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EFFECT OF DIFFERENT ACETIC ACID CONCENTRATIONS ON MICROBIAL QUALITY, COLOUR STABILITY, AND SENSORY ACCEPTABILITY OF BEEF SHAWARMA STORED UNDER REFRIGERATED CONDITIONS

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ABSTRACT

Effect of using different concentration of acetic acid (5, 7.5, 10 and 12.5%) on microbiological quality, pH, lipid oxidation and sensory properties of beef shawarma during storage at 4°C for 16 days were examined in this study. The results showed that addition of acetic acid at 10% concentration reduced and retard the growth of total number of microorganisms by one log cycle, prevented the growth of Enterobacteriaceae, Coliform, *E. coli* , Psychrotrophic bacteria, and *Staphylococcus aureus* . in beef Shawarma during storage at 4°C for 16 days. Both pH (6.1 to 6.14) and TBA (0.056-0.97 malonaldhyde absorbance /kg oil) did not much affected with increasing the concentration of acetic acid added to beef Shawarma. Meanwhile the increasing of acetic acid concentration caused a noticeable reduction in redness, slight changes in wave length, purity, visual density and brightness of beef Shawarma colour. Panelists showed that the dark grayish colour, sourish aroma, sourness taste, toughness texture of cooked beef Shawarma increased with increasing acetic acid concentration added to this product. Storage at 4°C for 16 days caused slight changes in the above measured properties.

INTRODUCTION

Minimizing product contamination and delaying growth of spoilage and pathogenic microorganisms in the product are major keys for improving fresh meat shelf life and increasing consumer safety **(Sallam and Samejima, 2004)**. Recently, there has been an increase interest in application of various chemical additives in order to minimize and control undesirable changes in fresh meats and their products. **(Friedrich *et al* 2008)**.

Acetic acid is a food acidulant and also used in food preservation. It is used in two forms, vinegar with 5-10% acetic acid, or an artificial acid containing 22-80% acetic acid. It classifies as a generally accepted food additive for food products **(EC, 1995)**. The primary effect of acetic acid as an antimicrobial agent is to lower cytoplasmic pH and the undissociated acid may have specific effects on the microbial metabolism that amplify it's action. Below pH value of 4.5, *Clostridium botulinum* is not able to grow in food, while at pH 4.2 most of other food poisoning microorganisms are well controlled **(Yusop *et al* 2010)**. pH also affects colour, flavor and texture of meat. Bovine α-lactoalbumine is insoluble at pH range between 4-5, near from it's isoelectric point. At this point, charge-based contribution to repulsion will be minimized since the proteins well be in their least expanded state and it's emulsion stability well be great.

This is probably due to the great surface converge at the isoelectric point with compact protein structure **(Burke and Monahan, 2003)**.

Generally acetic acid tends to be more inhibitory in combination with using lwo temperature, pasteurization, water activity reduction, salt, spices … etc. **(Bang *et al* 2011)**.

Although extensive researches have examined the effect of acetic acid on the viability of pathogens such as *Listeria monocytogenes.*, *Salmonella spp*. during refrigerated storage of caresses and meat products **(Barmpalia *et al* 2005 and Smith *et al* 2005)**, the study of the antimicrobial effects of

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acetic acid on the growth of spoilage microorganisms by incorporating it during meat product formulation is limited. Furthermore, the application of this acid may have adverse effects on a product colour, odour, flavor and appearance which can be quality indicators to consumers. The objective of this research, therefore, was to determine the influence of four of acetic acid concentration 5, 7.5, 10 and 12.5% on microbial quality, colour stability, pH, fat oxidation and sensory acceptability (Consumer) of beef Shawarma stored under chilled temperature, 4°C.

MATERIALS AND METHODS

Materials: Untied State frozen imported back rip boneless beef, fresh lamb fat, Ultra High Temperature sterilized (UHT) skimmed cow milk, spices blend (Cinnamon,white, red and black pepper, thyme, sage, nutmeg, rosemary, and Cardamom) of El-Motaheda. Co., fresh onion, refined fine iodized common salt, an edible grade acetic acid were obtained from local markets at Alexandria City, Egypt. All reagents and chemicales used in this study were analytical grades.

Methods

1-Technological methods

1-1. Manufacture of Shawarma: Frozen meat was thawed at room temperature (22 ± 3°C) for 4-5 hr., dressed by removing their surrounded fat layers, cut into 10 cm thickness portions then into small thin slices with 4-5 mm thickness using stainless steel knives, kept in stainless stell nets to separate drip, then well mixed with 6.7% chopped onions, 1% spices blend, 1% salt, 7% water, 6.6% UHT skimmed milk of meat weight in bowl and stored at 4°C for 24 hrs. Lamb fat was trimmed to remove inedible parts, cut into small portions, chopped in meat mincer to pass through 3 mm before adding to cool meat mixture at 10% of meat weight. Four different formulation of beef Shawarma containing 5, 7.5, 10 and 12.5% concentration of acetic acid of meat weight were prepared. The commercial product usually contain 5% acetic acid. The prepared Shawarma was packed in polypropylene pouch, stored at 4°C and subjected for analysis every 3 days.

1-2. Cooking of Shawarma: Raw product was cooked propely at 425°F(2185C)for 6 min. after spreading on the surface of hot clean move grill.

2- Analytical methods: The pH was determined using Testo pH meter, type 230, at room temperature (22 ± 3°C) as described in **AOAC (2000)**. Thiobarbituric acid (TBA) was colourimetrically estimated according to **Park *et al* (2007)** using Spectronic 601 Spectrophotometer and expressed as malonaldehyde absorbance per kilogram oil. Objective colour measurements of raw Shawarma was assayed as described by **Mackinnery and Little (1962)** using Lovibond Schofield Tintometer. The obtained lovibond values were converted into CIE units, hue, purity and lightness, using the visual density graph and an instructions in manual supplied with apparatus.

3- Microbiological methods: Ten grams of Shawarma was blended with 90 ml of sterilized peptone water for 5 min in sterilized glass jar of a blender.

Appropriate dilution was prepared for enumeration using standard microbiological pour plate technique and the recommended culture media of **Oxoid (2002)**. Plate count agar medium was used for enumerating the Total Viable Count (TVC) and Psychrotrophic count (PC) bacteria after incubating at 35-37°C for 48 hrs and 7°C for 10 days respectively. Violet red bile agar with methyl umbelliferyl glucouronide (VRB-MUG) selective media was used to isolate Coliform, gram negative enteric bacteria and rapid detection of*E. coli*. The proper dilution of the Shawarma homogenate was inoculated in sterile petri dishes then medium was poured and plates were incubated at 37°C for 18-24 hrs. Colonies of lactose negative Enterobacteriaceae are colourless and those of lactose positive are red and often surround by a forbid zone due to precipitation of bile acid. Light blue fluorescent colonies under UV-lamp (336 nm.) denote as *E. coli*. The recommended Difico Barid Parker agar medium by **ICMSF (1978)** was used to detect *Staphylococcus aureus* after incubating the plates at 35-37°C for 48 hrs. The black shiny colonies with narrow white margin and surrounded by clear zones were counts as *Staphylococcus aureus.*

4-Organoleptic methods: Colour, texture, flavor and overall acceptability of cooked Shawarma were organoleptically evaluated using 10 trained panelists and 5 point scale ranging from 1 (poor) to 5 (excellent) as mentioned by **El-Sahn *et al* (1995)**.

5- Statistical methods: The standard deviation was calculated using the method described by **Steel and Torrie (1980)**.

RESULTS AND DISCUSSIONS

1- Microbiological quality: Results in **Table (1)** showed that the total viable bacterial count of beef Shawarma containing different concentration of acetic acids ranged from 4 x 10^4 to 6.4 x 10^5 cfu/g. This population did not exceed the critical limit of 5 x 10^6 cfu/g. referred by the relevant 95/2 EC and 2073/2005 EC regulations for the production and placing on market of minced meat and meat preparation in addition to this microbiological criteria for food stuffs **(EC, 1995; EC, 2005)**. Addition acetic acid at 10% concentration in the beef Shawarma formula reduced the count of TVC from 10^5 to 10^4 cfu/g., one logermatic cycle. Increasing this concentration to 12.5% did not affect this load. Generally the antimicrobial effect of acetic acid as an organic acids depends on it's concentration, temperature, method and time of application **(Ozdemir *et al* 2006)**. During 16 days of storage at 4°C, slight changes in the count of TVC of beef Shawarma containing acetic acid with different concentration were noticed **(Table, 1)**. This may be due to the combination effects of acetic acid, spices mixture, common salt (NaCl) added to Shawarma in addition to refrigerated temperature (4°C) used for storing this product. Enterobacteriaceae bacteria were detected only in beef Shawarma made with 5 and 7.5% acetic acid in load varied from 5.4 to 7.9 x 10^2 cfu/g. Slight reduction in such count was observed with extending storage period of this product to 16 days at 4°C **(Table, 1)**. Also, Coliform bacteria were detected only in low count, 4-4.5 x 10^2 cfu/g., in fresh Shawarma containing 5 and 10% acetic acid concentration in it's formula. During refrigeration storage, Coliform bacteria was not detected. This means that both increasing of concentration of acetic acid to 10% or more and storage at 4°C caused disappearing of this type of bacteria from beef Shawarma. According to **Stivarius *et al* (2002)** adding 5% lactic acid to beef trimmings prior to grinding was effective in reducing *Salmonella typhimurium*, *Escherichia c*oli., Coliform and aerobic plate count. **Tan and Shelef (2002)**, found that the microbial stability of ground pork stored at 2°C was enhanced by the combination of 2% sodium lactate and l or 2% NaCl. This treatment extended the shelf life of meat from 7 to 14 days. It was also found that incorporation of acetic acid in the recipe of beef Shawarma at a concentration varied from 5 to 12.5% was able to prevent the presence of Psychrophilic bacteria*, E. coli* and *Staphylococcus aureus.* These types of bacteria were also not detected in Shawarma through the 16 days of refrigerated storage at 4°C.The above results indicated that the efficacy of acetic acid as antimicrobial agent depends on it's ability to equilibrate in it's undissociated from, acrosses the microbial cell membrance, and interfere with the pH ingredient that is normally maintained between the cytoplasm of the bacterial cell and food matrix surrounded it **(Gould, 1995)**.

2- pH and TBA: Data in **Table (2)** illustrated that the pH of beef Shawarma ranged from 6.10 to 6.14. This means that the pH of this product did not strongly change either with increasing concentration of acetic acid addition and/or during storage at 4°C. This may be due to the weak dissociation constant of acetic acid. It is also confirmed that the antimicrobial action of this acid in such product is due to it's undissociated from and it's ability to dissociate within the microbial cell. **Seyfert *et al* (2007)** found that moisture, fat and pH of ground beef were not appreciably impacted by addition of lactic acid salts and sodium acetate. As seen from data in **Table (2)** incorporating different concentration of acetic acid from 5 to 12.5% into the formula of beef Shawarma did not affect it's TBA values. The range of TBA was varied from 0.056 to 0.057 malonaldehyde absorbance/kg oil in fresh beef Shawarma. This means that acetic acid concentrations did not consistently affect the fat and it's rancidity in beef Shawarma. Chilled storage of Shawarma at 4°C for 16 days caused slight rise in it's TBA values. This can contribute to the saturated fat (Lamb fat rich in saturated triglycerides) in this product and refrigeration storage conditions (low temperature, 4°C, inside closed poly propylene pouches, far from light and air). According to **Jimenez-Villarreal *et al* (2003)**. use of chlorine dioxide followed by cetylpyridinium chloride and lactic acid followed by chlorine dioxide as multiple antimicrobial intervention had little impact on lipid oxidation and may improve shelf stability of ground beef.

3- Objective colour measurements: As shown in **Table (3)** increasing the concentration of acetic acid in formula of beef Shawarma caused reduction in redness of it's colour. This observation was also noticed with extending the storage period of this product at 4°C for 16 days. This may be attributed to the effect of acetic acid on denaturation of meat protein which usually associated with more light back scattering to the observer. This led to appear the colour of Shawarma pale or dark. **(Alvarado and Sams, 2003 and Swatland, 2008).**

Table 3. Effect of concentration of acetic acid on colour measurements of Shawarma during storage at 4°C.

Storage period (day)	Acid concentration of	Colour measurements						
		R*	Y**	Dominant colour	Wave length (hue)	Purity	Visually density	Brightness
Zero time	5 %	8.7	7.0	1.7 R	592	0.66	0.42	40.2
	7.5 %	8.0	7.0	1.0 R	591	0.66	0.42	40.2
	10 %	7.9	7.0	0.9 R	591	0.66	0.42	40.2
	12.5 %	7.3	7.0	0.3 R	589	0.67	0.40	39.8
4 Days	5 %	9.1	9.4	0.3 Y	589	0.67	0.42	40.2
	7.5 %	8.9	9.5	0.6 Y	590	0.65	0.42	40.2
	10 %	8.8	9.4	0.6 Y	590	0.65	0.42	40.2
	12.5 %	8.2	9.9	1.7 Y	591	0.66	0.42	40.2
8 Days	5 %	8.9	9.3	0.4 Y	590	0.65	0.42	40.2
	7.5 %	8.9	9.3	0.4 Y	590	0.65	0.42	40.2
	10 %	8.7	9.3	0.6 Y	590	0.65	0.42	40.2
	12.5 %	8.6	9.3	0.7 Y	591	0.66	0.42	40.2
12 Days	5 %	8.9	9.4	0.5 Y	590	0.65	0.42	40.2
	7.5 %	8.9	9.4	0.5 Y	590	0.65	0.42	40.2
	10 %	8.6	9.4	0.8 Y	591	0.66	0.42	40.2
	12.5 %	8.6	9.4	0.8 Y	591	0.66	0.42	40.2
16 Days	5 %	8.8	9.4	0.6 Y	590	0.65	0.42	40.2
	7.5 %	8.8	9.5	0.7 Y	590	0.65	0.42	40.2
	10 %	8.7	9.6	0.9 Y	591	0.66	0.42	40.2
	12.5 %	8.7	9.6	0.9 Y	591	0.66	0.42	40.2

R=Red **Y=Yellow

Also the results in **Table (3)** showed slight changes in the wave length, purity, visual density and brightness of Shawarma colour due to either the rise in the added acetic acid concentration and / or extending chilled period at 4°C. **Seyfert *et al* (2007)** illustrated that adding acetate only to ground beef created the most visual colour stability, lightness (L*), redness (a*) and yellowness (b*) values of CIE. **Jensen *et al* (2003)** found that acetate improved colour stability in injection enhanced pork. **Friedrich *et al* (2008)** found significant reduction in redness (a*) and lightness (L*) values of ground beef due to lactic acid treatment and storage under modified atmosphere at chilled storage.

4- Sensory properties: The effect of acetic acid concentration and chilled storage period on the sensory properties of cooked shawarma are shown in **Table (4)**. All attributes including colour, aroma, taste, texture and overall acceptability were affected by acetic acid concentration. The panelist observed that, the pale or dark greyish brownish colour, sourish aroma, aciduleus or sourness taste, and toughness textures of cooked shawarma increased with increasing the concentration of acetic acid added during preparing the formula of this product. According to **Sedaroglu *et al* (2007)** acidic substances play a major role in sensory properties of treated meat. It leads to an increase of

muscle protein denaturation causing high light scattering and appearing the dark colour. Also dropping pH of muscle protein below the isoelectric point pH (5.1-5.3) improved from it's tenderness. During cooking, acidic substances cause swelling and weakening of muscle structures, increasing proteolysis by cathepsins and increasing conversion of collagen to gelatin **(Sedaroglu *et al* 2007)**. Generally extending storage at 4°C for 16 days led to slight changes in panelists preference of the sensory properties of 10% acetic acid containing shawarma more than the other products as seen from their scoring for overall acceptability of this product, **Table (4)**.

Conclusion: Treatment beef shawarma with 10% concentration of acetic acid retarded the growth of aerobic bacteria, resulted slight discoloration of colour, sourness taste, sourish aroma and elastic but hard texture of beef shawarma. Therefore this concentration of acetic acid was preferred than 5% which usually use during preparing commercial shawarma product.

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