

EFFICIENCY OF PROPOLIS AND TURMERIC POWDERS AS NATURAL PRESERVATIVES IN MINCED BEEF

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ABSTRACT

Because of the great need of using natural food additives nowadays, so, this work was conducted to utilize of propolis and turmeric powders at different levels (1.5, 2 and 2.5 %) as natural preservatives during preparation of minced beef stored at refrigeration temperature ($7 \pm 1^\circ\text{C}$). The obtained results revealed that, propolis and turmeric had considerable effectiveness in decreasing aerobic plate count (APC) as well as some chemical indices as pH and thiobarbituric acid (TBA). Also, results indicated that the bacterial counts, pH and TBA values decrease as a function of increasing the concentration of the tested powder since the concentration of (2.5%) gives the best effectiveness. The antioxidant and antibacterial activities of the added propolis powder were higher than those of turmeric powder. In conclusion, propolis and turmeric can play an important role as antioxidant and antibacterial agents in refrigerated minced beef, but propolis powder is seems to be better one.

Keywords: Propolis, turmeric, refrigerated storage and minced beef.

INTRODUCTION

Meat is considered the major source of protein and valuable qualities of vitamins for most people in many parts of the world, thus they are essential for the growth repair and maintenance of body cells and necessary for our everyday activities (Hassan *et al.*, 2006). Due to the chemical composition and biological characteristics, meats are highly perishable foods which provide excellent source for growth of many hazardous microorganisms that can cause infection in humans and spoilage of meat and economic loss (Kim and Rajagopal, 2001).

Chemical preservatives are used to prevent the growth of food spoiling microbes in the food industry (Rasooli, 2007). There has been constant increase in search of alternative and efficient compounds for food preservation aimed at a partial or total replacement of antimicrobial chemical additives (Sağdıç and Özcan, 2003). Also there has been increase of consumers about foods free or with lower level of chemical preservative because these could be toxic for humans (Yoshida *et al.*, 1998). The use of natural preservatives to increase the shelf-life of meat products is a promising technology since many herbs, plants, vegetable and fruits extracts or powders have antioxidant and antimicrobial properties (Biswas *et al.*, 2012).

Recently attention has been focused on the use of propolis as a health supplement suited to consumers in developed countries. Propolis, a natural honey bee product, has different biological activities. It is a resinous substance collected by *Apis mellifera* L. from various tree buds, and used for coating hive parts and also sealing cracks and crevices in the hive. Chemical analyses revealed that propolis contains more than 300 constituents among them phenolic compounds, including flavonoids as major components (Bankova *et al.*, 2000).

Propolis has been much popular as an agent in traditional medicine and food supplementary material for human health in the world (Pereira *et al.*, 2008). It is a commercial resinous product that contains phenols and many other preventive agents. Propolis is produced

by honeybees using collected extracts from leaves, buds and exudates of various plant floras. Propolis is used as a building material in order to strengthen the borders of combs and as a chemical weapon against the pathogen microorganisms (Wollenweber *et al.*, 1990). Its antibacterial, antiviral, antitumor, anti-inflammatory, anticancer and immunomodulatory effects have been reported (Banskota *et al.*, 2002; Murad *et al.*, 2002 and Zhou *et al.*, 2009).

Turmeric is a spice that comes from the root of *Curcuma longa*, a member of the ginger family, Zingiberaceae (Pierce, 1999). It is bright yellow and has been used as a coloring and flavouring agent in foods. In India, it has been used for centuries as a spice and a food preservative, and also for its various medicinal properties. In Ayurveda (Indian traditional medicine), turmeric has been used for various purposes and through different routes of administration. It has been used topically on the skin for wounds, blistering diseases such as pemphigus and herpes zoster, for parasitic skin infections, and for acne. It has been used via oral administration for the common cold, liver diseases, urinary tract diseases, and as a blood purifier. For chronic rhinitis and coryza, it has been used via inhalation (Majeed *et al.*, 1996; Eigner and Scholz, 1999). Turmeric contains phenolic compounds called curcuminoids that possess all the bio-protective properties of turmeric (Goel, 2009). Turmeric extracts are found to show antibacterial activity against methicillin resistant *Staphylococcus aureus* (Kim *et al.*, 2005). The objective of the present study was to investigate the antimicrobial properties of propolis and turmeric in order to use them in augment the shelf life of minced beef as natural preservatives. Also their effect on microbiological and chemical attributes of the product under refrigeration ($7 \pm 1^\circ\text{C}$) storage was evaluated.

MATERIALS AND METHODS

1. Materials:

Propolis was brought from National Research Center in Cairo, Egypt. Turmeric and minced beef were obtained from the local market in Kafrelsheikh city,

Egypt. All microorganisms strains (*Listeria monocytogenes* – *Pseudomonas aeruginosa* - *Fusarium oxysporum* – *Saccharomyces cerevisiae*) were kindly provided by the Plant Pathology Department, Faculty of Agriculture, Kafr El-sheikh University. In addition, nutrient agar medium (NAM) and potatoes dextrose agar (PDA) used in the microbiological examination were purchased from Merck Co. Ltd. (Darmstadt, Germany). The applied reagents were of the highest purity available and purchased from the Sigma-Aldrich Chemical Company (St. Louis, Mo., USA).

2. Methods:

Determination of bioactive compounds:

Bioactive compounds of propolis and turmeric aqueous extracts were performed using GC- MS analysis according to the method described by Mahalingan *et al.* (2012). Perkin Elmer GC clarus 500 system comprising AOC – 20i auto- sampler and Gas chromatograph interfaced to a Mass spectrum meter (GC- MS). The relative percentage amount of each compound was calculated by comparing its average peak area to the total areas.

Preparation of aqueous extracts from propolis and turmeric:

Extraction was carried out as described previously by Gulcin *et al.* (2010). Propolis (25gm) was ground into a fine powder in a blender and mixed with 400 ml boiling water by magnetic stirrer for 15 min. Then the aqueous extract was filtered over cheese-cloth and Whatman No. 1 paper, respectively.

Turmeric (100 gm each) was crushed and sieved through mesh cloth to get the fine powder. Powdered spices were soaked in 200ml of distilled water and were kept at room temperature for 24 hours, then were filtered using Whatman No. 1 filter paper. These extracts were evaporated under vacuum in rotary evaporator at 45°C and stored at 4°C in refrigerator.

Antimicrobial activity of propolis and turmeric aqueous extracts :

Antimicrobial activity of propolis and turmeric aqueous extract was carried out by disc diffusion method against some microorganisms as described by Shihabudeen *et al.* (2010). Some bacterial strains represent gram positive bacteria namely *Listeria monocytogenes* and gram negative bacteria *Pseudomonas aeruginosa* were used. In addition, fungi isolate was also examined in this study, namely *Fusarium oxysporum*. Moreover, one strain of yeast namely *Saccharomyces Cerevisiae* was also tested.

The appropriate media were poured into sterile plates (12 cm diameter), left to solidify, at room temperature. The organisms were inoculated on the surface of the prepared media. A sterile disc, 6 mm diameter of Whatman No. 1 filter paper were dipped in the appropriate solutions, blotted and then placed on the surface of inoculated plates. The inhibitory effect of the distilled water and phenol solution 1% (w/v) was also tested by placing saturated disc with only distilled water and placing saturated discs with phenol solution on each inoculated plate. The plates of bacteria were kept for incubation at 37° C for 48 hrs, whereas the plates of

fungi and yeast were incubated at 25°C for 5 days. At the end of incubation period, inhibition zones formed around the disc were measured with transparent ruler in millimeter. All tests were performed in triplicate with four discs per plate. The bacteria were cultured on nutrient agar, while the fungi were inoculated on potatoes-dextrose agar (PDA).

Propolis and turmeric antimicrobial activity in minced beef:

Powder of propolis or turmeric at 1.5%, 2% and 2.5% levels were added in minced beef separately. Similar to experiment I, a reference with BHT and control product were prepared. After preparation of the test products and control, they were packed in polyethylene bag, and stored at (7 ± 1°C) for 9 days. The samples were analyzed chemically and examined microbiologically every three days during storage period (Najeeb *et al.*, 2014).

Some Chemical attributes of minced beef:

pH values were measured using a digital pH-meter (HAANA, HI902 meter, Germany). Two readings were taken from each of three minced beef samples (Yassin, 2003).

Thiobarbituric acid (TBA) was carried out according to the method recommended by Vyncke (1970).

Microbiological examination:

Determination of aerobic plate count (APC) was performed according to APHA (1992) method.

Statistical Analysis:

Each experiment was replicated thrice and each parameter was analyzed in duplicate. The data recorded were analyzed using SPSS version 17.0 (SPSS, Chicago, III, and U.S.A). Two way analysis of variance was applied and the data were tabulated. The level of significant effects were tested by comparing mean values using the least significant difference (LSD) test at 1 and 5% level as outlined by (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Bioactive compounds in propolis and turmeric powders:

Many bioactive compounds were extracted from propolis and turmeric powders. These compounds were identified and determined using GC-MS and the results are listed in Tables (1) and (2), respectively. It could be noted that propolis powder contains 6 bioactive compounds. 4-aminocyclohepta[f]thieno[2,3-b]pyridine (66.02%), A-Neoleana-3(5), 12-diene (22.99%) and Trans-cyclohexanol,2-(methylaminomethyl) (7.30%) were the major bioactive compounds presented and identified in propolis powder. While, turmeric powder contains 16 bioactive compounds, where the most predominant compounds presented and identified were Beta-Tumerone (51.17), Alpha- Tumerone (22.64%), AR-Curcumene (4.47%) and beta-Farnesene (4.15%). These results are in the same line with those of Ruby *et al.* (1995), who stated that, Curcumin, demethoxycurcumin, bis-demethoxycurcumin, and aromatic-turmerone were the major bioactive compounds of turmeric.

Table (1): Bioactive compounds in Propolis powder.

Compound Name	%
Pentacosane	0.36
5,5-D2-Trans-4,3-Dihydroxycyclopentene	0.64
Dodecane	1.04
Trans-cyclohexanol,2-(methylaminomethyl)	7.30
4-aminocyclohepta[f]thieno[2,3-b]pyridine	66.02
A-Neooleana-3(5), 12-diene	22.99
4-Methoxyamphetamine	1.66

Table (2): Bioactive compounds in turmeric powder.

Compound Name	%	Compound Name	%
2,3-Butanediol	3.47	Para-cymene	2.04
Cuminal	0.69	Alpha-Curcumene	2.40
Trans-Caryophyllene	0.53	Beta-Tumerone	51.17
Amphetamine	0.39	Alpha- Tumerone	22.64
AR-Curcumene	4.47	Amidephrine	0.44
Beta-bisabolene	0.84	Alpha-Atlantone	3.29
beta-Farnesene	4.15	Beta-Tumerone	0.63
1,2,4,5-tetramethylbenzene	0.53	Cinnamyl Tiglate	0.71

Antimicrobial activity of propolis and turmeric aqueous extract:

Both of propolis and turmeric aqueous extract gave an inhibition zones against *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Fusarium oxysporum* and *Saccharomyces cerevisiae*. Data in Table (3) show that, propolis aqueous extract gave the highest wide inhibition zones (11.2mm) with *Listeria*

monocytogenes, (12.5mm) with *Pseudomonas aeruginosa*, (13.0mm) with *Fusarium oxysporum* and (9.1mm) with *Saccharomyces cerevisiae*. Generally, propolis showed effective antimicrobial activity against gram negative bacteria higher than that of gram positive bacteria. Meanwhile, the opposite was found in the case of turmeric. These results were in accordance with those of Asimi *et al.* (2013).

Table (3): Antimicrobial activity of propolis and turmeric toward some microorganisms.

The tested aqueous solutions	Diameters of inhibition zones (mm) for some microorganisms			
	<i>Listeria monocytogenes</i> (G+)	<i>Pseudomonas aeruginosa</i> (G-)	<i>Fusarium oxysporum</i>	<i>Saccharomyces cerevisiae</i> .
Distilled water	0.00	0.0	0.0	0.0
Propolis aqueous extract	11.2 ^{cA}	12.5 ^{bA}	13.0 ^{aA}	9.1 ^{dA}
Turmeric aqueous extract	10.8 ^{bC}	10.7 ^{bB}	11.8 ^{aB}	8.7 ^{cB}
Phenol 1%	11 ^{aB}	10.3 ^{cC}	0.00	7.7 ^{bC}

Means with different superscripts (capital letters in the same column and small letters in the same row) are significantly different at P ≤ 0.01.

Chemical and microbiological qualities assessment of minced beef treated with propolis or turmeric powders: pH changes:

The effect of adding of propolis and turmeric at different levels on the pH values of minced beef stored at (7 ± 1°C) for 9 days is shown in Table (4).

The results in Table (4) showed that the pH value of the control and all tested samples at zero time had the same value (5.32). Furthermore, there was a significant (at P≤0.01) increase in pH mean values for different treatments during storage by using different rates of the tested propolis and turmeric and the highest incremental rates (pH values) were found in the untreated (control) samples.

Table (4): pH values of minced beef treated with propolis and turmeric powder during storage at 7 ± 1°C for 9 days.

Storage period (days)		pH values			
		0	3	6	9
Treatment					
Control		5.32 ^{dA}	6.27 ^{cA}	6.98 ^{bA}	7.20 ^{aA}
BHT		5.32 ^{dA}	5.40 ^{cF}	5.49 ^{bF}	5.57 ^{aF}
With propolis	1.5%	5.32 ^{dA}	6.09 ^{cB}	6.62 ^{bC}	6.75 ^{aB}
	2.0%	5.32 ^{dA}	5.92 ^{cC}	6.10 ^{bE}	6.63 ^{Ad}
	2.5%	5.32 ^{dA}	5.47 ^{cE}	5.99 ^{bE}	6.58 ^{aE}
With turmeric	1.5%	5.32 ^{dA}	6.11 ^{cB}	6.72 ^{bB}	6.78 ^{aB}
	2.0%	5.32 ^{dA}	5.97 ^{cC}	6.50 ^{bD}	6.74 ^{aB}
	2.5%	5.32 ^{dA}	5.50 ^{cD}	6.10 ^{bE}	6.70 ^{aC}

Means with different superscripts (capital letters in the same column and small letters in the same row) are significantly different at P ≤ 0.01.

The samples treated with 2.5% propolis and 2.5% turmeric powder, showed the highest significant (at $P \leq 0.01$) effect on pH lowering its values than those of untreated samples, followed by samples treated with 2% propolis and turmeric powder, respectively, lowering pH values of treated minced beef can enhance microbial inhibition. Finally the samples treated with 1.5% propolis and turmeric, respectively, till reaching the end of the storage period. There was a significant (at $P \leq 0.01$) increase in pH mean values of all untreated and treated samples with propolis or turmeric at all concentrations at the 9th day of the storage period.

Similar findings were found in pork patties and ground buffalo meat containing BHA/ BHT antioxidants during refrigerated and frozen storage, that reported by (McCarthy *et al.*, 2001). The increase in pH could be attributed to the activation effect of microbial load which may cause protein hydrolysis with the appearances of alkyl groups (Yassin, 2003).

TBA changes:

Data presented in Table (5) shows the changes of TBA values in minced beef containing different concentrations of propolis and turmeric powder and stored at ($7 \pm 1^\circ\text{C}$) for 9 days.

Table (5): TBA values of minced beef treated with propolis and turmeric powder during storage at $7 \pm 1^\circ\text{C}$ for 9 days.

Treatment		Storage period (days)	TBA values			
			0	3	6	9
Control			0.03 ^{dA}	0.44 ^{cA}	0.59 ^{bA}	0.62 ^{aA}
BHT			0.03 ^{dA}	0.08 ^{cF}	0.12 ^{bF}	0.16 ^{aG}
With propolis	1.5%		0.03 ^{dA}	0.21 ^{cC}	0.32 ^{bC}	0.40 ^{aC}
	2.0%		0.03 ^{dA}	0.18 ^{cD}	0.27 ^{bD}	0.33 ^{aD}
	2.5%		0.03 ^{dA}	0.12 ^{cE}	0.23 ^{bE}	0.28 ^{aF}
With turmeric	1.5%		0.03 ^{dA}	0.25 ^{cB}	0.38 ^{bB}	0.46 ^{aB}
	2.0%		0.03 ^{dA}	0.21 ^{cC}	0.30 ^{bC}	0.35 ^{aD}
	2.5%		0.03 ^{dA}	0.16 ^{cD}	0.25 ^{bD}	0.31 ^{aE}

Means with different superscripts (capital letters in the same column and small letters in the same row) are significantly different at $P \leq 0.01$.

The evaluation of TBA mean values of control and treated samples during storage at ($7 \pm 1^\circ\text{C}$) are shown in Table (5). The highest incremental rate was recorded in the untreated (control) samples, while the lowest one was recorded in samples treated with 2.5% propolis and turmeric powder, followed by samples treated with 1.5% propolis and turmeric powder, respectively. The lowest values were found in samples treated with 1.5% propolis, turmeric powder and BHT, respectively, till the end of the storage period. The incremental pattern in TBA values for all the stored samples with advancing the chilling storage time may be due to the auto-oxidation of meat lipids, bacteriological and/or oxidative rancidity. TBA value is routinely used as an index of lipid oxidation in meat products in stores (Raharjo and Sofos, 1993) and the

rancid flavor is initially detected in meat products that had TBA values in the range of 0.5 and 2.0 (Gray and Pearson, 1987).

Aerobic count of minced beef treated with propolis and turmeric powders:

Meat is prone to both microbial and oxidative spoilage, therefore it is important to use a preservative with both antioxidant and antimicrobial properties (Kanatt *et al.*, 2008). The growing concern about the safety of foods has led to the development of natural antimicrobials to control food borne pathogen (Nevas *et al.*, 2004).

Table (6) shows the effect of adding different concentrations of propolis and turmeric powders to minced beef stored at ($7 \pm 1^\circ\text{C}$) for 9 days on aerobic plate count (APC).

Table (6): Aerobic plat count of minced beef treated with propolis and turmeric powder during storage at $7 \pm 1^\circ\text{C}$ for 9 days.

Treatment		Storage period (days)	TBA values			
			0	3	6	9
Control			4.98 ^{dA}	5.76 ^{cA}	6.04 ^{bA}	6.45 ^{aA}
BHT			4.98 ^{dA}	5.21 ^{cG}	5.58 ^{bG}	5.66 ^{aF}
With propolis	1.5%		4.98 ^{dA}	5.52 ^{cC}	5.92 ^{bC}	6.20 ^{aC}
	2.0%		4.98 ^{dA}	5.40 ^{cE}	5.63 ^{bF}	6.01 ^{aD}
	2.5%		4.98 ^{dA}	5.37 ^{cF}	5.69 ^{bE}	5.94 ^{aE}
With turmeric	1.5%		4.98 ^{dA}	5.59 ^{cB}	6.07 ^{bB}	6.30 ^{aB}
	2.0%		4.98 ^{dA}	5.48 ^{cD}	5.75 ^{bD}	6.18 ^{aC}
	2.5%		4.98 ^{dA}	5.57 ^{cB}	5.61 ^{bF}	5.99 ^{aE}

Means with different superscripts (capital letters in the same column and small letters in the same row) are significantly different at $P \leq 0.01$.

The control samples showed the highest aerobic plate count APC counts comparing to the others containing propolis, turmeric and BHT as shown in

Table (6). Insausti *et al.* (2001) reported that meat spoilage cannot be occur until total viable counts reach 10^6 - 10^8 CFUg⁻¹ (limit of microbiological

acceptability) The relatively high initial counts of control samples may be attributed to the grinding process, which compounds the problem by introducing the pathogens into the interior of the meat and contributes to the increase of total viable counts of meat (Mead and Griffin, 1998). Aerobic plate count (APC) was gradually increased during cold storage for all the tested samples with different ratios depending on the concentration of propolis or turmeric powder. The incremental pattern in aerobic plate count (APC) can be arranged in a descending order as follows: samples treated with BHT, propolis powder and finally turmeric at 1.5%, 2.0% and 2.5% concentration levels, respectively. Data in Table (6) showed that aerobic plate count at 9th day in samples treated with propolis powder at 1.5, 2.0 and 2.5 % were 6.20, 6.01 and 5.94, respectively. Also, from the same table it could be noticed that the aerobic plate count at 9th day in samples treated with turmeric powder at 1.5, 2.0 and 2.5 % were 6.30, 6.18 and 5.99, respectively. In general, as the concentration of added substance decreased, aerobic plate count (APC) increased.

CONCLUSION

Based on the for mentioned results, it could be concluded that propolis and turmeric powders and their aqueous extracts can be considered good natural antioxidants and antimicrobial agents and can be used for extending the shelf life of minced beef especially when kept under refrigeration temperature.

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كفاءة البروبوليس ومسحوق الكركم كمواد حافظة طبيعية لحوم البقر المفروم ميرفت إبراهيم الدميري^١, عصام محمد السباعي^٢, نهله صلاح زيدان^١ و رويدا عيسى^٢ ^١ قسم الإقتصاد المنزلي - كلية التربية النوعية - جامعة كفرالشيخ - مصر ^٢ قسم تكنولوجيا الأغذية - كلية الزراعة - جامعة كفرالشيخ - مصر

بسبب الحاجة الماسة لاستخدام المضافات الغذائية الطبيعية في الوقت الحاضر، أدى ذلك، إلى العمل على معرفة إمكانية استخدام البروبوليس ومسحوق الكركم عند مستويات معينة (١.٥ و ٢ و ٢.٥٪)، كمضادات أكسدة ومضادات ميكروبية أثناء إعداد لحم البقر المفروم و تخزينها في درجة حرارة التبريد ($7 \pm 1^{\circ}\text{C}$) كشفت النتائج أن، البروبوليس والكركم ذو فعالية كبيرة في خفض العد الكلي للميكروبات الهوائية (APC) وكذلك بعض التقديرات الكيميائية مثل قيم الـ pH و الـ TBA. كما أشارت النتائج إلى انخفاض في العد الميكروبي، وقيم الـ pH و قيم الـ TBA عند زيادة تركيز المسحوق المختبر والذي اعطى افضل تأثير عند تركيز (2.5%). كانت الأنشطة المضادة للأكسدة ومضادة للميكروبات من مسحوق البروبوليس المضاف أعلى من مسحوق الكركم، وبالتالي يمكن ان يلعب البروبوليس والكركم دورا هاما كعامل مضاد للأكسدة ومضاد للميكروبات وبالتالي في اطالة فترة صلاحية لحم البقر المفروم المبرد، ولكن مسحوق البروبوليس كان أكثر تأثيرا.