservatives, and other ingredients. On the 5th day of storage, an increase of injured yeast by the effect of heat, and the count increased gradually till it reached $> 10^3$ cfu/g (the recommended count by Egyptian standards) on the 10th day of storage. The addition of sodium nitrite caused a faster increase in total yeast count than what occurred in T2 and T3, as the count reached $> 10^3$ cfu/g on the 7th day.

The addition of sodium nitrite at 125 ppm (recommended by Egyptian and Codex standards) could keep the total yeast count below the maximum permissible limit until the end of the storage period of propolis alone at a concentration of 20 ppm (the obtained MIC during the preliminary experiment) in T1 and T4, which could keep the yeast count within the recommended limit till the 7th day.

Table 8: Comparison between the effect of local propolis extract and different concentrations of sodium nitrite on Total staphylococcal count in processed luncheon:

Sample No	Minced meat Before Mixing and Process cfu/g	Before Processing after Mixing cfu/g	After processing (cfu/g)						
			0d	1d	3d	5d	7d	10d	14d
STD	$5x10^3$	$1x10^5$	ND	ND	80x10	$13x10^2$	$20x10^3$	$17x10^4$	$40x10^4$
T1		$9x10^5$	ND	ND	ND	ND	ND	3x10	10x10
T2		$1x10^5$	ND	ND	ND	ND	ND	20x10	30x10
T3		$9x10^5$	ND	ND	ND	ND	ND	ND	ND
T4		$9x10^5$	30x10	13x10	20x10	$10x10^2$	$80x10^2$	$6x10^3$	$20x10^3$

Table 9: Comparison between the effect of local propolis extract and different concentrations of sodium nitrite on Total yeast count in processed luncheon:

Sample No	Minced meat Before Mixing and Process cfu/g	Before Processing after Mixing cfu/g	After processing (cfu/g)						
			0d	1d	3d	5d	7d	10d	14d
STD	$16x10^3$	$>10^3$	100x10	60x10	80x10	90x10	100x10	60x10	$5x10^2$
T1			ND	ND	ND	ND	8x10	$28x10^2$	$30x10^3$
T2			ND	ND	$18x10^3$	12x103	$>10^3$	$>10^3$	$>10^3$
T3			3x10	5x10	10x10	$14x10^3$	$>10^3$	$>10^3$	$>10^3$
T4			4x10	1x10	1x10	3x10	4x10	1x10	2x10

In Table 12, it was clear that no significant changes were observed either in colour, smell, or texture between T2, T3, and T4 compared with standard. On the other hand, there were significant differences in colour between T1 and standard. There were significant changes in taste between T2 and T3 compared with standard. It was noticed that the ingredients containing propolis (T1 and T4) had the acceptable taste of luncheon by all the participants in this panel test

Panel test

Table 10 Template of panel test score sheet

Sample No. Items	Control	1	2	3	4
Color					
Smell					
Texture					
Taste					

Table 11 Template of Comparison score sheet

Sample No.	There is a difference	There is No differences
1		
2		
3		
4		

.

Table 12: Statistical analysis of panel test results of luncheon with different compositions:

	Parameters			
Groups	Colour	Smell	Texture	Taste
STD	2.8 ± 0.42^{a}	2.7 ± 0.48^{a}	2.2 ± 0.42^{abc}	2.9 ± 0.56^{a}
T1	0.8 ± 0.78^{c}	2.4 ± 0.51^{b}	2.1 ± 0.73^{a}	2.5 ± 0.71^{ab}
T2	1.7 ± 0.67^{a}	2.6 ± 0.51^{a}	2.1 ± 0.56^{a}	2.4 ± 0.51^{b}
T3	1.6 ± 0.51^{ab}	2.7 ± 0.48^{a}	2.1 ± 0.56^{a}	2.4 ± 0.51^{b}
T4	2.6 ± 0.51^{a}	3 ± 0.66^{a}	2.3 ± 0.48^{abc}	2.7 ± 0.48^{a}

Mean values are expressed as means ± SD. Means with different superscript letters in the column are significantly different at P < 0.05.

3. Discussion

Recently, researchers have started to find natural preservatives that can be used in food processing safely. Propolis was tested as a food preservative. The antimicrobial and antioxidant activities of Propolis were attributed to its richness in flavonoids and phenolic compounds, which have a bactericidal effect against different types of food spoilage bacteria that cause deterioration of essential fatty acids and vitamins.

The main purpose of the microbiological tests of ethanolic extract of propolis (EEP) sample is to be an indicator of a good and reliable extraction technique. It is clear that it was free from all indicator parameters: total bacterial count (TPC), total coliform count (TCC), faecal coliform count (FCC), and total fungal count (TFC). In this study, we used a concentration of 20 ppm of propolis because it is the minimum inhibitory concentration of inhibition of selected bacteria Staphylococcus spp., B. cereus, and E. coli, which is the appropriate percentage that has been used in the luncheon industry. These results agreed with those reached by [4] that propolis at a concentration of 0.02 g/ml showed significant inhibition of pathogenic bacteria growth and recommended that it be used as a natural additive preservative.

In this study, it was found that the EEP sample was rich in many flavonoids and phenolic substances as measured by GC-MS/MS and LC-MS/MS. This data is consistent with the findings of a study that demonstrated that the antimicrobial effects of propolis are related to its flavonoid content and, as a result, vary depending on the botanical origin of the product. Products with higher concentrations of polyphenols and flavonoids were also the ones with higher antimicrobial activity [3, 25 and 26and27]. In the case of using propolis at a concentration of (20 ppm), this showed an extended shelf life till 7 days of storage. This result was similar to that obtained by [28], who concluded that using propolis is recommended as a natural antioxidant and a good alternative to chemical preservatives according to[29,30]. The results of the panel test showed that was the most acceptable preservative, and the results of the panel test were not significant compared with the traditional way of manufacturing luncheon meat using sodium nitrite as a chemical preservative. Furthermore, studies can be conducted to study how to benefit from the antimicrobial activity of propolis at a more effective concentration.

4. Conclusions

At the end of this research, we can summarize that propolis can be completely or partially replaced by sodium nitrite preservatives during luncheon processing. A luncheon containing 20 ppm of propolis without sodium nitrite could be considered an excellent treatment. Propolis, as a preservative, has a significant effect on food poisoning bacteria and can extend the shelf life of a variety of food categories.

5. Acknowledgements

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