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Full Length Research paper

Improvement of the quality and shelf-life of minced beef mixed with soyprotein by Sage (*Saliva officinalis*)

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The effect of various concentrations of Sage (*Saliva officinalis*) on the microbial quality and the shelf-life of minced beef mixed with soybean protein was investigated. For this purpose, minced beef mixed with soybean protein samples were divided into four groups. First group was kept as a control group, others ones were treated with 0.1, 0.3 and 0.5% sage extract. All samples were stored at 4°C and microbiological evaluation was conducted at intervals of 0, 1, 3, 5 and 7 days post-storage for aerobic, anaerobic, enterobacteriaceae, yeast-mould, *Bacillus cereus* and *Staphylococcus aureus* counts. Minced beef pose a risk of food-borne infection or intoxication due to existence and growth of *B. cereus* and *S. aureus*. Sage extract concentration of 0.3 and 0.5%, significantly reduced aerobic, anaerobic, enterobacteriaceae, yeast-mould, *B. cereus* and *S. aureus* counts in the samples. Control and 0.1% sage groups did not comply with the Egyptian standards criteria for total aerobic counts when compared to other groups within the seven days of shelf-life. The results indicated that 0.3 and 0.5% sage extract improved the microbiological quality and prolonged the shelf-life of the minced beef to seven days of retail displayed at 4°C.

Key words: Minced beef, sage, soybean, microbial quality and shelf-life.

INTRODUCTION

Minced beef mixed with soybean is one of the meat products widely consumed in Egypt. Microbial quality of meat products plays an important role in increasing public health issue all over the world. During the last decade, there were great improvements in hygienic technique for production of meat products with attention of a lot of consumers towards healthy nutrition. The use of antimicrobial ingredients is one of the widely used methods to maintain microbiological safety and prolong the shelf-life of food products (BIngöl and Bostan, 2007).

In the Egyptian organization for standardization and quality control (EOS, 2005) a minced beef mixed with soybean protein is defined as a fresh meat mixed with soybean protein with or without the addition of food salt or spices and stored at chilling or freezing condition. The use of soybean protein (food grade) as meat products extenders is widely spread in meat products industry because of their interesting nutritional and functional properties. Together with these, health diets and economical reasons are the major causes for the addition of soybean proteins to meat products (Belloque et al., 2002).

Herbs are used as substances enhancing the taste and varieties of regularly food. Some herbs are used as meat additives reporting to have bactericidal or bacteriostatic activities (Dyankova et al., 2009). The inhibitory effects of herbs are mostly because of the volatile oils (Vazgeçer et al., 2004; Ağaoğlu et al., 2007). Sage, *Saliva officinalis,* is a perennial herb, with gray green aromatic leaves and their name derived from the Latin word salvere, meaning "to save". Ancient Greek-Romans and ancient Egyptian used sage as meat preservative and as a medical herb. In the middle ages, it was used in treating cholera, common cold, fevers, and epilepsy (Tainter and Grenis, 1995). The variety used nowadays in cooking is the flowerless and broad leaves. Herbs have a very powerful flavor and can be used fresh or dried.

Minced beef is not only highly susceptible to spoilage, but also it frequently involved in the spread of food-borne illnesses. During slaughtering and processing of cattle, all

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potentially edible tissues are subjected to microbial contamination from various sources. Aerobic plate counts often are chosen as an indicator of the effectiveness of hazard analysis critical control point (HACCP) plans, because data for all aerobic bacteria are more easily collected than data for pathogens of concern or other indicator organisms (Chong-Hae et al., 2008). Enterobacteriaceae are very useful as indicators of bad hygiene or bad treatment of food products, and their presence in large number indicates a big possibility of their multiplication, implying multiplication of other pathogens (Nissen et al., 2001). It is suggested that yeast and mould play an important role in causing spoilage of meat (Deak, 1991; Fleet, 1992). *Bacillus cereus* and *Staphylococcus aureus* are two important food-borne pathogens. The most staphylococcal food poisoning and *B. cereus* food poisoning outbreaks may due to poorly cooked and extensively handled meat products (Mossel et al., 1995).

Several studies have been revealed to the antimicrobial properties of sage (Ahmed and Abd El-Rahman, 2002; Mitić-Ćulafić et al., 2005, Oral et al., 2008; Celikel and Kavas, 2008; Pokorny et al., 2008; Abdel-Hamied et al., 2009), but researchers did not pay their attention on the antimicrobial properties of sage on improving the microbial quality of minced beef mixed with soybean protein. Therefore, the objective of this study was to evaluate the efficacy of sage extract for improving the microbial quality and prolonging the shelf-life of minced beef mixed with soybean protein when stored at 4℃.

MATERIAL AND METHODS

Preparation of sage extracts

The dried herbs, *S. officinalis*, obtained from local market at Ismailia city, Egypt were individually ground by an electrical grinder to pass through 60 mesh sieve. About 5 g of ground herb was shaken for 30 min. with 50 ml of 96% ethanol at room temperature (22℃ ± 2). The mixture was allowed to stand for 24 h. Then it was filtered to obtain alcoholic particle free sage extract.

Experimental design

Firstly, fresh beef was purchased from a local market at Ismailia city, Egypt on the day of preparation. Then, meat was cut and minced with a grinder through a 4 mm plate diameter. Minced beef was mixed with 30% of their weight by soybean protein (Cargill, Food grade) previously soaked in potable water for at least 4 h. The samples were divided into four groups; each group included 25 samples, each one weighs 200 g. The four groups were thoroughly mixed in sterile mixer with 0% (control group), 0.1, 0.3 and 0.5 sage extracts eventually. Each sample was wrapped with saran wrap and placed in a chiller at 4℃ ± 1. During chilling at intervals of 0, 1, 3, 5 and 7 days, five samples were removed for microbiological evaluation.

Samples preparation

Minced beef samples of 25 g were removed aseptically and homogenized in 225 ml of 0.1% sterile peptone water for 2 min using a Stomacher 400 Lab Blender to provide dilution of 10^{-1}. From the original homogenate, 1 ml was transferred aseptically to a test tube containing 9 ml sterile 1% peptone water (*w/v*) to prepare dilution of 10^{-2}, then from which further ten fold decimal serial dilution up to 10^{-8} were prepared. From these serial dilutions, the bacteriological investigations were performed.

Microbiological evaluation

Appropriate diluents of each tube were placed on the following media in duplicate; plate count agar (Difco Co., Ltd.) for total aerobic count; reinforced clostridial medium (Oxoid, CM 0149) for total anaerobic count; violet red bile glucose agar (Oxoid, CM 485) for total enterobacteriaceae counts; malt extract agar (Oxoid, CM 0059) for total yeast and mould count; mannitol egg yolk polymixin agar (Oxoid, CM 0929) for total *B. cereus* count and Baird-Parker agar (Oxoid, CM 275) with egg yolk-tellurite emulsion (Oxoid, SR 54) for total *S. aureus* count.

Statistical analysis

Experimental trials were repeated twice. The statistical analysis for this study was performed using SAS for Windows (SAS, 1998). Data were analyzed for the significant differences due to samples treatment with different levels of sage extract and stored for different time periods. Data were subjected to one-way analysis of variance (ANOVA). Any significant differences ($P < 0.05$) were analyzed by the multiple comparisons procedure of LSD (least significant difference), using a level of significance of alpha = 0.05.

RESULTS AND DISCUSSION

Results are presented in Table 1. Generally, addition of different levels of sage extract decreased the initial microbial count (0 day) partially and slowed down the growth during the storage period parallel to the increasing sage concentrations. The microbiological quality of meat products as purchased by the consumer is relied on a number of factors, such as the quality of the raw materials, other materials used or added during processing operations to the products as extraneous contaminants, sanitation during processing and packaging. At concentration of 0.3 and 0.5%, sage significantly reduced the mean aerobic counts in the samples. The bactericidal effects of 0.5% sage extract in laboratory media was determined on various microorganisms, which commonly encountered in food industry (Grzybowski et al., 1990, Ahmed and Abd El-Rahamn, 2002 and Abdel-Hamied et al., 2009).

EOS (2005) set a standard maximum limit of 10^6 cfu/g for total aerobic count in minced beef mixed with soybean protein with maximum 7 days of storage at 4℃. Based on this standard, control groups and 2nd group (0.1% sage) did not comply with the Egyptian standards criteria for that product. The most important effect of 0.3 and 0.5% sage extract on treated minced beef is a significant improvement in its shelf-life up to 7 days of storage at 4℃.

Table 1. Effect of different concentrations of sage extract on microbial quality (counts in log_{10} cfu/g) of minced beef stored at 4°C.

Sage extract (%)	Sampling time (days)				
	0	1	3	5	7
			Aerobes		
0	4.29^{a*}	4.77^{a}	5.64^{a}	6.74^{a}	6.78^{a}
0.1	2.40^{a}	2.44^{b}	3.90^{b}	5.11^{b}	6.12^{b}
0.3	1.96^{b}	1.40^{bc}	2.20^{c}	3.20^{c}	3.87^{c}
0.5	1.64^{c}	0.55^{d}	1.57^{d}	2.04^{d}	3.58^{cd}
			Anaerobes		
0	1.39^{a*}	1.71^{a}	1.98^{a}	2.19^{a}	3.15^{a}
0.1	1.18^{ab}	1.22^{b}	1.90^{ab}	2.10^{ab}	2.79^{b}
0.3	0.80^{c}	0.97^{c}	1.18^{c}	1.27^{c}	1.50^{c}
0.5	0.34^{d}	ND	ND	0.35^{c}	0.98^{d}
			Enterobacteriaceae		
0	2.37^{a*}	2.51^{a}	2.92^{a}	3.99^{a}	4.95^{a}
0.1	1.11^{b}	1.12^{b}	1.90^{b}	2.81^{b}	3.35^{b}
0.3	0.60^{c}	0.67^{c}	1.15^{c}	1.96^{c}	2.50^{c}
0.5	0.32^{d}	0.20^{d}	0.87^{d}	1.85^{cd}	2.56^{cd}
			Yeast and mould		
0	3.01^{a*}	3.51^{a}	3.52^{a}	3.60^{a}	3.67^{a}
0.1	2.52^{b}	2.47^{b}	2.20^{b}	2.74^{b}	3.02^{b}
0.3	1.84^{c}	1.20^{c}	2.11^{bc}	2.50^{bc}	2.59^{c}
0.5	1.42^{d}	0.50^{d}	0.99^{d}	1.11^{d}	1.20^{d}
			B. cereus		
0	2.24^{a*}	2.67^{a}	2.84^{a}	3.66^{a}	4.68^{a}
0.1	1.85^{b}	ND	0.76^{b}	1.90^{b}	2.78^{b}
0.3	1.00^{c}	ND	ND	ND	ND
0.5	0.57^{d}	ND	ND	ND	ND
			S. aureus		
0	0.84^{a}	1.21^{a}	1.84^{a}	2.14^{a}	2.63^{a}
0.1	0.57^{b}	0.32^{b}	1.24^{b}	1.75^{b}	2.41^{b}
0.3	0.21^{c}	0.13^{c}	ND	ND	ND
0.5	0.20^{d}	ND	ND	ND	ND

Means in the same column (a, b, c, d) and in the same row (p, q, r, s) with different letters for each microbial group differ significantly ($P < 0.05$).
ND = not detected.

As sage extract concentration increased, the total anaerobic bacteria counts decreased. This reduction was even faster in samples containing 0.3 and 0.5% sage extract; no growth of anaerobic bacteria was observed in the analysis after 1 and 3 days of storage for samples treated with 0.5% sage extract. Generally, the significantly lower mean anaerobic counts obtained for treated samples reflect the inhibitory effect of the sage extract beside the effect of chilling temperature on the growth of anaerobic bacteria.

Enterobacteriaceae family includes facultative anaerobic gram negative straight bacilli, motile employ peritrichous flagella. Most members of this family are mainly mesophilic while some strains can grow at 0°C (Downes and Ito, 2001). Enterobacteriaceae family include some of food-borne genera as *Escherichia*, *Salmonella*, *Klebsiella*, *Serratia*, *Enterobacter*, *Citrobacter*, *Yersinia*, *Proteus*, *Providencia*, *Shigella* and *Erwinia* that pose a great health risks for consumers (Holt et al., 1994).

The results confirmed the significant (P < 0.05) inhibittory effect of different concentrations of sage extract with combined of chill temperature on the growth of enterobacteriaceae compared to control sample. 0.3 and 0.5% sage extract were more effective in lowering the enterobacteriaceae counts compared to 0.1% sage extract concentration.

Yeast and mould contribute a definite part of the natural microflora of meat. Their counts are used as an index of storability and sanitary quality of the products. The total yeast and mould counts of all sage treated groups were significantly lower than that of the control samples. Fungicidal activity of sage was reported by Farag et al. (1989) and Schmitz et al. (1993).

The presence of yeast and mould in meat products are objectionable, as they grow at a wide range of temperature and pH values, resulting in spoilage of the product (Pitt and Hocking, 1997). Such yeast and mould might cause gas and off flavor in chilled food on account of their proteolytic activity, rancidity of cold-stored meat products spoiled by yeast is mainly the cause of lipolytic activity by yeast (Viljoen and Greyling, 1995). Meanwhile some pathogenic yeast found in meat products, makes these products unsuitable for human consumption (Wolter et al., 2000).

Addition of 0.3 and 0.5% of sage extract to minced beef were effective in inhibiting the growth of *B. cereus*. During slaughtering process, meat is exposed to various sources of *B. cereus* contamination. Most gram positive bacteria were susceptible to the sage extract, but showed considerable variation in their extent of inhibition.

Most of *Bacillus* species kill at the 0.5% sage concentration, whereas, the minimum inhibitory concentrations of sage extract were 1.25 - 2.5 μL/mL for *S. aureus* and 0.15 - 2.5 μL/mL for *B. subtilis* (Grzybowski et al., 1990; Liu and Nakano, 1996; Mitić-Ćulafić et al., 2005). Sage at concentration 0.2% did not inhibit the growth of the majority of bacteria (Hammer et al., 1999).

Staphylococcus species are gram positive nonmotile, nonspore forming, facultative anaerobic cocci occurring in pairs or irregular clusters. The initial counts of *S. aureus* for control group were higher than treated groups. The inhibitory effect of sage extract against *S. aureus* was also reported (Liu and Nakano, 1996 and Mitić-Ćulafić et al., 2005).

The most important food-borne genera of staphylococci are *S. aureus* coagulase positive. The growth of *S. aureus* in foods is a potential public safety hazard since many of its strains produce enterotoxins that cause food poisoning when ingested. *S. aureus* can be isolated from meat grinders, knives, saw blades and cutting boards or tables (Downes and Ito, 2001).

Conclusion

Microbiological control in minced beef has been identified as an important factor in improving quality, extending the shelf-life of the product and protecting consumers from the hazards of food-borne illness. The results indicate that the minced beef mixed with soybean when stored at 4℃, are still contaminated with high levels of microorganisms. Sage extract were found to be a significant effective in improving microbiological quality and prolonging the shelf-life of the minced beef to day seven of retail display.

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