Extending the Shelf-Life of Processed Cheese Spread Using Propolis Extract

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Abstract: Propolis considered a natural product with an antimicrobial effect. Treating processed cheese spread with ethanol extract of propolis (EEP) was investigated by storage time. The treated with 200, 400, 600, 800 and 1000 mg of EEP/kg and control (non-treated) samples were stored at 5°C/days. The quality of samples with respect to microbial, mycotoxin and sensory analysis every 5 days were evaluated. Gradual decrease of total mesophilic aerobic bacterial count, spore-forming bacteria and fungal count of the treated samples [(7.6 ~ 0.7) x 10^2, (9.8 ~ 1.2) x 10^1 and (8.0 ~ 1.2) x 10^1 CFU/g)] showing substandard values throughout the storage time compared with the increasing control values [(7.6 ~ 9.3) x 10^2, (1.1 ~ 3.0) x 10^2 and (0.8 ~ 1.3) x 10^2 CFU/g], respectively. Pathogens (coliform group, Escherichia coli, Bacillus cereus, Staphylococcus aureus, Salmonella sp, Clostridium perfringens, and Listeria monocytogenis) were not detected in any of the samples. Aflatoxin M1 was affected by neither the concentration nor the storage time. Sensory evaluation showed a negative impact regarding propolis effect on color, taste, odor, texture and overall acceptance. EEP is recommended as a natural preservative in processed cheese spread regardless of its sensory effect.

Keywords: Processed cheese spread, Propolis, Microbiology, Mycotoxins.

INTRODUCTION: Processed cheese is produced by blending natural cheese of different ages and degrees of maturity in the presence of emulsifying salts and other dairy and nondairy ingredients followed by heating and continuous mixing to form a homogeneous product with an extended shelf life (Verma et al., 2013). The spread type has selected proportions of ingredients and manufacturing procedure varies according to the end product. The hydrolyzed casein loses its properties provides the structure of the cheese spread will be short and spreadable (Toro et al., 2016).

The preparation technology and the storage environment affect the extent of its microbial contamination. It ranks among nonacid foods (pH > 4) providing favorable conditions to many microorganisms including spore-forming bacteria. This kind of cheese harbors various spore-forming bacteria, i.e. Clostridium spp. and Bacillus spp., due to their presence in milk and environment in addition to their ability to produce spores (Lazárková et al., 2011 and Oliveira et al., 2016).

The Egyptian Standards coined the maximum coliform group should not exceed 10 cells/g and free of pathogenic bacteria, i.e. L. monocytogenis, Salmonella spp, E. coli, and Cl. perfringens (ES: 999-2/2005). Also, aflatoxins M1 should not exceed 50 ng/kg (ES: 7136/2010).

Propolis is the resinous mixture that honey bees collect from different sources to use it as a sealant for unwanted open spaces in hives (Badria et al., 2017). An antimicrobial activity attributed to the synergism effect of flavonoids, phenolic acids, and aldehydes, esters, ketones, hydroxyl and ses-qui-terpenes (Santos et al., 2018). Also, antiseptic, antibiotic, antifungal and antiviral features of propolis come from its galangin, caffeic and ferulic acid content (Yucel et al., 2017). It inhibits Gram-negative bacteria, Gram-positive bacteria and fungal growth (Skowron et al., 2019). EEP has higher bacterial activity at 25°C than at 4°C (Pobiega et al., 2019). The concentration of 4 wt% of propolis extract showed more than 50% inhibition against all tested microorganisms (Tzima et al., 2015). Propolis can be used to improve the quality of several kinds of cheese and as a natural preservative to increase its shelf life (Metwalli, 2011; Khaleel, 2006; Mehmetoğlu et al., 2017 and Verma et al., 2013).

The aim of this study was to evaluate the potential of EEP as decontaminant and to increase the shelf life of processed cheese spread.

MATERIALS AND METHODS: Ethanol extract of propolis (EEP):
Crude propolis was collected from private honey bee hives at El-Dakahlya governorate within 2018. Propolis was ethanol extracted according to Apaydin and Gümüş (2018) with modification as follow: The collected propolis samples were grounded and extracted (30% w/v) in ethanol 70% (Kubiliene et al., 2015), then incubated at 60°C/24h with rotary shaking at 150 rpm. The extract was centrifuged (Centurion Scientific, UK) at 4000 rpm/10 min, then the supernatant was filtered through Whatman no. 4 filter paper and subjected to the rotary evaporator (IKA, Germany) at 50°C.

Manufacture of processed cheese spread:
Processed cheese spread was manufactured by mixing Ras cheese (38.44%), Cheddar cheese (12.80%), skim milk powder (5.12%), butter (10.26%), emulsifying salt (2.50%, i.e. Sodium phosphate (65%) and Sodium citrate (35%)) and water (to complete 100% of the ingredients). The mixtures were processed in locally constructed double jacket ban at 80-85°C, poured in sterile screw capped glass jars (100±20g), cooled at room temperature then stored at 5±1°C in the refrigerator. The resultant processed cheese spread was divided into 6 portions to form control (non-treated) and 5 treated samples, i.e. 200, 400, 600, 800 and 1000 mg of EEP/kg. These portions were tested for microbiological and mycological analysis beside organoleptic properties at zero, 5, 10, 15, 20, 25 and 30 days (El-Dardiry et al., 2017 and Mehanna et al., 2017). Three replicates were manufactured and subjected for analysis, in which tests were performed in duplicate.

Microbiological analysis:
A mass of the manufactured cheese samples (10 g) was weight into 90 ml of sterile peptone water and homogenized in Stomacher (Seward 3500, Lab system, England) for 2 min. Subsequently, serial dilutions for detection and enumeration of the following microorganisms with media and incubation conditions in parenthesis subjected to standard media purchased from Oxoid and Difco and prepared following its instructions referring to Collins et al. (2004):

- Total mesophilic aerobic bacterial count (Standard Plate Count Agar at 37°C/48 h), fungal (molds and yeasts) count (Sabouraud Dextrose Agar at 20-24°C/3-5 days), coliform group with Most Probable Number (MPN); MacConkey’s Broth at 37°C/48 h, Escherichia coli (Eosin Methylene Blue (EMB) at 37°C/24 h, Tryptone water at 37°C/24 h + indol reagent, Methyl Red Voges Proskauer Broth (MRVP) at 37°C/5 days + Methyl red solution and at 37°C/48 h + α-naphthol solution, Simmon Citrate Agar at 37°C/48 h), Bacillus cereus (Polymyxin Egg Yolk Manitol Blue Agar (PEMBA) at 30°C/24 and 48 h), Staphylococcus aureus (Baird-Parker’s medium at 37°C/24 and 48 h and Brain Heart Infusion Broth at 37°C/1-24 h), Salmonella sp. (Lactose Broth, Lysine Iron Agar (LIA) and Triple Sugar Iron Agar (TSI) at 37°C/24 h; Selenite Cystine Broth and Tetraionate Brilliant Green Broth at 43°C/24 h and Bismuth Sulphate Agar and Brilliant Green Agar at 37°C/24 and 48 h in addition to serological kits of Bacto Salmonella O antiserum), Clostridium perfringens (Oleandomycin Polyoxin Sulfadiazine Perfringens (OPSP) + Selective Supplement A and B at 35°C/18-24 h) and Listeria monocytogenes (Listeria Enrichment Broth at 30°C/7 days and Oxford Medium at 35°C/48 h).

Mycotoxin analysis:
According to Shaneshin et al. (2018), 25 g of the cheese samples were prepared then subjected to Competitive Direct-Enzyme-Linked Immuno-Sorbent Assay (CD-ELISA) for aflatoxin M1 using RIDASCREEN® Aflatoxin M1 (Art. No. R1121) kit, following its instructions. The results were yielded in nanogram per kilogram (ng/kg) by microwell reader (MRX, UK) with software Version 1.2.

Sensory evaluation:
According to Verma et al. (2013), sensory attributes of the cheese samples conducted by 10 untrained independent panel assessors from the National Nutrition Institute. Numerical scores (20 points) for each color, taste, odor, texture and overall acceptance to observe total scores out of 100 points.

Statistical analysis:
The results were analyzed with two way ANOVA with interaction and multiple comparisons among the treatments with Tukey’s test (p<0.05) using SPSS program version 20 (Arkkelin, 2014).
RESULTS AND DISCUSSION:
The present study represents the effect of adding different concentrations 200, 400, 600, 800 and 1000 mg of EEP/kg to processed cheese spread during production compared with control samples were stored in the refrigerator at 5ºC/30 days on microbial, mycotoxin and sensory aspects.

The microbial quality of control and propolis treated processed cheese spread during different storage intervals are shown as follow:
Coliform group, *E. coli, B. cereus, Staph. aureus, Salmonella sp., Cl. perfringens* and *L. monocytogenes* were not detected in both control and treated samples during the different storage stages. These bacteria render cheese unacceptable following the Egyptian standards (ES: 999-2/2005). The absence of these food-borne pathogens influenced by ingredients, equipment sanitation, heat treatment during manufacturing plant, handler, environment, packaging material (Pintado et al., 2015 and Pal et al., 2016). Phosphate-based emulsifying salt has an inhibitory effect on various microbial growths in processed cheese spread (Kapoor and Metzger, 2008). Otherwise, propolis extract has antibacterial activity due to flavonoids, aromatic compounds and their esters against the above mentioned pathogens if present in the treated samples (Aida et al., 2016; Afrouzan et al., 2018 and Skowron et al., 2019).

The absence of these food-borne pathogens indicated poor sanitary conditions and consequence toxin which is not affected by propolis extract. However, these pathogens were not detected in the current cheese samples, an inhibitory effect of propolis extracts against *Salmonella spp, L. monocytogenes, Staph. aureus* and *E. coli* was found by Temiz et al. (2011), Rocha et al. (2013) and Apaydin and Gümüş (2018).

A significant gradual decrease (p<0.05) of total mesophilic aerobic bacteria, spore-forming bacteria and fungal count of treated samples showing substandard values throughout the storage time compared with the increasing control (non-treated) values.

Figure (1) depicted the total mesophilic aerobic bacterial count in control sample increased by prolonging storing time as (7.6 to 9.3) x10^2 CFU/g, while considerable reduction in direct proportion with propolis extract concentrations ranged between (7.6 to 6.6) x10^2 CFU/g in T1 and (6.3 to 0.7) x10^2 CFU/g in T5. The incidence of the colony counts was the same as that reported by Metwalli (2011).

![Fig. (1): Total mesophilic aerobic bacterial count in control and propolis treated processed cheese spread by time. (p<0.05)](image)

The propolis derivatives, flavonoids, disrupt cell wall, block the ion channels and affect the synthesis of ATP. Also, it forms complexes with the bacterial cell wall, surface-exposed adhesions, polypeptides and cell membrane enzymes (Skowron et al., 2019). Heating step during production in the pilot plant cannot guarantee the presence of food-borne microorganisms to follow the standard regulations.

Figure (2) revealed that there is a marked decrease in fungal count represent mold and yeast as the propolis percentage in the treated cheese samples increase. The highest fungal count was 1.3x10^2 CFU/g in control samples and then decreased to reach 1.2x10^1 CFU/g in T5 by the end of 30 days storage.
Antifungal activity of propolis has been reported by Matny et al. (2015), Badawy (2016) and Afrouzan et al. (2018). The concentration of 5% EEP contains 1% chitosan film that has an antifungal effect (Skowron et al., 2019). Low yeast count may be related to the metabolites excreted by the faster growing bacteria inhibits the yeast cells or to compete for utilization of growth substrates (Borelli et al., 2006). It decreases conidial production and germination in addition to mycelial growth. Flavonoids enhance saturated fatty acids accumulation causing cellular membrane resistance mechanism leading to decreasing its elasticity and fluidity (Hashem et al., 2012). The pinocembrin content inhibits the hyphal cells respiration, energy deficit, cell membranes damage then cell death (Peng et al., 2012). Propolis within selected concentrations prevents the production of mould (Yucel et al., 2017).

As shown in Figure (3), spore-forming bacteria were decreased with increasing EEP concentration during storage as follow: Control, T1, T2, T3, T4 and T5 samples contained 1.1x10^2 ~ 3.0x10^2, 9.8x10^1 ~ 8.7x10^1, 9.5x10^1 ~ 6.0x10^1, 8.2x10^1 ~ 4.0x10^1, 7.0x10^1 ~ 1.9x10^1 and 6.2x10^1 ~ 1.2x10^1 CFU/g, respectively.

Sporulated bacteria are more sensitive to propolis concentration than non-spore forming bacteria, which reduce spore loads (Ramauskiene et al., 2009 and Borba and Spivak, 2017). It reduces the spore viability; avoid germination, anti-sporeforming and chelating spores (Yang et al., 2017, Abd-El-Kareem et al., 2018, Quiroga et al., 2006, Kubiliene et al., 2015 and Diba et al., 2018).

Figure (4) reveals the levels of aflatoxin M1 in naturally contaminated cheese treated groups analyzed every 5 days in triplicate for a duration of 30 days. The values of naturally contaminated cheese samples with aflatoxin M1 tends to fluctuate during the storage time (ranged between 29 to 31 ng/kg), showing no significant difference (p>0.05), which may be due to analytical variation.
Propolis extract is very difficult to remove aflatoxins from the cheese product because of thermal resistance and stability, in addition, to resist the processing step and remain through the production chain (Al-Zoreky and Saleh, 2019). It inhibits the fungal growth which decreases toxin production beside it interacts with toxin production biosyntheses (Matny et al., 2015). Flavonoids have an anti-aflatoxigenic effect because of their antioxidant activity which inhibits aflatoxin production that is produced by peroxidation of fungal cells (Tian and Chun, 2017).

The panelists observed a marked difference in terms of color, taste, odor, texture and overall acceptance, among the control and treated freshly prepared cheese samples (Figure 5). The sensory quality adversely affected in which an inverse proportion between the added concentration of EEP and sensory acceptance score. However, the gradual concentrations of EEP in the treated cheese showed lower grades than the score of control samples.

**CONCLUSION:**

Addition of selected concentrations of ethanol extract propolis to processed cheese spread can be used as a natural preservative have distinct contribution: a positive aspect is to enhance the microbial quality of the cheese product while a negative aspect is to reduce its sensory evaluation by time.

**REFERENCES:**


xiv. Egyptian Organization for Standardization and Quality (ES): 2010: Maximum levels for certain contaminations in foodstuffs (No. 7136).


