Improvement the shelf life of minced beef

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Abstract : This study examined the effect of acetic acid, carbon dioxide and ground mustard, on the microbiological and chemical quality of raw minced beef storage at 4 °C. The results showed that addition of acetic acid, carbon dioxide and ground mustard significantly delayed the proliferation of aerobic plate counts, Enterobacteriaceae, psychrotrophic counts, and Total Coliform count (MPN) and extended the shelf life of the product up to 6,10 and 12 days, respectively, versus 4 days only for control. Ground mustard was the most effective to decontaminate the minced beef being effective in preventing the growth of a wide variety of microorganisms. Therefore, ground mustard could be utilized successfully to reduce the microbial growth, maintain the chemical quality, and extend the shelf life of minced beef held under proper refrigeration.
[Mohamed Tharwat Elabbasy, Kamal Ibrahim Eldesoky and Alaa Eldin Morshdy. **Improvement the shelf life of minced beef.** *Life Sci J* 2014;11(9):185-190]. (ISSN:1097-8135). http://www.lifesciencesite.com. 26

Keywords: Minced beef, Acetic acid, Carbon dioxide, Mustard and Shelf life.

1. Introduction

Minced meat products are commonly marketed at refrigerated temperatures (2–5 °C). The microbiological quality and safety of refrigerated minced beef are major areas of concern for retailers, consumers, and public health. However, many undesirable changes of the products can occur during refrigeration due to microbial growth, which give rise to quality reduction, meat spoilage, and economic loss. Minimizing product contamination and delaying or inhibiting growth of spoilage and pathogenic organisms in the product are major keys for improving fresh meat shelf life and increasing consumer safety **(Sallam and Samejima 2004**).

Essential oils are well-known as antimicrobial agents that could be used to control food spoilage and foodborne pathogenic bacteria **(Burt 2004**). It has been demonstrated that several spices, herbs and fruits containing essential oils effectively inhibit microbial growth, although different results are observed depending on test conditions, microorganisms, and the source of the antimicrobial compound (**Roller 2003)**. Among food grade antimicrobials, Mustard has shown antimicrobial activity by the effect of its primary component allyl isothiocyanate (AIT). AIT from natural sources is permitted for use as a food preservative in Japan, and as a GRAS flavoring agent in the US **(ISSHIKI, et al. 1992**, **Delaquis and Mazza 1995**) **(Kim, et al. 2002**) and Several studies have shown the potential of AIT as a natural antimicrobial in different food matrices, including meat products such as ground beef **(Mari, et al. 2002, Muthukumarasamy, et al. 2003)**. The antibacterial activity of mustard EO is probably due to the ability of its components to disrupt the membranes of bacterial cells, causing lysing of the cell **(Burt 2004).**

Carbon dioxide (CO_2) enriched atmospheres are known to extend the shelf life of product by inhibiting the growth of aerobic spoilage bacteria **(FDA 2001)**. CO_2 , which can readily pass through cell membranes, forms carbonic acid within the cell with a resultant decrease in intracellular pH which slows intracellular enzyme activities such as decarboxylating enzymes which are specifically inhibited by a mass action effect of CO_2 **(KING and NAGEL 1975).** dissolution of CO_2 in the cell membrane alters membrane properties and inhibits membrane functions **(Al-Nehlawi, et al. 2013).**

Acetic acid is an organic acid that has been frequently studied on meat tissues **(Hardin, et al. 1995)**. Acetic acid has effective antimicrobial capabilities due to its ability to lower the pH and cause instability of bacterial cell membrane **(Luck and Jager 1998).**

Shelf life is the period of time between packaging of the product and its use that the product properties remain acceptable to the product user, with shelf life properties being appearance, texture, flavor, color, and nutritive value **(Singh and Singh 2005).** The shelf life of meat and meat products thus depends on level of its microbial contamination, therefore, enhancing the keeping quality, reducing or killing spoilage–causing and food-borne pathogens of meat were very important objectives of food technologists and microbiologists **(Okolocha and Ellerbroek 2005).**

Considering the above, the aim of the present study was to investigate the effect of acetic acid, co_2

and freshly ground mustard on the shelf-life extension of fresh minced beef stored at 4 ±1 °C.

2. Material and methods

Sampling:

Minced beef of normal pH was transported to the laboratory within 30 min of purchase (two kilogram) and was divided into four batches (500 g for each group). One was left as the control batch (without treatment). The second batch was mixed with acetic acids 2% by 5 ml /100 g minced beef. The third batch was gas-flushed with co_2 (Sodastream carbonator 60 liter co_2 cylinder/425 g co_2) immediately prior packaging. While the fourth group was mixed with Mustard (the freshly ground Mustard seeds 5 g/ 100 g minced beef). Each group samples were formed into 20 g portions and were packed individually into plastic pouches and stored at 4 ±1 °C without light. Analyses were carried out on the fresh raw material and then every two days during the 15-day storage period.

Organoleptic examination :

The color, odor, consistency and taste were determined for each sample of minced meat which was tested according to **(Pearson and Tauber 1984) and (Varnam and Sutherland 1995)** at each inspection day**.**

pH determination

Ten grams of sample were homogenized with 40 ml distilled water in a blender for 30s. The homogenate was filtered and the pH value of the filtrate was determined using a digital pH meter (model HM-5S; TOA Electric Industrial Co. Ltd., Tokyo, Japan) standardized at pH 4 and 7 **(Sallam and Samejima 2004).**

Microbiological analysis

At each sampling time, minced beef meat samples (5 g) in the stomacher bag were aseptically filled with 45 ml of peptone water 0.1%. The contents were homogenized in the stomacher for 2 min at room temperature. Such homogenate represents the dilution of (1:10), and then ten folds serial dilution were done.

The microbiological analysis included determination of Total Viable Counts (TVC) which determined on Plate count agar (Himedea lab. Pvt. limited, India) incubated for 48 ± 2 h at 35 °C in accordance with **(BAM, et al. 1998),** Total psychrotrophic count was determined according to **(GREER 1981)** methodology where Plate count agar were incubated at 25 ºC for 24 hours. Enterobacteriaceae were determined using the plate counting method on Violet Red Bile Glucose agar (VRBG) as a medium after 24 hours' incubation at 37 °C **(Mercuri and Cox 1978)**. . All different counts were plated onto growth media in duplicate. Total Coliform count (MPN) was determined according to the Three tubes most probable number (MPN) method recommended by **(ICMSF 1978)** using MacConkey broth tubes which incubated at 37 ºC, and then examined after 24 hours and 48 hours for acid and gas production in inverted Durham's tubes.

Statistical analysis:

Results of microbiological analysis were reported as mean values ± standard error of mean (S.E). Microbial counts were converted to log CFU/g values. Statistical analysis of Data was done by using the statistical package for social sciences (SPSS Inc.; Chicago, IL, USA) software. One way analysis of variance (ANOVA) at 95% level of confidence was done to determine significant differences in the microbial counts in meat (Statistical analysis was done using SPSS-17 statistical software package and ANOVA test). $P<0.05$ was considered as significant.

3. Result and discussion

Fresh minced meat is a highly perishable product due to its biological composition and the shelf life of refrigerated minced meat is limited by the growth and biochemical activities of aerobic and psychrotrophic strains of bacteria. Minced beef can be easily contaminated with microorganisms and, if not properly handled and preserved, support growth of spoilage and pathogen bacteria, leading to loss of quality and potential public health problems **(Vernozy‐Rozand, ‐ et al. 2002)**. Refrigeration storage is usually the most common preservative method of minced meat. The shelf life of minced meat can be extended by the use of preservatives and other antimicrobial treatments **(Fik and Leszczyńska-Fik 2007) (Fik, et al. 2008).** However, consumers increasingly demand use of natural products as alternative preservatives in foods, as the safety of synthetic additives has been questioned in last years **(Imaida, et al. 1983).**

The microbial stability of minced beef stored at 4 °C was enhanced by the using of acetic acid, co_2 and ground mustard and these treatments extended the shelf life of the meat from 4 to 6, 10 and 12 days, respectively.

The results of organoleptic examination of minced beef stored at 4 °C revealed that control samples, acetic acid treated samples, co_2 and ground mustard samples were organoleptically rejected (shown extreme discoloration and off-odor) after fourth, six, ten and twelve days of refrigeration storage, respectively.

Changes in pH values in minced beef during storage at 4 °C (Table 1) revealed that the initial pH value of untreated samples (control) and treated samples with acetic acid, co2 or ground mustard was 5.82±0.34, 5.35±0.125, 5.79±0.09 and 5.8 ±0.16, respectively at zero day of refrigeration storage.

Obtained data showed, that the pH did not change significantly during the storage at 4 °C. In general, the addition of acetic acid resulted in significantly decreased pH compared to the other treated and untreated samples. By more time of refrigeration storage pH values increased in different degrees within untreated and treated meat samples as shown in table (1) due to endogenous enzymes , bacterial metabolites as hydrogen sulfides , organic sulfides , other volatile organic compounds like amines **(Gill 1986)**. The antimicrobial activity of acetic acid, co_2 and ground mustard in minced beef samples stored at 4 °C for 15 days, are shown in Figs. 1,2,3 and 4. Initial contamination of the meat in terms of total viable count (TVC) of bacteria in control, acetic acid, co_2 and ground mustard samples was 5.82, 5.77, 5.72 and 5.75 log cfu/g respectively. The statistical analysis of TVC revealed no significant difference ($p > 0.05$) between examined minced beef samples at zero day of refrigeration storage, while the increase in the storage time produce significant proliferations in TVC ($P < 0.05$), whatever the treatment conditions. However, TVC reached and exceeded a value of 7 log cfu/g, considered as the upper microbiological limit for good quality minced meat, as defined by the **(ICMSF 1986)**, on the fourth day for the control samples, indicating a shelf life of about 4 days. Compared with the control, all other Treatments significantly reduced the initial TVC with the increase in the storage time ($P < 0.05$), and caused inhibition of the growth of bacteria in minced beef samples and extension of the shelf life under storage at 4 °C. The use of the freshly ground mustard was the most effective, achieving the greatest initial reduction and TVC never reached the limit of 7 log cfu/g during the 12 day storage period resulting in a shelf life extension of 12 days as compared to the control samples.

With respect to Enterobacteriaceae (Fig. 2), considered as a hygiene indicator **(Zeitoun, et al. 1994)**, the initial counts were 4.12±3.16, 3.97±3.07, 3.9 ± 2.46 and 3.85±2.5 log cfu/g in control, acetic acid, co_2 and ground mustard samples at zero day of refrigeration storage, respectively. Compared to control, Enterobacteriaceae grew in ground mustard samples at a slower rate and exceeded the 5 log cfu/g at 12 day of storage while thus value was exceeded in control samples, acetic acid samples and co_2 samples at 4 , 6, 10 days of refrigeration storage, respectively.

At storage day 0, the initial Psychrotrophic bacteria in minced beef samples were 5.7±3.44, 4.6±2.9, 4.3±3.1 and 4.16±2.9 log cfu/g in control, acetic acid, co_2 and ground mustard samples, respectively. The changes in psychrotrophic count were approximately similar to those of TVC, with control also being the highest at day 4 followed by samples treated with acetic acid and co_2 while much lower counts was detected in samples treated with ground mustard. Significant differences were detected in psychrotrophic count between samples treated with mustard and those of controls, also between samples treated with ground mustard and samples treated with acetic acid.

The detection of coliforms is widely used as a mean of measuring the effectiveness of decontamination **(Lues and Van Tonder 2007).** Total coliforms counts (MPN) are shown in Fig. 4. The initial coliform count were 27, 20, 14 and 15 MPN/g in control, acetic, co_2 and ground mustard samples, respectively at zero day of refrigeration storage. Coliform counts (MPN) were approximately similar to those of Enterobacteriaceae that grew at a slower rate in the treated samples versus control samples while ground mustard samples had the lowest coliforms count (MPN).

Acetic acid antimicrobial activity is based primarily on its pH lowering effect, and it exhibits this antimicrobial activity only in an undissociated form **(Sofos, et al. 1998**). The inhibitory effect of CO_2 on microbial growth, and therefore on the extension of meat shelf life, is well documented **(Clark and Lentz 1972, Silliker and Wolfe 1980, Luno, et al. 2000).**

Such result declared that the most effective decontaminant of minced beef was using the freshly ground mustard. Mustard Essential oils affected the microbial cell membrane integrity , resulting in a loss of cell homeostasis and also affected the concentration of intracellular component, such as ATP and the pH, so mustard Essential oils can be used as an effective antimicrobial agent **(Turgis, et al. 2009).**

The results of the present work with regard to the use of mustard to decontaminate minced beef are in general agreement with those of **(Nadarajah, et al. 2005)** who stated that More direct contact between AIT produced by glucosinolate hydrolysis in the hydrated mustard flour when mixed with meat was presumed to have improved antimicrobial effectiveness and he also mentioned that mustard flour having greater antimicrobial activity than the concentrated liquid AIT. **(Chacon, et al. 2006)** reported that the delay in the growth was observed in all AIT treatments after the first 6 days of storage while Inhibition of mesophilic bacterial populations growth in finely chopped beef was more evident during the first 12 days when using AIT levels ranged from 1500 to 2828 ppm.

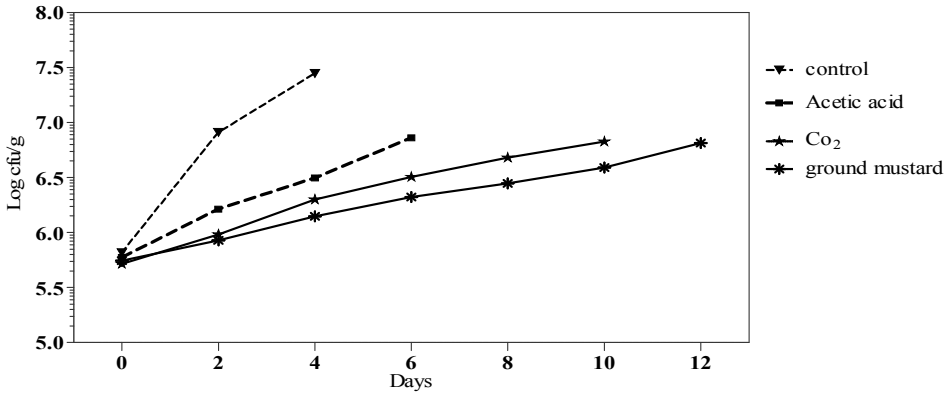

Fig. 1.: Changes in total viable count (TVC) in minced beef samples during storage at 4 °C.

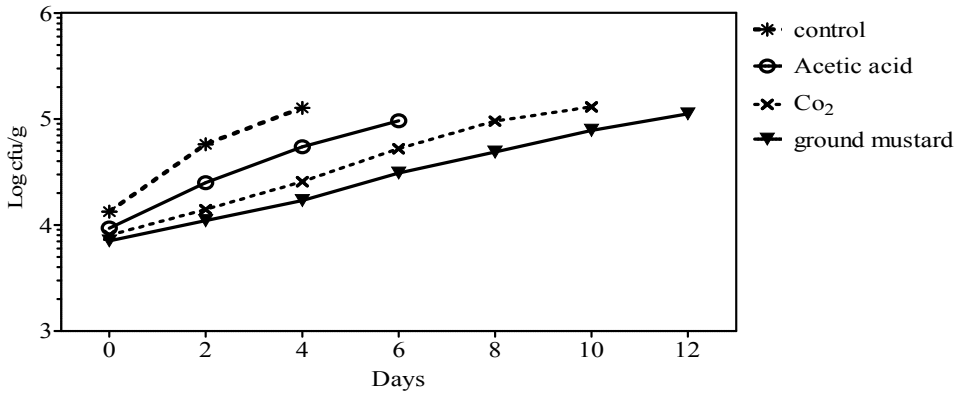

Fig. 2.: Changes in Enterobacteriaceae count in minced beef samples during storage at 4 °C.

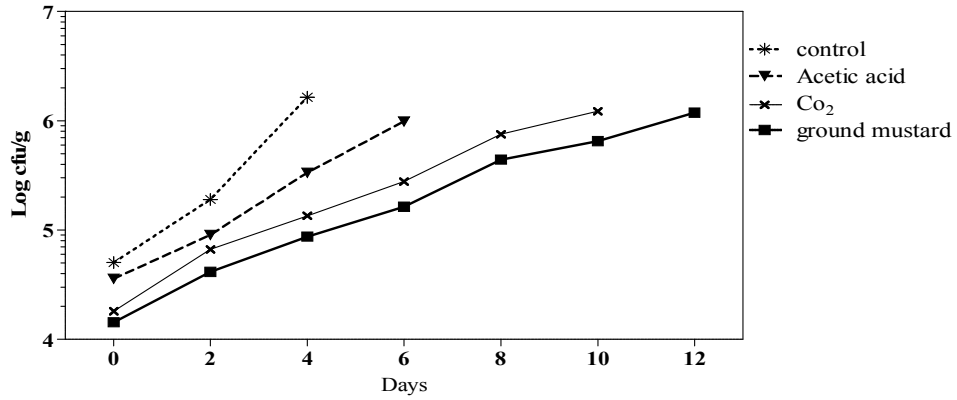

Fig. 3.: Changes in total Psychrotrophic count in minced beef samples during storage at 4 °C.

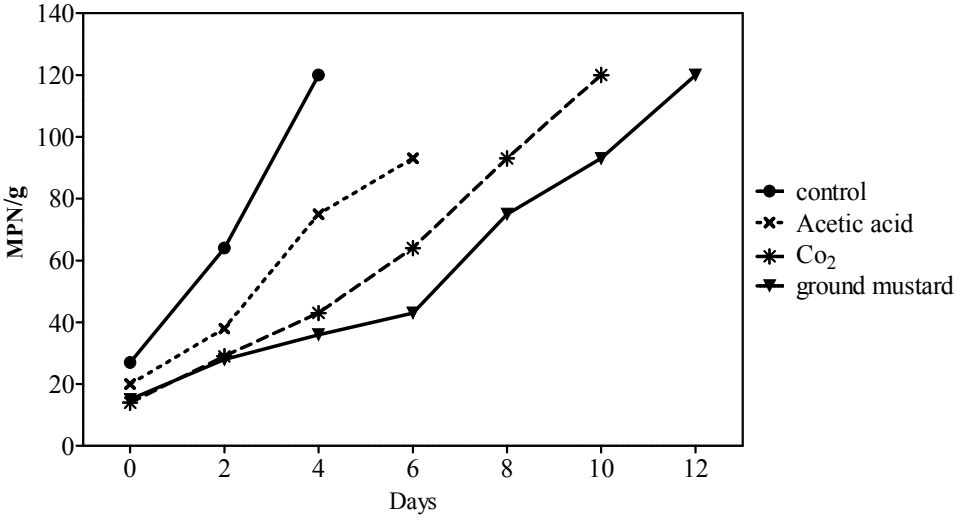

Fig. 4.: Changes in Total Coliform count (MPN) in minced beef samples during storage at 4 °C.

Table (1): The mean values of pH in examined untreated and treated samples of minced beef samples stored at 4± 1°C.

Groups / *Days of storage*	control	Acetic acid	Co_2	ground mustard
Zero time	5.82±0.34	5.35±0.125	5.79±0.09	5.8 ±0.16
2nd day	6.32±0.215	5.91 ±0.12	6.11±0.13	6.18±0.19
4th day	6.78±0.26	6.27±0.135	6.29±0.23	6.35±0.08
6th day	**AD**	6.75±0.145	6.4±0.29	6.42±0.17
8th day		**AD**	6.64±0.126	6.51±0.34
10th day			6.81±0.11	6.69±0.25
12th day			**AD**	6.85±0.194
14th day				**AD**

AD: Apparent Decomposition

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5/23/2014