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Effect of Bee Honey in Safety and Storability of Beef Sausage

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Abstract: The effect of bee honey addition on the safety and storability of beef sausage was evaluated. Beef sausage was processed by the addition of different concentrations (0.0, 2.5, 5.0 and 7.5%) of bee honey. The processed beef sausage was packaged in poly ethylene bags and refrigerated at $4^{\circ}\text{C}\pm 2$ for up to 9 days. Several variables were determined, using subjective and objective measurements, to evaluate the effects of concentration of bee honey and storage periods on the quality attributes of the processed sausage. These included proximate composition, pH, Peroxide Value (PV), total viable count, total coliform, *Escherichia coli*, *Staphylococcus aureus* and psycho trophic bacteria as well as sensory attributes of the sausage. The evaluations were carried out immediately after processing, three, six and nine days post processing. Among all treatments, the control sausage (0% bee honey) had the highest ($P<0.05$) PV, total viable count, total coliform, *Escherichia coli*, *Staph aureus* and psycho trophic bacteria. Sausage sample with 7.5% bee honey had the lowest ($P<0.05$) PV, total viable count, total coliform, *Escherichia coli*, *Staph aureus* and psycho trophic bacteria. Generally and regardless of storage period PV, total viable count, total coliform, *Escherichia coli*, *Staphylococcus aureus* and psycho trophic bacteria decreased with the increase of bee honey concentrations added to the sausage. Incorporation of bee honey in sausage formulation led to substantial improvement in the sensorial and keeping qualities of beef sausage.

Key words: Sausage, bee honey, peroxide value, microbial, storage

INTRODUCTION

Meat and meat products are highly perishable materials so sanitation and cooling is essentials in handling, marketing and processing of meat. The sanitation in the Sudan, in general is very poor with regard to slaughtering, handling, marketing and processing of meat, except for very few meat plants and slaughter houses.

The assurance of inventory and the shelf life of meat and meat product represent an important challenge for the meat industry.

The spoilage of refrigerated meat caused by microbial agent such as bacteria which are responsible for the off odors, off flavors, discoloration, gas production and slime production such problem necessitated the usage of artificial preservatives to prolong the shelf life of meat and insure its safety. In earlier times food was preserved with salt, sugar, spices and wood smoke. With the development of new products, chemical antimicrobial agents and many organic acids were relied on to achieve a longer shelf life and greater assurance of protection from microbial spoilage.

With growing concern over the presence of chemical residues in foods the demand for nontoxic natural preservatives is increasing.

Many natural substances in honey bee species with different plant origins may play an important role in functional properties such as anti oxidative and anti

bacterial activities. Honey is nectar collected from many plants and processed by honey bees (*Apis mellifera*). This natural product is widely appreciated as the only concentrated form of sugar available worldwide (FAO, 1996). and is also used as food preservative (Cherbuliez *et al.*, 2003).

Honey has been reported to contain about 200 substances (complex mixture of sugar but also small amounts of other constituents such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids and other phytochemicals) It's also contains a number of components to act as preservatives; these include tocopherol, ascorbic acids flavonoids, other phenolics and enzymes such as glucose oxidase, catalase and peroxidase (Crane, 1975; Ferreres *et al.*, 1993 and Lyrish, 1974). It is suggested that any of these substances owe their preservative properties to their anti oxidative activity (Cerutti, 1994).

Development of natural preservatives with both antioxidants and antibacterial activities, that prolong the shelf life of meat and prevent food-borne illness, is desirable. We suggest that honey can act as a natural antibacterial and anti oxidant which is important with the recent emphasis on decreasing the use of artificial preservation in food.

Honey as a source of anti oxidant has been proven to be effective against deteriorative oxidation reactions in food, caused by light, heat and some metals (Mickibben and

Engeseth, 2002), such as enzymatic browning of fruit and vegetables (Chen *et al.*, 2000), lipid oxidation in meat (Nagai *et al.*, 2006) and inhibit the growth of food borne pathogens and food spoilage (Taomina *et al.*, 2001). Over all honey serves as source of natural anti oxidants (Al-Mamary *et al.*, 2002; Aljadi *et al.*, 2004; Beretta *et al.*, 2005; Cheldof *et al.*, 2002; Küçük *et al.*, 2007; Nagai *et al.*, 2001) which play an important role in food preservation and human health by combating damage caused by oxidizing agents e.g., oxygen, namely reducing the risk of heart disease, cancer, immune system decline, cataracts, different inflammatory processes, etc. (The national Honey Board, 2003).

The antioxidants present in honey include both enzymatic: catalase (Schepartz, 1966) glucose oxidase, peroxidase (Loyrish, 1974) and non enzymatic substances: Ascorbic acid, alpha-tocopherol (Crane, 1975), carotenoids, amino acids, proteins, organic acids, Millard reaction products (Al-Mamary *et al.*, 2002; Aljadi *et al.*, 2004; Baltrusaityte *et al.*, 2007; Cheldof *et al.*, 2001, 2002; Schramm *et al.*, 2003; The National Honey Board, 2003) and more than 150 poly phenolic compounds including flavonols, flavonoids phenolic acids, catechins and cinnamic acid derivatives.

Although it has already been demonstrated that it has anti oxidant activity and different anti oxidant compounds nothing is reported about the different contributions of the entire honeys and their phenolic extracts to those properties.

The anti microbial properties of honey have been known for thousands of years (Zumla *et al.*, 1989). Honey has been studied to clarify which components are responsible for antagonistic activity against pathogenic microorganisms including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Cooper *et al.*, 2002 and Willix *et al.*, 1992).

The anti microbial activity of honey has been attributed to hydrogen peroxide, osmolarity, acidity aromatic acids and phenolic compounds (Molan, 1992a, b) the high osmolarity of honey is due to high content of sugars (average over 85% of honey) including fructose, glucose, maltose sucrose and other types of carbohydrate (White, 1975). Hydrogen peroxide, by glucose oxidase originating from the honey bees (White *et al.*, 1963), honey was tested against six food borne pathogens by (Taomina *et al.*, 2001) it was shown that varying levels of anti microbial activity of honey were present depending on the variety of honey. The activity of honey was attributed to not only hydrogen peroxide but also anti oxidant compounds in honey. Recently, propolis, a mixture of viscous compounds including herbal poly phenolics found in honey and honey combs, showed anti staphylococcus activity (Lu *et al.*, 2005). It was reported that poly phenolics of propolis inhibited glucosyl transferase activity and the growth of

streptococcus mutans which is associated with dental caries (Koo *et al.*, 2002 and Park *et al.*, 1998).

In New Zealand, Manuka honey has been consumed as a medicinal product due to it is high level of anti microbial activity (Allen *et al.*, 1991). Manuka honey has been reported to exhibit anti microbial activity against pathogenic bacteria including multi-drying resistant *S. aureus* strains and *Helicobacter pylori* from gastric ulcers (Al-Somal *et al.*, 1994; Cooper *et al.*, 2002; Willix *et al.*, 1992). An alternative to traditional antibiotic therapy, gama irradiated manuka honey is commercially available as a topical ointment for burned or wounded skin to protect from opportunistic bacterial infections or cure chronic wounds (Cooper *et al.*, 2002). The unusually high antimicrobial activity of manuka honey has been attributed to aromatic acids or phenolic compounds derived directly from the honey sources, the manuka bushes (Weston *et al.*, 1999).

Objectives: The effect of bee honey on safety and storability of beef sausage.

MATERIALS AND METHODS

Food materials: The fresh beef meat was obtained from Khartoum North butcher shops. The beef meat was transferred immediately stored frozen at -18°C in freezer Food Research Center, Shambat.

Bee honey was obtained from Department of Crop Protection, Faculty of Agriculture, University of Khartoum and stored at room temperature. Spices, salt, sugar, potato were obtained from local markets.

The additional fat needed for the sausage formulation was obtained from the butcher shops. However, for ease of the calculation, only uniform rendered fat, free of protein and moisture content was used.

Casings: Animal casings were obtained from Khartoum North butcher shops.

Raw materials preparation

Meat preparation: Stored beef was allowed to thaw over night in a refrigerator at 4±2°C and sliced then ground through a 0.375 inch, plate using a meat grinder and stored refrigerated at 4±2°C.

Sausage preparation: A basic sausage formula shown in Table 1 was used in the preparation of sausage. Minced meat, salt, minced fat, potato, spices and half of calculated ice water were chopped using a Meat Chopper for about 4 minutes there after the rest of the ingredients were added. The mixture was well homogenized. Then the entire mass was divided in to four equal parts. Each part was assigned randomly to one of the bee honey treatments i.e., 0, 2.5, 5 and 7.5%

Table 1: Basic sausage formula

Component	(%)
Protein	14
Fat	10
Moisture	63
Starch	8
Salt	1.5
Spices	1.4
Binder	2.0

Table 2: Sausage formulation for all treatments

Ingredients	Level of bee honey			
	0%	2.5%	5%	7.5%
Beef meat	1125	1125	1125	1125
Bee honey	0	28.125	56.25	84.375
Potato	125	125	125	125
Fat	125	125	125	125
Water/ice	250	250	250	250
Salt	23	23	23	23
Sugar	4.5	0	0	0
Black pepper	4.5	4.5	4.5	4.5
Nut meg	1.25	1.25	1.25	1.25
Garlic	5	5	5	5
Milk powder	32.25	32.25	32.25	32.25
Coriander	4.5	4.5	4.5	4.5
Chinese kabab	1.25	1.25	1.25	1.25

* Wight in gram

(Table 2). After homogenization the mixture was transferred to a manual stuffer to be stuffed into animal casing, linked at lengths of 10cm, packed in poly ethylene bags and stored refrigerated for up to 9 days and assessed immediately after processing (0 day) and at an intervals of 3, 6 and 9 days post storage.

Peroxide Value (PV): Peroxide value of the beef sausage sample was determined according to AOAC (1995). One gram of extracted was accurately weighed into 250 ml conical flask. Thirty ml of a mixture of glacial acetic acid and chloroform (3:2) were added to the conical flask. One gram of saturated solution of potassium iodide was added. The flask was vigorously shaken for 1 min and kept away from the light for exactly 5min then titrated with accurately standardized solution of 0.01N sodium thiosulphate. Titration continued until the yellow color almost disappeared. A 0.05 ml of starch indicator solution was added. Titration was performed with continuous shaking till the end point. A drop of thiosulphate was added until the blue color has just disappeared. PV was calculated as shown below:

$$PV (\%) = (A-B) \times N \times 1000/S$$

Where:

- B = Reading of blank in mls
- A = Reading of sample mls
- S = Weight of oil sample.
- N = Normality of sodium thiosulphate.

Microbiological analysis

Preparation of serial dilution: Ten grams of meat sample were weighed aseptically and added to conical flask containing 90 ml sterile 0.1% peptone water and sufficiently shaken for homogenization. This dilution was referred as stock solution (dilution 10^{-1}). One ml of stock solution was pipetted aseptically, with sterilized pipette into 9ml sterile peptone water (dilution 10^{-2}) and serial decimal dilutions up to 10^{-6} were prepared as described by Harrigan (1998).

Determination of microbial load of beef sausage: Total viable count was carried out using the pour-plate methods as described by Harrigan (1998). One ml of each dilution was transferred aseptically sterile Petri dish. To each dilution 10-15ml of melted and cooled (45°C) Plate Count Agar were added. The inocula were mixed with medium and allowed to solidify. The plates were then incubated aerobically in an incubator at (37°C) for 48hr. A colony counter was used to count the viable bacterial colonies. The count was expressed as colony-forming unit (cfu) per gram.

Determination of coliform bacteria

Presumptive coliform test: One ml of each of three first dilutions (10^{-1} , 10^{-2} and 10^{-3})³ was inoculated aseptically in triplicates of 9 ml sterilized MacConkey broth using the three-tube technique with Durham tubes. The tubes were incubated at 37°C for 48 hours. Positive tubes gave acid and gas in the Durham tubes.

Confirmed coliform test: All tubes of the dilutions showing gas fermentation in 24 hours were subjected to the confirmation test using brilliant green bile lactose broth fermentation tubes with Durham tubes and then incubated at 37°C for 48 hours. The Most Probable Number (MPN) was recorded. The most probable number (MPN) Tables were used to record the coli forms number.

Faecal coli form test: At least 3 loops full of each confirmed positive tube were sub-cultured into EC broth medium and then incubated at 44.5°C for 24 hours. Tubes showing any amount of gas production were considered positive. The Most Probable Number (MPN) was recorded.

Differentiation of faecal coli form test: For further confirmation of faecal coliform, tubes giving positive reaction at 44.5°C for 24 hours were streaked on Eosin Methylene Blue (EMB) agar. Colonies with green metallic sheen confirmed a positive test.

Staphylococcus aureus: 0.1ml was transferred from suitable dilutions by means of sterile pipette and spread on solidified manitol salt agar plates. The plates were

incubated at 37°C for 36 hours. Then the plates were examined. Presumptive coagulase-positive *Staphylococci*. Produce colonies with bright yellow zones whilst coagulase-negative *Staphylococci* are surrounded by a red or purple zone. Suspect colonies were picked off and were sub cultured in a Nutrient Broth media to be ready for carry out the coagulase test.

Tube coagulase test: Half ml of diluted fresh human plasma was added to small test tube and then 0.5ml of an 18-24hrs broth culture was added and incubated at 37°C. Coagulase was examined after an hour then at intervals up to 24 hrs.

Psychotropic bacteria: One ml was transferred aseptically from every dilution to sterile Petri dish. Fifteen milliliter of sterile nutrient agar were added to every Petri dish after the inoculum was mixed with the medium and allowed to be solid. The plates were incubated for 4°C at 2-3 days. Then by using a colony counter the viable bacterial colonies were counted.

Statistical analysis: The data collected were subjected to analysis of variance and whenever appropriate the mean separation procedure of LSD was employed (Steel and Torrie, 1980). The SAS program (SAS Institute, 2002) was used to perform the GLM analysis.

RESULTS AND DISCUSSION

Peroxide Value (PV): PV value of treated and untreated beef sausage is presented in Fig. 1. PV value of the untreated (0%) beef sausage was higher than all the treatments tested (P<0.05). However it's noted that as the level of bee honey added to beef sausage increased the PV value of beef sausage decreased significantly (P<0.05) of the lowest PV (less than 1 meq/Kg) was observed in the beef sausage treated with 7.5% bee honey. The decrease in PV with the increase in bee honey level may be due to antioxidants found in bee honey. This result agree very well with Antony *et al.* (2002) who reported the effect of dry honey on oxidation of turkey breast meat and they showed that addition of up to 15% dry honey inhibited the development of oxidation compounds in cooked turkey meat. Also this result is supported by Chen *et al.* (2000) who concluded that addition of honey reduced lipid oxidation in meat.

The effect of storage period on PV of beef sausage is shown in Fig. 2. The highest PV was found on day 0 where as beef sausages stored for 3, 6 and 9 days had similar peroxide values.

Microbial analysis

Total Viable Count (TVC): The effect of bee honey on the Total Viable Count is presented in Fig. 3. Bee honey, regardless of the level, reduced substantially the TVC of

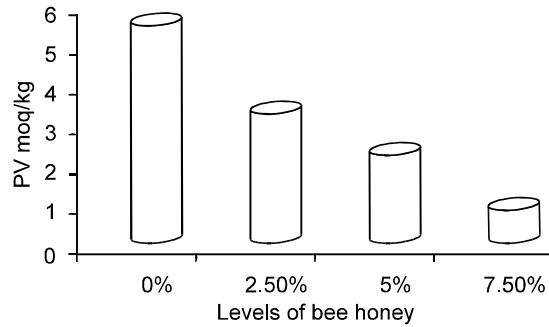


Fig. 1: Effect of bee honey on the PV of beef sausage

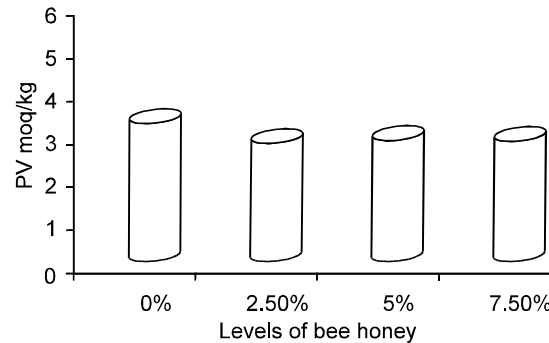


Fig. 2: Effect of the storage period of the PV of beef sausage

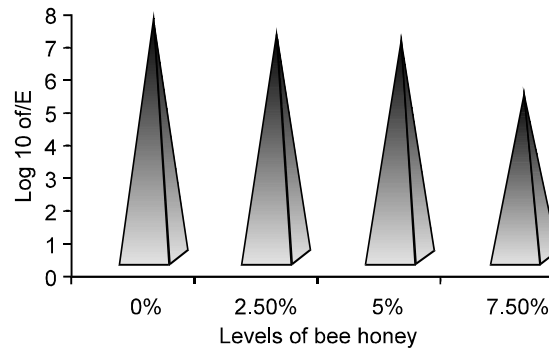


Fig. 3: Effect of the bee honey on the TVC (log 10 cfu/g) of beef sausage

beef sausage. Bee honey on 2.5, 5 and 7.5% level reduced in a 6.82, 6.64 and 4.98 log reduction in TVC respectively Fig. 3. clearly the effect of bee honey increased with the increase in the level of bee honey. Compared with the other treatments (0, 2.5 and 5%) 7.5% treatment gave the lowest TVC. The report of Lee *et al.* (1998) acknowledges to the results reported here in that honey may be useful for inhibiting bacterial growth in either meat products that are less stable or require longer storage time.

As Shown on Fig. 4. The TVC showed similar values over the entire storage period.

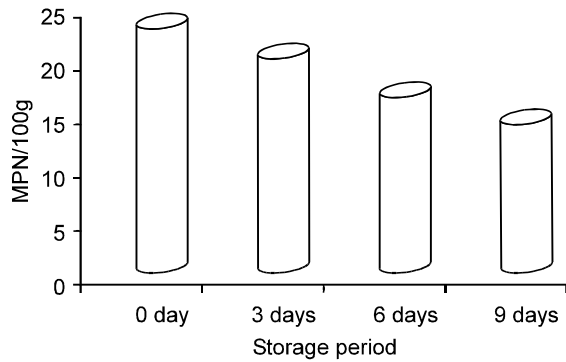


Fig. 4: Effect of the storage period on the TVC (log 10 cfu/g) of beef sausage

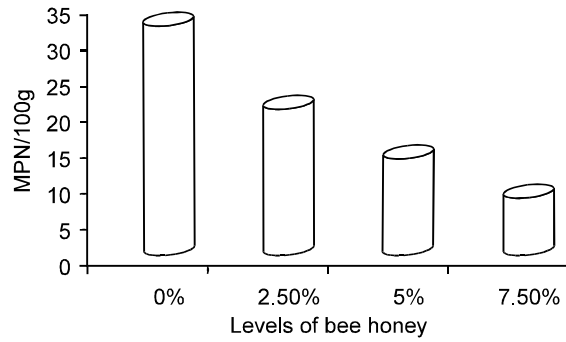


Fig. 6: Effect of the storage period on the Total coliform count of beef sausage

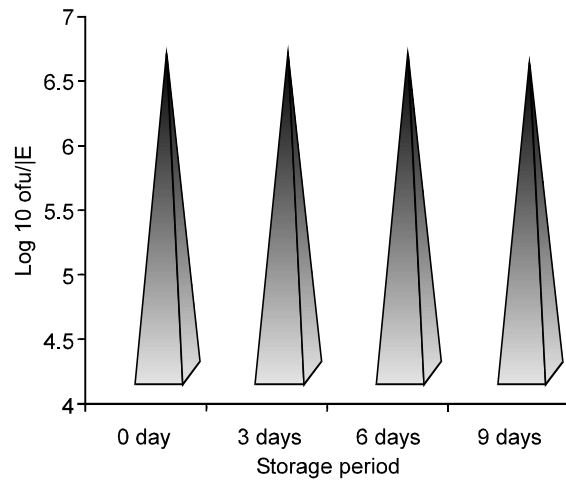


Fig. 5: Effect of bee honey on the Total coliform count of beef sausage

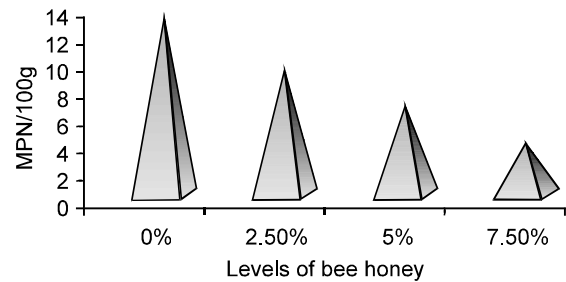


Fig. 7: Effect of bee honey on the *E. coli* of beef sausage

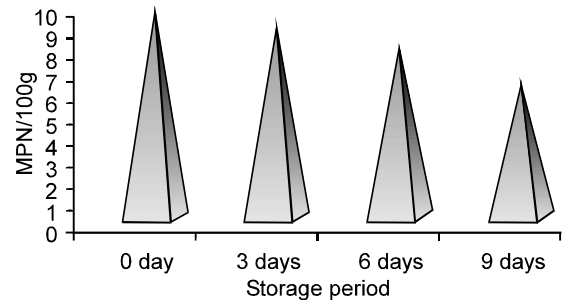


Fig. 8: Effect of the storage period on the *E. coli* of beef sausage

Total coli form: As shown on Fig. 5. The highest total coli form was reported for the beef sausage without bee honey (0%). Obviously as the level of bee honey added to beef sausage increased the total coli form decreased. The poor total coli form growth in the honey treated beef sausage samples (2.5, 5 and 7.5%) supported the findings of Molan (1992) that honey treated meat and poultry products resulted in lower levels of bacterial growth. Also Belewu and Morakinyo (2009) reported that the 15% honey treated cheese sample had no bacterial growth throughout the experimental periods. The total coli form for all treatment during storage period is shown in Fig. 6. Initially (0 day) the total coli form was reported highest value and with increased storage period the total coli form decreased significantly ($P < 0.05$).

***E. coli*:** The effect of bee honey on the *E. coli* is shown in Fig. 7 *E. coli* decreased significantly ($P < 0.05$) with the increase of bee honey levels. Comparison of untreated

and treated beef sausage show that untreated had higher *E. coli* than treated ones. Regardless of the treated and untreated beef sausage *E. coli* decreased with the increase in the storage period (Fig. 8). However at day 0 the *E. coli* was reported the highest value while at day 9 were reported the lowest value with significant difference ($P < 0.05$).

***Staphylococcus aureus*:** As shown in Fig. 9. The highest *Staphylococcus aureus* was reported for the beef sausage without bee honey (0%) while the lowest *Staphylococcus aureus* was reported for the 7.5%, however the 2.5 and 5% bee honey treated beef sausage had similar ($P > 0.05$) *Staphylococcus aureus* count.

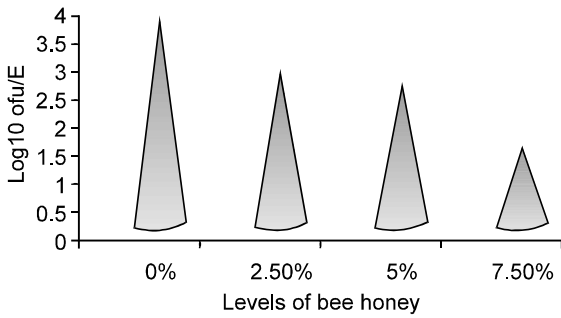


Fig. 9: Effect of bee honey on the *Staphylococcus aureus* of beef sausage

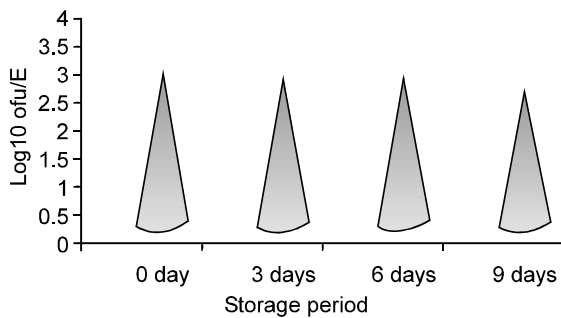


Fig. 10: Effect of the storage period on the *Staphylococcus aureus* of beef sausage

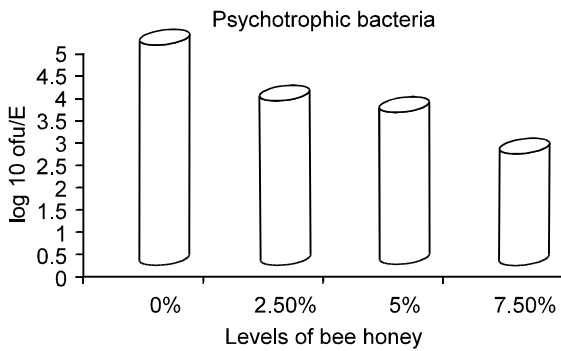


Fig. 11: Effect of the bee honey on the Psycho trophic bacteria of beef sausage

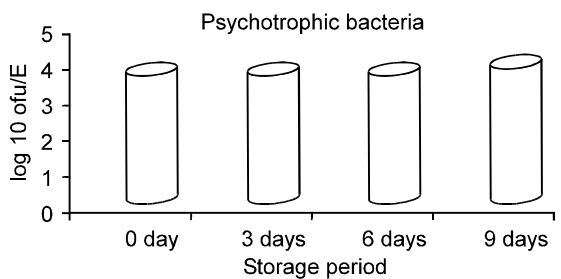


Fig. 12: Effect of the storage period on the Psycho trophic bacteria of beef sausage

The effect of the storage period on *Staphylococcus aureus* as shown in Fig. 10. Obviously there was no significant difference ($P>0.05$) of all treatments.

Psycho trophic bacteria: As shown on Fig. 11, obviously the treatment (0%) noted the highest count and 7.5% was reported the lowest count while the 2.5 and 5% treatment was reported the similar count. Initially and throughout the storage period the psycho trophic count. Fig. 12 Showed similar values over the entire storage period.

Conclusions: Utilization of bee honey in processing of beef sausage usage lead to a significant decrease in PV with the increase of bee honey levels.

Utilization of bee honey resulted in substantial reduction of the Total viable count, total coliform, *E. coli*, *Staphylococcus aureus* and psychotrophics count according it could improves the quality of beef sausage.

REFERENCES

- Aljadi, A.M. and M.Y. Kamaruddin, 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chem.*, 85: 513-518.
- Allen, K.I., P.C. Molan and G.M. Reid, 1991. A survey of the antibacterial activity of some New-Zealand Honeys. *J. Pharmacy Pharmacol.*, 43: 817-822.
- Al-Mamary, M., A. Al-Meerri and M. Al-Habori, 2002. Antioxidant Activities and Total Phenolics of Different Types of Honey. *Nutr. Res.*, 22: 1041-1047.
- AL-Somal, N., K.E. Coley, P.C. Molan and B.M. Hancock, 1994. Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *J. Royal Soc. Med.*, 87: 9-12.
- Antony, S.R.I., M.J.R. Rieck and P.L. Dawson, 2002. Ant oxidative effect of maillard product added to turkey meat during heating by addition of honey. *J. Food Sci.*, 67: 1719-1724.
- AOAC, 1995. Official Method of Analysis. 17th Edn., Association of official analytical chemists, Washington D.C., 7: 65-68.
- Baltrusaityte, V., P.R. Venskutonis and V. Ceksteryt, 2007. Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chem.*, 101: 502-514.
- Belew, M.A. and O.A. Morakinyo, 2009. Nutritional, microbiological and sensory evaluation of honey treated and untreated soft cheese. *Eur. J. Sci. Res.*, 26: 131-138.
- Beretta, G., P. Granata, M. Ferrero, M. Orioli and R.M. Facino, 2005. Standardization of antioxidant properties of honey by a combination of spectrophotometric/Fluorimetric Assays and Chemometric Analytica Chimica Acta, 533: 185-191.
- Cerutti, P., 1994. Oxy-radicals and Cancer. *Lancet*, 344: 862-863.

- Cheldof, N., X. Wang and N. Engesetg, 2001. Characterization of the Antioxidant in Honeys from Different Floral Sources. Presented at Annual Meeting, Institute of Food Technologists, Dallas, USA. 11-14 June.
- Cheldof, N., X.H. Wang and N.J. Engeseth, 2002. Identification of antioxidant components of honeys from various floral sources. *J. Agric. Food Chem.*, 50: 5870-5877.
- Chen, L., A. Mehta, M. Berenbaum, A. Zangerl and N. Engeseth, 2000. Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *J. Agric. Food Chem.*, 48: 4997-5000.
- Cherbuliez, T. and R. Domerego, 2003. L'apitherapie, Medecine des abeilles. Editions Amyris.
- Cooper, R.A., P.C. Molan and K.G. Harding, 2002. The Sensitivity to honey of gram-positive Cocci of Clinical significance isolated from wounds. *J. Applied Microbiol.*, 93: 857-863.
- Crane, E., 1975. Honey a comprehensive survey. New York: Crane Russak and Co
- FAO 1996. Value-added products from bee keeping. FAO. Agricultural Services Bulletin. Rome, Italy: FAO.
- Ferreres, F., C. Garcaviaguera, F. Tomaslorente and F.A. Tomasbarberan, 1993. Hesperetin C a marker of the floral origin of citrus honey. *J. Sci. Food Agric.*, 61: 121-123.
- Harrigan, W.F., 1998. Laboratory methods in food microbiology, 3rd Edn., Academic press, San Digo, London, Boston, New York, Sydney, Tokyo and Toronto.
- Koo, H., B.P.F.A. Gomes, P.L. Rosalem, G.M.B. Ambrosano, Y.K. Park and J.A. Cury, 2002. *In vitro* antimicrobial activity of propolis and arnica montana against oral pathogens. *Arch. Oral Biol.*, 45: 141-148.
- Kücük, M., S. Kolayli, S. Karaoglu, E. Ulusoy, C. Baltaci and F. Candan, 2007. Biological activities and chemical composition of three honeys of different types from anatolia. *Food Chem.*, 100: 526-534.
- Lee, B.J., D.G. Hendricks and D.P. Cornforth, 1998. Antioxidant effects of carnosine and phytic acid in a model beef system. *J. Food Sci.*, 63: 394-398.
- Loyrish, N., 1974. Bees and people. Russia: MIR Publishers, Moscow.
- Lu, L.C., Y.M. Chen and C.C. Chou, 2005. Antibacterial Activity of Propolis Against *Staphylococcus aureus*. *Int. J. Food Microbiol.*, 102: 213-220.
- Mickibben, J. and N.J. Engeseth, 2002. Honeys as a protective agent against lipid oxidation in ground turkey. *J. Agric. Food Chem.*, 50: 592-595.
- Molan, P.C., 1992b. The Antibacterial Activity of Honey: 2. Variation in the Potency of the Antibacterial Activity. *Bee World*, 73: 59-76.
- Molan, P.C., 1992a. The antibacterial activity of honey: 1. the Nature of the Antibacterial Activity. *Bee World*, 73: 5-28.
- Nagai, T., R. Inoue, N. Kanamori, N. Suzuki and T. Nagashima, 2006. Characterization of honey from different floral sources. its functional properties and effects of honey species on storage of meat. *Food Chem.*, 97: 256-262.
- Nagai, T., M. Sakai, H. Inoue and N. Suzuki, 2001. Antioxidant activities of some commercially honeys, Royal Jelly and Propolis. *Food Chem.*, 75: 237-240.
- Park, Y.K., M.H. Koo, J.A.S. Abreu, M. Ikegaki, J.A. Cury and P.L. Rosalen, 1998. Antimicrobial activity of propolis on oral microorganisms. *Current Microbiol.*, 36: 24-28.
- SAS, 2002. SAS User Guide, Release 6.03 Edition SAS.
- Schepartz, A.I., 1966. Honey Catalase: Occurrence and some kinetic properties. *J. Agric. Res.*, 5: 167-176.
- Schramm, D.D., M. Karim, H.R. Schrader, R.R. Holt, M. Cardeti and C.L. Keen, 2003. Honey with high levels of antioxidants cans provide protection to healthy human subjects. *J. Agric. Food Chem.*, 51: 1732-1735.
- Steel, R.G. and J.H. Torrie, 1980. Principle and procedures of statistics, Mc Grow Hill, New York, U.S.A.
- Taomina, P.J., B.A. Niemira and L.R. Beuchat, 2001. Inhibitory activity of honey against food borne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *Int. J. Food Microbiol.*, 69: 217-225.
- The National Honey Board, 2003. Honey-Health and therapeutic qualities. Lashley Street Longmont, <<http://www.nhb.org>>.
- Weston, R.J., 1999. Antibacterial phenolic components of New Zealand Manuka Honey. *Food Chem.*, 64: 295-301.
- White, J.W., 1975. Minerals in Honey. In: Crane, E. (Ed.), Honey, a Comprehensive study, 9th Edn., London: Heinemann.
- White, J.W. and M.H. Sabers, 1963. Studies on honey inhibited a chemical assay. *J. Apick. Res.*, 15: 23-28.
- Willix, D.J., P.C. Molan and C.G. Harfoot, 1992. A comparison of the sensitivity of wound-infecting species of the bacteria to the antibacterial activity of manuka honey and other honey. *J. Applied Bacteriol.*, 73: 388-394.
- Zumla, A. and A. Lutat, 1989. Honey a remedy rediscovered. *J. Royal Soc. Med.*, 82: 384-385.