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

Author Details: Emad A. H. Guirguis
Department of Food Hygiene, National Nutrition Institute, General Organization of Teaching Hospitals and Institutes, Egypt

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 5ºC /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 monocytoginis) 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. monocytoginis, 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-quiterpenes (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 25ºC than at 4ºC (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 60ºC /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 50ºC.

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 80-85ºC, poured in sterile screw capped glass jars (100±20g), cooled at room temperature then stored at 5±1ºC 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 37ºC/48 h), fungal (molds and yeasts) count (Sabouraud Dextrose Agar at 20-24ºC/3-5 days), coliform group with Most Probable Number (MPN); MacConkey's Broth at 37ºC/48 h), *Escherichia coli* (Eosin Methylene Blue (EMB) at 37ºC/24 h, Tryptone water at 37ºC/24 h + indol reagent, Methyl Red Voges Proskauer Broth (MRVP) at 37ºC/5 days + Methyl red solution and at 37ºC/48 h + α-naphthol solution, Simmon Citrate Agar at 37ºC/48 h), *Bacillus cereus* (Polymyxin Egg Yolk Manitol Blue Agar (PEMBA) at 30ºC/24 and 48 h), *Staphylococcus aureus* (Baird-Parker's medium at 37ºC/24 and 48 h and Brain Heart Infusion Broth at 37ºC/1-24 h), *Salmonella sp.* (Lactose Broth, Lysine Iron Agar (LIA) and Triple Sugar Iron Agar (TSI) at 37ºC/24 h; Selenite Cystine Broth and Tetrathionate Brilliant Green Broth at 43ºC/24 h and Bismuth Sulphate Agar and Brilliant Green Agar at 37ºC/24 and 48 h in addition to serological kits of Bacto Salmonella O antiserum), *Clostridium perfringens* (Oleandomycin Polymxin Sulfadiazine Perfringens (OPSP) + Selective Supplement A and B at 35 ºC/18-24 h) and *Listeria monocytogenes* (Listeria Enrichment Broth at 30ºC/7 days and Oxford Medium at 35ºC /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 presence of pathogens indicates 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 these colony counts was the same as that reported by Metwalli (2011).

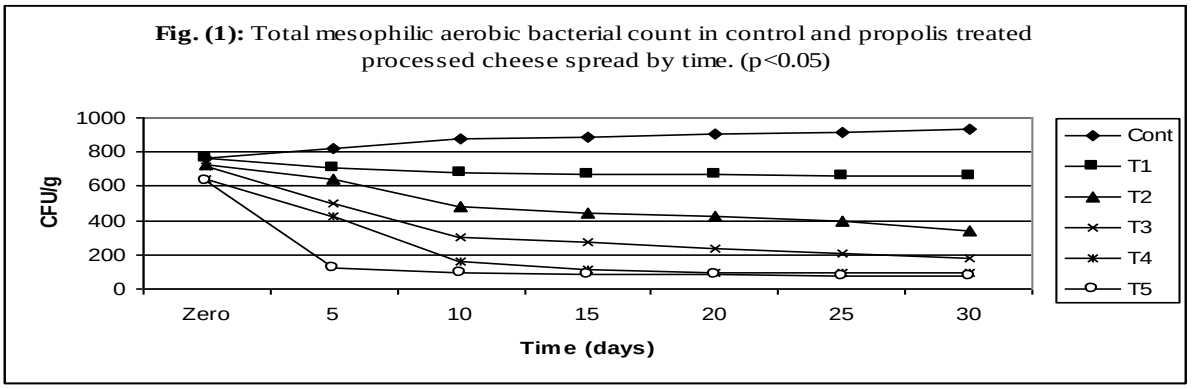
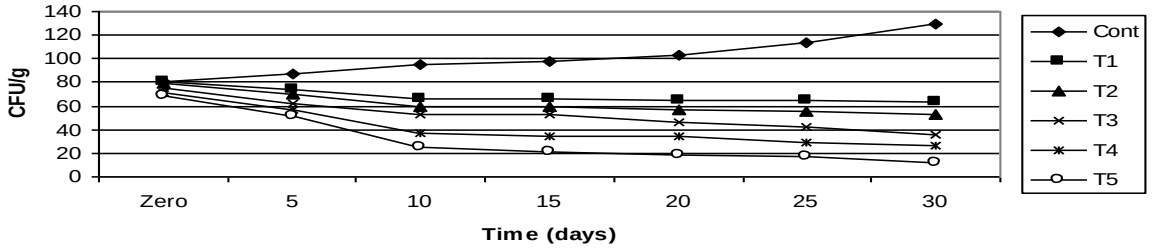


Fig. (1): Total mesophilic aerobic bacterial count in control and propolis treated processed cheese spread by time. ($p<0.05$)

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.

Fig. (2): Fungal (moulds and yeasts) count in control and propolis treated processed cheese spread by time. ($p<0.05$)

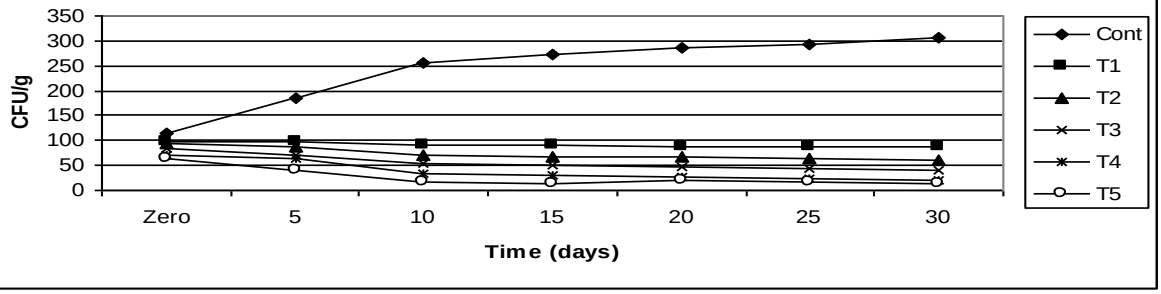


Antifungal activity of propolis has been reported by Matny et al. (2015), Badawy (2016) and Afrouzan et al. (2018). The concentration of 5% EEP contains 1% chitosan film that has an antifungal effect (Skowron et al., 2019). Low yeast count may be related to the metabolites excreted by the faster growing 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 $1.1x10^2 \sim 3.0x10^2$, $9.8x10^1 \sim 8.7x\ 10^1$, $9.5x10^1 \sim 6.0x10^1$, $8.2x10^1 \sim 4.0x10^1$, $7.0x10^1 \sim 1.9x10^1$ and $6.2x10^1 \sim 1.2x10^1$ CFU/g, respectively.

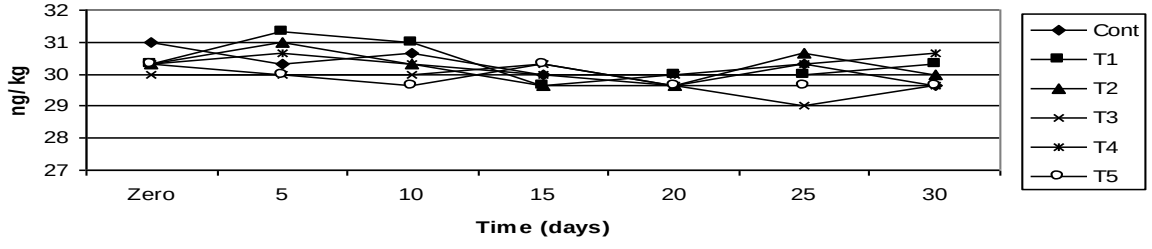
Fig. (3): Spore-forming bacteria in control and propolis treated processed cheese spread by time. ($p<0.05$)



Sporulated bacteria are more sensitive to propolis concentration than non-spore forming bacteria, which reduce spore loads (Ramanauskienė 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.

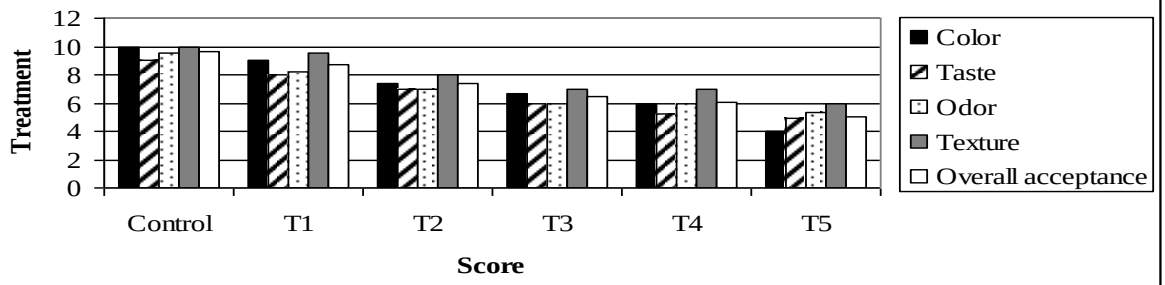
Fig. (4): Aflatoxin M1 in control and propolis treated processed cheese spread by time. ($p>0.05$)



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.

Fig. (5): Sensory evaluation of control and propolis treated processed cheese spread. ($p<0.05$)



The sensory acceptance of control samples as highly desirable, while treated samples showed decreased scores by increasing the EEP concentrations. These results concurred with those obtained by Temiz et al. (2013), El-Mossalami and Abdel-Hakeim (2013) and Haščik et al. (2014). The characteristic taste and flavor of the propolis, which exerts an adverse effect on the end-product sensory features (Pobiega et al., 2019). Propolis has a characteristic strong odor which confers its odor and color and consequently affects the acceptance of the products (Gonçalves et al., 2011).

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:

i. *Abd-El-Kareem, F.; Saied, N.M. and El- Mohamedy, R.S.R. (2018): Seed Treatment With Chitosan and Ethanol-Extracted Propolis for Suppression Bean Root Rot Disease Under Greenhouse Conditions. J. Mater. Environ. Sci., 9(8): 2356-2361.*

ii. *Afrouzan, H.; Tahghighi, A.; Zakeri, S. and Es-haghi, A. (2018): Chemical composition and antimicrobial activities of Iranian propolis. Iran. Biomed. J., 22(1): 50-56.*

iii. *Aida, M.; Agron, M.; Emiljano, T. and Mutlu, Ö. (2016): Anti-inflammatory and Regenerative Effects of Albanian Propolis in Experimental Vital Amputations in Piglets (Histological assessment). IJSR, 5(7): 2041-2045.*

iv. *Al-Zoreky, N.S. and Saleh, F.A. (2019): Limited Survey on aflatoxin contamination in rice. Saudi J. Biolog. Sci., 26: 225-231.*

v. *Apaydin, H. and Gümüş, T. (2018): Inhibitory effect of propolis (Bee gum) against Staphylococcus aureus bactria isolated from instant soups. J. Tekirdag Agric. Fac., 15(01): 67-75.*

vi. *Arkkelin, D. (2014): Using SPSS to Understand Research and Data Analysis. Psychology Curricular Materials. Book 1. pp. 194. http://scholar.valpo.edu/psych_oer/1*

vii. *Badawy, I.F.M. (2016): Effect of ethanol-extracted propolis on fruit quality and storability of balady oranges during cold storage. Assiut J. Agric. Sci., 47(4): 156-166.*

viii. *Badria, F.A.A.; Fathy, H.M.; Fatehe, A.S.; Elimam, D.M.A. and Ghazy, M.G.M. (2017): Evaluate the cytotoxic activity of honey, propolis and bee venom from different localities in Egypt against liver, breast and colorectal cancer. J. Apither., 2(1): 1-8.*

ix. *Borba, R.S. and Spivak, M. (2017): Propolis envelope in Apis mellifera colonies supports honey bees against the pathogen, Paenibacillus larvae. Scientific Reports, 7: 11429-11435.*

x. *Borelli, B.M.; Ferreira, E.G.; Lacerda, I.C.A.; Franco, G.R. and Rosa, C.A. (2006): Yeast populations associated with the artisanal cheese produced in the region of Serra da Canastra, Brazil. World J. Microbiol. Biotechnol., 22: 1115-1119.*

xi. *Collins, C.H.; Lyne, P.M.; Grange, J.M. and Falkinham, J.O. (2004): Microbiological Methods. 8th ed., Arnold, London, UK, pp. 456.*

xii. *Diba, K., Mahmoodi, M. and Hashemi, J. (2018): In vitro activity of propolis alcoholic extract on opportunistic pathogenic fungi. Int. J. Res. Appl. Basic Med. Sci., 4(2): 68-73.*

xiii. *Egyptian Organization for Standardization and Quality (ES: 2005): Processed cheese and spreadable processed cheese. Part: 2. Spreadable processed cheese. (No. 999-2).*

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

xv. *El-Dardiry, A.I.; Al-Ahwall, R.I. and Gab-Allah, R.H. (2017): Preparation and properties of processed cheese spread containing Quinoa paste. Egypt. J. Dairy Sci., 45(2): 171-180.*

xvi. *EL-Mossalami, H.H.A. and Abdel-Hakeim, Y.A. (2013): Using of propolis extract as a trial to extend the shelf-life and improving the quality criteria of fresh Egyptian sausage. Assiut Vet. Med. J., 59(139): 23-33.*

xvii. *Gonçalves, G.M.S.; Srebernich, S.M. and Souza, J.A.M. (2011): Stability and sensory assessment of emulsions containing propolis extract and/or tocopheryl acetate. BJPS, 47(3): 585-592.*

xviii. *Haščík, P.; Garlík1, J.; Elimam1, I.O.E.; Kňazovická1, V.; Čuboň1, J. and Kročko1, M. (2014): Sensory evaluation of hubbard jv chickens meat after propolis application in their diet. J. Microbiol. Biotech. Food Sci., 3(3): 14-17.*

xix. *Hashem, A.; Abd-Allah, E.F. and Alwathnani, A. (2012): Effect of propolis on growth, aflatoxins production and lipid metabolism in Aspergillus parasiticus spear. Pak. J. Bot., 44(3): 1153-1158.*

xx. *Kapoor, R. and Metzger, L.E. (2008): Process Cheese: Scientific and Technological Aspects- A Review. Comprehensive reviews in food science and food safety (CRFSFS), 7:194-214.*

xxi. *Khaleel, E.M.A. (2006): Studies on yeasts and molds and their role in dairy products. Ph.D. Thesis, Department of Food Science, Faculty of Agriculture, Zagazig University, pp. 259.*

xxii. *Kubiliene, L.; Laugaliene, V.; Pavilonis, A.; Maruska, A.; Majiene, D.; Barcauskaite, K.; Kubilius, R.; Kasparaviciene, G. and Savickas, A. (2015): Alternative preparation of propolis extracts: Comparison of their composition and biological activities. BMC Complementary and Alternative Medicine, 15: 156-162.*

xxiii. *Lazárková, Z.; Buňka, F.; Buňková, L.; Holán, F.; Kráčmar, S. and Hrabe, J. (2011): The effect of diffferentheat sterilization regimes on the quality of canned processed cheese. Journal of Food Process Engineering, 34:1860-1878.*

xxiv. *Matny, O.N.; Al-Warshan, S.H.S. and Ali, A.M. (2015): Antifungal Evaluation of Iraqi propolis against Penicillium expansum and mycotoxin production in apple. Int. J. Curr. Microbiol. App. Sci., 4(11): 399-405.*

xxv. *Mehanna, N.S.; Hassan, F.A.M.; El-Messery, T.M. and Mohamed, A.G. (2017): Production of Functional Processed Cheese by Using Tomato Juice. Int. J. Dairy Sci., 12(2): 155-160.*

xxvi. *Mehmetoğlu, S.; Tarakçi, Z.; Demirkol, M.; Çakici, N. and Güney, F. (2017): Propolis as a food ingredient. Aricilik Araştirma Dergisi, 9(1): 32-39.*

xxvii. *Metwalli, S.A.H. (2011): Extended shelf life of Kareish cheese by natural preservatives. Egypt. J. Agric. Res., 89 (2): 639-649.*

xxviii. *Oliveira, R.B.A.; Margalho, L.P.;Nascimento, J.S.; Costa, L.E.O.; Portela, J.B.; Cruz, A.G. and Sant'Ana, A.S. (2016): Processed cheese contamination by spore-forming bacteria: A review of sources, routes, fate during processing and control. Trends in Food Science & Technology, 57(A):11-19.*

xxix. *Pal, M.; Mulu, S.; Tekle, M.; Pintoo, S.V. and Prajapati, J.P. (2016): Bacterial Contamination of Dairy Products. BEVERAGE & FOOD WORLD, 43(9): 40-43.*

xxx. *Peng, L.; Yang, S.; Cheng, Y.J.; Chen, F.; Pan, S. and Fan, G. (2012): Antifungal Activity and Action Mode of Pinocembrin from Propels against Penicillium italicum. Food Sci. Biotechnol., 21(6): 1533-1539.*

xxxi. *Pintado, M.; da Cruz, A.G. and de Sá, P.B.Z.R. (2015): Cheese Microbiology. In: Özer, B.H. and Akdemir-Evrendilek, G. (Eds), Dairy Microbiology and Biochemistry. Taylor and Francis Group, LLC.*

xxxii. *Pobiega, K.; Kraśniewska, K. and Gniewosz, M. (2019): Application of propolis in antimicrobial and antioxidative protection of food quality – A review. Trends in Food Science & Technology, 83: 53-62.*

xxxiii. *Quiroga, E.N.; Sampietro, D.A.; Sobero´ n, J.R.; Sgariglia, M.A. and Vattuone, M.A. (2006): Propolis from the northwest of Argentina as a source of antifungal principles. J. Appl. Microbiol., 101: 103-110.*

xxxiv. *Ramanauskienė, K.; Inkėnienė, A.M.; Savickas, A.; Masteikova, R. and Brusokas, V. (2009): Analysis of the antimicrobial activity of propolis and lysozyme in semisolid emulsion system. Acta Poloniae Pharmaceutica – Drug Research, 66(6): 681-688.*

xxxv. *Rocha, B.A.; Bueno, P.C.P.; Vaz, M.M.O.L.; Nascimento, A.P.; Ferreira, N.U.; Moreno, G.P.; Rodrigues, M.R.; Costa-Machado, A.R.M.; Edna Aparecida Barizon, E.A.; Campos, J.C.L.; de Oliveira, P.F.; Acésio, N.O.; Martins, S.P.L.; Tavares, D.C. and Berretta, A.A. (2013): Evaluation of a Propolis Water Extract Using a Reliable RP-HPLC Methodology and In Vitro and In Vivo Efficacy and Safety Characterisation. Evidence-Based Complementary and Alternative Medicine, pp. 1-11. http://dx.doi.org/10.1155/2013/670451*

xxxvi. *Santos, M.S.; Estevinho, M.L.M.F.; de Carvalho, C.A.L.; Magalhães-Guedes, K.T.; Schwan, R.F. and Almeida, R.C.C. (2018): Propolis as natural additive: A systematic review. Afr. J. Biotechnol., 17(41): 1282-1291.*

xxxvii. *Shaneshin, M.; Noursalehi, H.; Mousavi, Z.; Pourjabbar, Z. and Hajimahmoodi, M. (2018): Determination of aflatoxin M1 in whey powder by HPLC and ELISA methods. J. Food Safe & Hyg., 4(1-2): 21-26.*

xxxviii. *Skowron, K.; Kwiecińska-Piróg, J.; Grudlewska, K.; Gryń, G.; Wiktorczyk, N.; Balcerek, M.; Załuski, D.; Wałecka-Zacharska, E.; Kruszewski, S. and Gospodarek-Komkowska, E. (2019): Antilisterial activity of polypropylene film coated with chitosan with propolis and/or bee pollen in food models. BioMed Research International, pp. 1-12.*

xxxix. *Temiz, A.; Mumcu, A.S.; Tüylü, A.Ö.; Sorkun, K. and Salih, B. (2013): Antifungal activity of propolis samples collected from different geographical regions of Turkey against two food-related molds, Aspergillus versicolor and Penicillium aurantiogriseum. GIDA, 38(3): 135-142.*

xl. *Temiz, A.; Şener, A.; Tüylü, A.Ö.; Sorkun, K. and Salih, B. (2011): Antibacterial activity of bee propolis samples from different geographical regions of Turkey against two foodborne pathogens, Salmonella Enteritidis and Listeria monocytogenes. Turk. J. Biol., 35: 503-511.*

xli. *Tian, F. and Chun, H.S. (2017): Natural products for preventing and controlling aflatoxin contamination of food. In: Abdulra'Uf, L. (Ed.), Aflatoxin- Control, Analysis, Detection and Health Risks. INTECH, pp: 13-44.*

xlii. *Toro, E.E.B; Valencia, J.U.S. and Molina, D.A.R. (2016): Characterization of a processed cheese spread produced from fresh cheese (quesito antioqueño). Rev. Fac. Nac. Agron., 69(2): 8015-8022.*

xliii. *Tzima, K.; Makris, D.; Nikiforidis, C.V. and Mourtzinos, I. (2015): Potential use of rosemary, propolis and thyme as natural food preservatives. J. Nutri. Health, 1(1):1-6.*

xliv. *Verma, S.K.; Upadhyay, S.; Chandra, R. and Paul, A. (2013): Preparation of Processed Cheese Spread Using Tofu, Mozzarella and Cheddar Cheese. IJFANS, 2(2): 19-23.*

xlv. *Yang, W.; Wu, Z.; Huang, Z.Y. and Miao, X. (2017): Preservation of orange juice using propolis. J. Food Sci. Technol., 12(Sep.): 1-9.*

xlvi. *Yucel, B.; Topal, E. and Kosoglu, M. (2017): Bee Products as Functional Food. In: Superfood and Functional Food – An Overview of Their Processing and Utilization. Waisundara, V. and Shiomi, N. (Eds.). INTECH, pp. 15-33.*