Veterinaria Italiana, **46** (2), 167-172

Propolis as a natural decontaminant and antioxidant in fresh oriental sausage

Fatma H. Ali[1], Gehan M. Kassem[2] & Osama A. Atta-Alla[2]

Summary

The authors evaluate the effect of propolis on the shelf-life and different quality criteria of fresh oriental sausage. Experimentally processed fresh oriental sausage treated with 0.6% ethanolic extract of propolis and control samples were kept in a refrigerator at 5°C and examined every 3 days until signs of spoilage were observed. Both groups were investigated for sensory, chemical and microbiological deteriorative criteria. The results revealed that control samples decomposed after 12 days, while treated samples had longer shelf-life as they decomposed after 21 days. In general, the thiobarbituric acid (TBA) value (mg malondialdehyde/kg) and total volatile bases nitrogen (TVB-N) mg/100 g increased gradually in all samples examined, with a significantly lower level for treated samples than for controls. In addition, a gradual increase in the microbiological load of control samples throughout the storage time was observed, whereas proteolytic, lipolytic and total mould and yeast counts were 6.39 ± 0.41, 6.0 ± 0.57 and 6.6 ± 0.53 $\log_{10}$ cfu/g, respectively, at day 12 and were rejected. Such counts were slightly lower in treated samples up to day 15 of storage, followed by a gradual increase until the end of storage. Propolis is recommended as a preservative in fresh oriental sausage processing.

Keywords

Antioxidant, Decontaminant, Egypt, Food, Meat, Preservative, Propolis, Safety, Sausage.

La propoli come decontaminante e antiossidante nella salsiccia orientale fresca

Riassunto

Gli autori hanno valutato l'effetto della propoli sulla durata di conservazione della salsiccia fresca orientale e hanno preso in esame i criteri di qualità relativi al prodotto. I campioni di salsiccia fresca trattati con estratto di propoli al 0,6% di etanolo e quelli di controllo sono stati mantenuti alla temperatura di 5°C ed esaminati ogni 3 giorni fino alla rilevazione di segnali di deterioramento. Lo studio dei campioni ha previsto esami organolettici, chimici e microbiologici. I risultati hanno rilevato che i campioni di controllo si sono deteriorati dopo 12 giorni, quelli trattati con propoli dopo 21 giorni. I valori del malondialdeide (tiobarbiturico-TBA) mg/kg e dell'azoto volatile (TVB-N) mg/100 g sono aumentati gradualmente in tutti i campioni esaminati ma con un livello significativamente più basso nei campioni trattati con propoli. Un aumento graduale della carica microbiologica è stata osservata nei campioni di controllo in tutto il periodo dello studio, a differenza della carica batterica proteolitica e lipolitica e a muffe e lieviti che hanno raggiunto la concentrazione rispettiva di 6,39 ± 0,41, 6,0 ± 0,57, 6,6 ± 0,53 $\log_{10}$ ufc/g al 12 giorno di osservazione. I risultati sono stati leggermente inferiori nei campioni trattati con propoli fino al 15° giorno, con un graduale aumento fino alla fine dello studio. La propoli è da considerare un efficace conservante nella lavorazione della salsiccia fresca orientale.

(1) Department of Food Hygiene, Faculty of Veterinary Medicine, Beni-Suef University, 62511 Beni-Suef, Egypt
fatma111969@yahoo.com
(2) Department of Food Hygiene, Faculty of Veterinary Medicine, Cairo University, 15125 Giza, Egypt

© IZS A&M 2010

Parole chiave
Alimenti, Antiossidante, Carne, Conservanti, Decontaminante, Egitto, Propoli, Salsiccia, Sicurezza.

Introduction

Fresh sausage is one of the most popular processed meat products. It is made using a combination of raw ingredients which yield a final product of acceptable quality at a competitive price. A high microbiological quality is necessary to improve processed meat products (25).

The oxidative rancidity of fats during the storage of meat and meat products causes the development of an objectionable odour and flavour and may also give rise to deleterious nutritional effects, such as the destruction of essential fatty acids and the loss of vitamins. Moreover, free radicals produced during the oxidation process have been considered to be carcinogenic substances (29). The rate and extent of oxidative deterioration can be reduced by various means, such as curing to preserve the meat tissues, vacuum packaging to remove the oxygen source, or adding antioxidants to scavenge the oxidants (31). The application of antioxidants is one of the simplest ways of reducing lipid oxidation. Antioxidants minimise lipid peroxidation, act as oxygen scavengers, react with free radicals and chelate catalytic metals and consequently retard oxidative deterioration (28).

Propolis is a resinous, rubbery and balsamic substance collected by honey bees from the buds of flowers, trees and other plant sources. Propolis contains resins, aromatic and ethereal oils, flavonoid pigment, vanillin, isovanilin, caffeic, benzoic and ascorbic acids as well as benzyl alcohol and cinnamic acid (11, 14, 27). These components possess antimicrobial, antifungal and antioxidant properties (4, 16, 17, 20, 29). Moreover, propolis has both bacteriostatic and bactericidal properties, depending on the concentration, type of propolis and type of bacteria tested (10).

It is worth mentioning that propolis can be used as a water or ethanolic extract; both extracts can reduce the total volatile basic nitrogen content in fresh sausage and can thus serve as a good preservative and can contribute to promote human health because they are produced naturally (12). However, water-extracted propolis has a weaker antibacterial, antioxidant and antifungal action than ethanolic extract (10). In recent years, propolis has been taken for health reasons but has had limited use in meat processing and food preservation. Consequently, our study was designed to evaluate the potential of ethanolic extract of propolis as a decontaminant and antioxidant for fresh oriental sausage during refrigerated storage and to estimate the possibility of using propolis to increase the shelf-life of fresh oriental sausage.

Materials and methods

Purification of propolis

Crude propolis (honey bee glue) samples were obtained from the Department of Economic Entomology and Pesticides at the Faculty of Agriculture, Cairo University. Propolis samples were manually purified from impurities (wood, straw, fragments and insects), then blended to fine particles in a Waring blender and stored in a dark bottle at room temperature until use (5, 27).

Preparation of ethanolic extract of propolis

Ethanolic extract of propolis (EEP) was prepared by extracting 100 g of propolis with 200 ml of ethanol (95%). A 0.6% dilution of EEP was prepared and then kept in a dark bottle until use (13).

Sausage manufacture

Fresh oriental sausage was processed according to the quality criteria recommended by the Egyptian Organisation for Standardisation and Quality Control (ESS) (7), where the formula used is as follows:

- fresh lean meat (7 kg)
- beef fat (1.5 kg)
- soy hydrate (0.3 kg)
- skimmed milk (0.15 kg)
- bread (0.2 kg)
- water (1 kg)

 © IZS A&M 2010

- sodium chloride (0.2 kg)
- black pepper (0.035 kg).

Fresh beef and fat were minced and the other ingredients were added thereafter. The paste obtained was divided into two portions; the first was stuffed in natural mutton casing as control samples and the second portion was treated with 0.6% EEP/kg and stuffed in the same manner as the control samples. Both control and treated samples were then packed in polyethylene bags and chilled in a refrigerator at 5°C. During refrigerated storage, the control and treated samples were examined every three days for sensory, chemical and microbiological deteriorative criteria until spoilage occurred, as described below.

Sensory evaluation

Sensory attributes for raw and cooked sausage samples (form, binding, colour, odour and taste) were examined according to the scheme adopted by Mohamed (23) and are shown in Table I. Sensory examinations of samples were conducted by an untrained independent panel consisting of 10 members from the Food Hygiene Department until the end of the study.

Table I
Scheme used for sensory evaluation of oriental sausage

Sensory attributes	Desirable	Undesirable
Form	Normal	Deformed and burst casing
Binding	Firm	Poor
Colour	Bright red, dark red	Brown, grey
Odour	Fresh, spicy	Rancid, putrid
Taste	Fleshy	Bitter and decomposed

Chemical examination

An estimation of the thiobarbituric acid (TBA) value using the distillation method was applied according to a technique described by the Food and Agriculture Organization (FAO) (9) and determination of total volatile bases nitrogen (TVB-N) using the distillation method was performed as recommended by the Analytical Methods Committee (AMC) (3). The TBA values were expressed as mg-malondialdehyde/kg, while TVB-N were expressed as mg/100 g.

Microbiological examination

Samples homogenate was prepared by homogenisation of 10 g of examined sample with 90 ml sterile peptone water 0.1%. From sausage homogenate, serial decimal dilutions up to 10^8 were performed. The microbiological procedures recommended by the American Public Health Association (APHA) (2) were applied as follows:

- the proteolytic count was performed using skimmed milk agar 10% (Oxoid, CML 31), lipolytic count on tributyrine agar (Oxoid, PM4) and total mould and yeast count on Sabouraud dextrose agar (Oxoid, CM 41).

Statistical analysis

The results are presented as the mean of three replicates. The analytical test used included unpaired Student *t*-test to compare means for the two groups at $p<0.05$ (19).

Results and discussion

Table II presents sensory acceptance of control samples as highly desirable for the first six days and for the following five days, samples became just desirable, while on day 12 of storage, spoilage signs (offensive odour, unpleasant taste and formation of a greenish colour) were clear and the samples were discarded. Treated samples were accepted by the team of panelists for nine days more than control samples and were rejected after 21 days of storage. These results concurred with those obtained by Hemeida and Kobeassy (18).

In regard to the deteriorative criteria of chemicals, the TBA-value was determined as an index of lipid oxidation taking place in sausage samples during refrigerated storage. Table III indicates that the TBA value mg-malondialdehyde/kg was subjected to a gradual increase during the experimental period. In addition, TBA values in control samples were the highest in comparison to

Table II
Sensory evaluation of control and treated sausage samples during refrigerated storage

Parameters	Desirable	Undesirable
Control	Days 0-11	Day 12
Treated samples	Days 0-20	Day 21

treated samples (EEP 0.6%). On day 12 of storage, the TBA value in the control samples was 0.74 ± 0.043. This value was slightly below the rejection limit (0.9 mg-malondialdehyde/kg) stipulated by the EES (7) and correlated well with sensory attributes (poor colour and taste); the control samples were therefore rejected. A significantly (p<0.05) lower value (0.58 ± 0.032) was recorded in treated samples. The data we obtained were very similar to those obtained by Han and Park (13) and Hemeida and Kobeassy (18).

The addition of ethanolic extract of propolis (EEP 0.6%) to sausage samples significantly (p<0.05) retarded the oxidative changes of lipids and this retardation of lipid oxidation may be related to its content of flavonoids and phenolic compounds (6, 15, 16, 21, 22, 24, 30). In addition, its antibacterial effect against lipolytic bacteria may enhance antioxidant properties.

TVB-N mg/100 g content (Table III) for the control samples (19.8 ± 1.52) on day 12 of refrigerated storage is the highest value obtained, while treated (EEP 0.6%) samples showed a significantly (p<0.05) lower level than those of the corresponding control samples on days 9 and 12 of storage and did not exceed the permissible limit of 20 mg/100 g stipulated by the EES (7). This could be attributed to the preservative action of propolis on proteolytic bacteria. These results agree with those obtained by Han *et al.* (12) and Hemeida and Kobeassy (18).

TVB-N is considered to be an important factor when measuring the extent of protein degradation to amino acids and putrefaction of meat and meat products (12); it is also considered to be an index of deterioration in regard to the sourness of sausages (26). In this respect, Abd El-Salam (1) stated that TVB-N content was found to increase in frozen meat during storage, which lowered the shelf-life of sausages prepared from frozen meat.

Table IV reveals that no change in the mean proteolytic, lipolytic and total mould and yeast counts in control samples for the first 3 days of refrigerated storage. However, a gradual increase in the organisms examined had been observed throughout the storage period and signs of spoilage began to appear on day 9. Finally, on day 12, significantly higher counts of proteolytic, lipolytic and total mould and yeast (6.39 ± 0.41, 6.0 ± 0.57 and 6.6 ± 0.53 $\log_{10}$ cfu/g, respectively) were noted and signs of spoilage were more pronounced; the samples were rejected. On the contrary, Table IV shows a decrease in microbial load of treated samples during refrigerated storage till day 15 as proteolytic, lipolytic and total mould and yeast counts were 3.47 ± 0.23, 2.9 ± 0.21 and 3.6 ± 0.35 $\log_{10}$ cfu/g, respectively,

Table III
Thiobarbituric acid value mg-malondialdehyde/kg and TVB-N mg/100 g in control and treated sausage samples during refrigerated storage

Samples	Day 0	Day 3		6		9		12		15	18	21
		C	T	C	T	C	T	C	T	T	T	T
TBA value	0.23 ± 0.019	0.29 ± 0.012	0.28 ± 0.021	0.38 ± 0.024	0.31 ± 0.02	0.56 ± 0.031	0.42 ± 0.013	0.74± 0.043*	0.58 ± 0.032	0.69 ± 0.03	0.80 ± 0.05	0.89 ± 0.06
TVB-N	9.8 ± 0.51	10.4 ± 0.71	10.2 ± 0.64	12.9 ± 0.91	11.2 ± 0.87	16.2± 1.01*	12.2 ± 0.85	19.8± 1.52*	12.4 ± 0.76	14.6 ± 1.06	16.8 ± 1.31	19.3 ± 0.86

* significant differences between control and treated samples at p<0.05

C control sausage sample
T treated sausage sample
TBA thiobarbituric acid
TVB-N total volatile bases nitrogen

 © IZS A&M 2010

Table IV
Mean values of proteolytic, lipolytic and total mould and yeast count log_{10} cfu/g of control and treated sausage samples during refrigerated storage

Micro-organisms counted	Day 0	Day 3 C	3 T	6 C	6 T	9 C	9 T	12 C	12 T	15 T	18 T	21 T
Proteolytic	3.77 ± 0.13	3.77 ± 0.26	3.30 ± 0.28	4.00 ± 0.36	3.30 ± 0.27	4.47 ± 0.36	3.69 ± 0.31	6.39 ± 0.41*	3.47 ± 0.21	3.47 ± 0.23	4.00 ± 0.33	6.47 ± 0.42
Lipolytic	3.00 ± 0.24	3.30 ± 0.32	2.95 ± 0.19	3.95 ± 0.33	2.90 ± 0.14	4.00 ± 0.27	3.00 ± 0.25	6.00 ± 0.57*	2.77 ± 0.19	2.90 ± 0.21	3.60 ± 0.29	4.00 ± 0.27
Mould and yeast	3.99 ± 0.31	3.95 ± 0.37	3.47 ± 0.24	4.30 ± 0.41	3.47 ± 0.28	4.69 ± 0.42	3.90 ± 0.32	6.60 ± 0.53*	3.47 ± 0.33	3.60 ± 0.35	4.00 ± 0.37	6.00 ± 0.56

* significant differences between control and treated samples at $p<0.05$

C control sausage sample
T treated sausage sample

followed by a gradual increase on days 18 and 21 of storage to be 6.47 ± 0.42, 4.0 ± 0.27 and 6.0 ± 0.56 log_{10} cfu/g for proteolytic, lipolytic and total mould and yeast counts, respectively, and the samples were rejected on day 21 of storage. The results obtained agreed with those recorded by Hemeida and Kobeassy (18).

The data obtained during this study indicated that the treated samples (EEP 0.6%) had a longer shelf-life than the control sample; this confirmed the reports of Ashour (4), El- Deib *et al.* (8), Hegazi and Abd El-Hady (17), Hemeida and Kobeassy (18), Lu *et al.* (20), Said *et al.* (27) and Trusheva *et al.* (29), namely, that propolis has antibacterial and antifungal properties and that propolis obtained from different sources has different antibacterial and antifungal effects.

Conclusion

In conclusion, propolis is considered to be a good decontaminant and antioxidant as it reduces the microbial load, lowered TVB-N content and TBA value and improved the sensory quality of fresh sausage. Finally, it also extended the shelf-life of the experimentally formulated oriental sausage. Consequently, propolis is recommended as a natural decontaminant and antioxidant in oriental sausage processing to substitute chemical preservatives.

Acknowledgements

The authors would like to thank Professor A. Tawfic for his assistance in the manufacturing of sausage in the Food Technology Laboratory and Dr M.M. Farag of the Faculty of Agriculture of the Cairo University for the supply of propolis.

References

1. Abd El-Salam M.A. 1978. Chemical and technological studies on some Egyptian meat. MV Sc thesis, Faculty of Agriculture, El-Azhar University, El-Azhar, 92-106.
2. American Public Health Association (APHA) 1992. Compendium of methods for the microbial examination of food, 3rd Ed. APHA, Washington, DC, 183-198.
3. Analytical Methods Committee (AMC) 1979. Analytical Methods Committee: report prepared by the fish products sub-committee. Recommended general methods for the examination of fish and fish products. *Analyst*, **104** (1238), 434-450.
4. Ashour Azza T. 1989. Studies on propolis gathering with special reference to its antimicrobial properties. PhD thesis, Faculty of Agriculture, Cairo University, Cairo, 72-84.

5. Bonvehi J.S., Coll F.V. & Jorda R.E. 1994. The composition, active components and bacteriostatic activity of propolis in dietetics. *J Am Oil Chem Soc*, **71** (5), 529-532.
6. Dessouki T.M., El-Dashlouty A.A., El-Ebzary M.M. & Heikal H.A. 1980. Propolis and some other natural antioxidants for fats of frozen meat. *Agri Res Rev*, **58** (3), 311-320.
7. Egyptian Organisation for Standardisation and Quality Control (ESS) 1991. Egyptian standard specifications for frozen sausage. ESS, Cairo, ES: 3492-2000, 3-6.
8. El-Dieb S.M., Abd Al-Fattah M.A. & Abd Al-Fattah A.M. 1997. The antibacterial and antifungal effect of honeybee propolis and its use on Ras cheese. *Bull Fac Agri Cairo Univ*, **48**, 95-103.
9. Food and Agriculture Organization (FAO) 1986. 8. Food analysis. *In* Manuals of food quality control (P.G. Martin, ed.). FAO, Food and Nutrition Paper 14/5, Rome, 248.
10. Garedew A., Schmolz E. & Lamprecht I 2004. Microbiological and calorimetric investigations on the antimicrobial actions of different propolis extracts: an *in vitro* approach. *Thermochimica Acta*, **422** (1-2), 115-124.
11. Greenaway W., Scaysbrook T. & Whately F.R. 1990. The composition and the plant origins of propolis. A report of work at Oxford. *Bee World*, **71** (3), 107-118.
12. Han S.K., Yamauchi K. & Park H.K. 2001. Effect of nitrite and propolis preservative on volatile basic nitrogen changes in meat products. *Microbios*, **105** (411), 71-75.
13. Han S.K. & Park H.K. 2002. Accumulation of thiobarbituric acid-reactive substances in cured pork sausages treated with propolis extracts. *J Sci Food Agric*, **82** (1313), 1487-1489.
14. Harman N.W. 1983. Hive products for therapeutic use. *Am Bee J*, **17** (1), 39-43.
15. Hayashi K., Komura S., Isaji N. & Ohishi N. 1999. Isolation of antioxidative compounds from Brazilian propolis: *Chem Pharm Bull*, **47** (11), 1521-1524.
16. Hegazi A.G. & Abd El-Hady F.K. 2001. Egyptian propolis: 1. Antimicrobial activity and chemical composition of Upper Egypt propolis. *Z Naturforsch C*, **56** (1-2), 82-88.
17. Hegazi A.G. & Abd El-Hady F.K. 2002. Egyptian propolis: 3. Antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. *Z Naturforsch C*, **57** (3-4), 395-402.
18. Hemeida H.H. & Kobeassy M.I.M. 2003. Chemical composition and active components of Egyptian honeybee propolis as a natural substance for preservation of bastrami. *J Agric Sci Mansoura Univ*, **28** (5), 3747-3764.
19. Ingelfinger J.A., Mosteller F., Thibodeau L.A. & Ware J.H. 1994. Biostatistics in clinical medicine, 3rd Ed. McGraw-Hill Inc., New York, 340 pp.
20. Lu L.-C., Chem Y.-W. & Chou C.-C. 2005. Antibacterial activity of propolis against *Staphylococcus*. *Int J Food Microbiol*, **102**, 213-220.
21. Marcucci M.C. 1995. Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie*, **26** (2), 83-89.
22. Marquele F.D., Stracieri K.M., Fonseca M.J.V. & Freitas L. 2006. Spray-dried propolis extract: physicochemical and antioxidant properties. *Pharmazie*, **61** (44), 325-330.
23. Mohamed Manal M. 2002. Quality improvement of some Egyptian frozen meat products. PhD thesis, Faculty of Veterinary Medicine, Cairo University, Cairo, 42-48.
24. Nagai T., Inoue R. & Suzuki N. 2003. Preparation and antoxidant properties of water extract of propolis. *Food Chem*, **80**, 29-33.
25. Nouman T., El-Nawawy F.A., El-Ashmawy A.M. & Sorur A. 1982. The microbial association in cool-stored fresh sausage. *Assiut Vet Med J*, **10** (19), 137-146.
26. Olivant J.M. 1957. An introduction to laboratory methods in veterinary food control. Department of Veterinary Pathology, Liverpool University, Liverpool, 20 pp.
27. Said S.A., Khan S.A., Ahmad I. & Ali H.S. 2006. Chemical composition of Egyptian and UAE propolis. *Pak J Pharm Sci*, **19** (1), 58-61.
28. Shahidi S. & Wanasundara B.K. 1992. Phenolic antioxidants. *Crit Rev Food Sci Nur*, **32**, 67-103.
29. Trusheva B., Popova M., Bankova V., Simova S., Marcucci M.C., Miorin P.L., da Rocha Pasin F. & Tsvetkova I. 2006. Bioactive constituents of Brazilian red propolis. *Evid Based Complement Alternat Med*, **3** (2), 249-254 (ecam.oxfordjournals.org/cgi/content/short/3/2/249 accessed on 11 May 2010).
30. Wang B.-J., Hui-Lien Y. & Ran, Y Z. 2003. Supercritical fluid extractive fraction – study of the antioxidant activities of propolis. *J Food Chem*, **86** (2), 237-243.
31. Wong J.W., Hashimoto K. & Shibamoto T. 1995. Antioxidant activities of rosemary and sage extracts and vitamin E in a model meat system. *J Agric Food Chem*, **43** (10), 2707-2712.

 © IZS A&M 2010