EFFECT OF ONION (*ALLIUM CEPA* L.) EXTRACT ON MICROBIOLOGICAL QUALITY OF REFRIGERATED BEEF MEAT

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ABSTRACT

*The effect of onion (*Allium cepa *L.) extract on the fresh beef fillet meat was investigated. Beef fillet samples were cut into pieces and treated with 5, 10, 20 and 50 % onion-water extract (v/v) and stored in refrigeration conditions at 4C. Microbiological quality of the samples was investigated during storage for (0, 1, 3, 6 and 9) 9 days. Increasing concentrations of onion extract significantly affected* Escherichia coli *and yeast-mould counts ($P < 0.05$, $P < 0.01$);* Pseudomonas spp., *aerobic mesophilic bacteria and total coliforms were not affected significantly ($P > 0.05$) for some concentrations and days. The count of bacteria of the samples reached and exceeded the spoilage limit after 9 days at 4C. High concentrations of onion extract were effective in protecting beef meat, depending on the reduction of some microbial contaminations.*

PRACTICAL APPLICATIONS

The effects of onion extracts on beef meat microbial spoilage were evaluated. Microbial spoilage of meat is an important factor influencing consumer health directly as well as influencing the cost and food availability. Antimicrobial activity of onion juice in different researches was previously reported. In this study, onion extract reduces some microbial contamination of beef fillet meat by inhibiting *Escherichia coli* and yeast-mould counts during 9 days at refrigeration conditions. Onion extract can find applications especially with service preservation of meat meals, with the aid of refrigeration, which is also prepared with onion flavor (döner kebap, meat balls, cig köfte, etc.).

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INTRODUCTION

Fresh meat is a highly perishable product because of its biological and biochemical composition. The shelf life of precooked sliced beef fillet is limited by the lack of barriers to microbial growth and the development of off-flavors. The quality of precooked beef fillet is highly variable and microbiological safety is very critical for it (Sachindra *et al.* 1998; Yashoda *et al.* 2000). Great emphasis is being placed on the microbiological quality of meat as well as searching for alternative mechanisms to reduce natural and cross contamination, thus avoiding major public health problems. Although the destruction of pathogenic microorganisms is of primary importance, the prevention of growth of meat spoilage organisms is also important to maintain the initial quality of meat during storage (Delaquis *et al.* 1999).

Onion is usually consumed as fresh, in powder or as essential oil forms, and its properties were well documented not only for flavoring but also for antibacterial and antifungal activities against a variety of gram-negative and gram-positive bacteria. Onion juice and extracts have also been shown to have antimicrobial activity in different studies *in vitro* models. The results indicate that high concentrations of onion juice-water has a slowing-down effect on microbiological spoilage of beef meat and on prevention of pathogenic microorganisms (Mau *et al.* 2001; Benkeblia 2004; Souza *et al.* 2005; Irkin and Korukluoglu 2007; Martinez-Corzo *et al.* 2007; Wilson and Demming-Adams 2007).

It was reported that fresh onion extracts have a strong antimicrobial effect due to both methyl-cysteine sulfoxide and S-n-propyl cysteine sulfoxide, from which the corresponding thio-sulfinates are formed enzymatically. Also, the flavonoids in onions exhibit various antimicrobial activities (Griffiths *et al.* 2002; Taran *et al.* 2006). In the preparation of some traditional meat dishes (in meat balls, ground beef) in some countries such as Turkey, onion is the most popular seasoning agent, thus, waiting for the beef meat in the onion extract process can give storage stability by inhibiting spoilage microorganisms as well as provide good sensory quality (Kilic 2003; Jang and Lee 2005).

The low temperature in common meat distribution systems adds barriers to microbial growth and natural preservatives could improve the stability and safety of precooked meats. The improving quality of refrigerated beef meat with onion (*Allium cepa* L., 5, 10, 20, 50% [v/v]) was tested in this study. Beef meat was obtained from a slaughterhouse and stored under refrigeration conditions evaluated at selected time intervals (0, 1, 3, 6 and 9 storage days). The effect of the onion juice on the microbial contaminants of the beef (aerobic mesophilic bacteria, *Pseudomonas* spp., total coliforms, *Escherichia coli* and mould-yeasts) was evaluated.

MATERIALS AND METHODS

Meat

The naturally contaminated beef fillet was obtained from a meat-processing plant (Dört Mevsim Meat Industry Company in Susurluk, Balikesir, Turkey), 24 h after slaughter (stored at 4C). The beef fillet meat was trimmed of external fat and cut into 50-g (approximately $4 \times 3 \times 1.5$ cm dimensions) pieces aseptically. A total of 102 (34 [for 1 trial] ×3) beef fillet portions (5,100 g) were obtained for microbiological evaluation.

Onion Extracts and Treatments

Onion extract was prepared based on the modified method by Singh and Shukla (1984). The onion bulbs (*Allium cepa* L.) were purchased from a local market in Balikesir and it was peeled, washed in sterile water, dried and weighed. Onions were homogenized in a sterile blender (BKK 2159, Hotmix Blender, BEKO, Istanbul, Turkey) containing sterile distilled water to make 1:1 (w/v, 50%) aqueous extract. The aqueous extract (50%), contains 4.85% dry matter, was calculated according to AOAC (1990). The aqueous homogenate was filtered by using Whatman filter paper (No. 1) and then filtered from a membrane filter (Cole-Parmer–47 mm, 45 μm pore size) by the aid of nitrogen gas for sterilization. This sterilized extract was diluted with sterile distilled water to obtain 5, 10 and 20% (v/v) diluted extracts, and they were used immediately. Sliced beef fillets were dipped in the sterile glass jars and filled at a ratio of 1:1 (meat : onion homogenate) for each concentration (5, 10, 20 and 50%) of onion extracts separately and closed aseptically. Beef fillets immersed in sterile distilled water were the control group. All groups were stored under refrigeration conditions (+4C) during the 9-day period. Three trials were analyzed for each group.

Microbiological Analysis

The samples for analysis were taken from the control (Dort Mevsim Company, Susurluk, Balikesir) and treated groups on 0, 1, 3, 6 and 9 days of storage. For microbiological analysis, a 10-g meat sample was added to 90 mL of sterile saline peptone water (0.1% peptone and 0.85% NaCl) and homogenized in a stomacher (Stomacher 80, Seward Medical, London, U.K.) for 2 min at low speed at room temperature. Serial decimal dilutions were prepared from each treated groups: total aerobic mesophilic bacteria were determined using Plate Count Agar (Merck, Darmstadt, Germany) at 35C for 48 h; *Pseudomonas* spp. were grown on (Glutamate Starch Phenol Red Agar [GSP], Merck) GSP agar at 28C for 72 h; total coliforms and *E. coli* were grown on

Violet Red Bile Agar (Merck) at 35C for 48 h; and the total yeast and moulds were enumerated on potato dextrose agar (Merck) at 28C 2–5 days (Halkman 2005). Afterwards, incubation plates having 30–300 colony forming unit (cfu) were counted and multiplied by the dilution factor to determine cfu/g of meat.

Statistical Analyses

Results were converted to logarithms and statistically analyzed using the two-way analysis of variance, SPSS 12.0 program, and differences between the groups were determined by Duncan test (Ozdamar 2004).

RESULTS AND DISCUSSION

The evolution of the microbiological counts of the beef fillet stored in onion extract in the refrigerator is presented in Tables 1, 2 and 3. As shown in

TABLE 1.
THE COUNTS OF AEROBIC MESOPHILIC BACTERIA, TOTAL COLIFORMS IN BEEF MEAT TREATED WITH VARIOUS CONCENTRATIONS OF ONION EXTRACTS AT REFRIGERATION CONDITION

Aerobic mesophilic bacteria						
Days	Control	5%	10%	20%	50%	*P*
0	$4.67 \pm 0.04_{B}$	Nd	Nd	Nd	Nd	
1	$4.56 \pm 0.23_{B}$	$5.35 \pm 0.19_{B}$	$4.6 \pm 0.045_{B}$	$4.48 \pm 0.12_{B}$	4.42 ± 0.14	–
3	$5.43 \pm 0.08_{B}$	$5.53 \pm 0.065_{B}$	$5.48 \pm 0.16_{AB}$	$4.85 \pm 0.17_{B}$	4.78 ± 0.06	–
6	$5.85 \pm 0.46_{B}$	$6.61 \pm 0.13_{AB}$	$5.6 \pm 0.026_{AB}$	$6.49 \pm 0.19_{A}$	5.24 ± 0.59	–
9	$8.33 \pm 0.43_{A}$	$7.79 \pm 0.17_{A}$	$7.72 \pm 0.97_{A}$	$7.51 \pm 0.2_{A}$	6.77 ± 0.097	–
P	**	*	*	*	–	
Total coliforms						
Days	Control	5%	10%	20%	50%	*P*
0	$3.43 \pm 0.10_{B}$	Nd	Nd	Nd	Nd	
1	$4.50 \pm 0.03_{AB}$	4.39 ± 0.06	4.33 ± 0.06	4.21 ± 0.06	4.02 ± 0.07	–
3	$4.66 \pm 0.04_{AB}$	4.43 ± 0.102	4.42 ± 0.07	4.42 ± 0.02	4.14 ± 0.06	–
6	$5.57 \pm 0.01_{A}$	5.19 ± 0.1	5.16 ± 0.05	5.09 ± 0.06	5.12 ± 0.08	–
9	$5.62 \pm 0.04_{A}$	5.46 ± 0.1	5.4 ± 0.045	5.26 ± 0.04	5.26 ± 0.03	–
P	*	–	–	–	–	

Mean values within the same row with different capital letters are different.
Mean values within the same column with different superscript small letters are different; * $P < 0.05$, ** $P < 0.01$, $-P > 0.05$.
Nd, means not determined.

TABLE 2.
THE COUNTS OF *ESCHERICHIA COLI* AND *PSEUDOMONAS* SPP. IN BEEF MEAT TREATED WITH VARIOUS CONCENTRATIONS OF ONION EXTRACTS AT REFRIGERATION CONDITION

Escherichia coli

Days	Control	5%	10%	20%	50%	*P*
0	2.22 ± 0.08	Nd	Nd	Nd	Nd	
1	2.27 ± 0.08^{a}	$1.88 \pm 0.06^{ab}{}_{B}$	$2.03 \pm 0.13^{ab}{}_{AB}$	$1.32 \pm 0^{b}{}_{B}$	$1.16 \pm 0.27^{b}{}_{B}$	*
3	2.34 ± 0.04^{b}	$3.04 \pm 0.054^{a}{}_{A}$	$1.36 \pm 0.08^{b}{}_{B}$	$2.14 \pm 0.06^{b}{}_{A}$	$1.71 \pm 0.34^{b}{}_{AB}$	*
6	2.38 ± 0^{ab}	$3.15 \pm 0.12^{a}{}_{A}$	$1.86 \pm 0.4^{b}{}_{AB}$	$2.34 \pm 0.03^{ab}{}_{A}$	$2.18 \pm 0^{b}{}_{A}$	*
9	3.38 ± 0.05^{a}	$3.38 \pm 0.054^{a}{}_{A}$	$2.55 \pm 0.16^{b}{}_{A}$	$2.38 \pm 0^{b}{}_{A}$	$2.32 \pm 0^{b}{}_{A}$	*
P	–	*	*	*	*	

Pseudomonas spp.

Days	Control	5%	10%	20%	50%	*P*
0	$3.20 \pm 0.02_{B}$	Nd	Nd	Nd	Nd	
1	$4.23 \pm 0.05_{B}$	$4.20 \pm 0.08_{B}$	4.81 ± 0.05	$3.18 \pm 0.06_{B}$	4.18 ± 0.61	–
3	$4.83 \pm 0.06_{AB}$	$4.7 \pm 0.04_{AB}$	4.86 ± 0.03	$4.34 \pm 0.03_{AB}$	4.24 ± 0.1	–
6	$5.71 \pm 0.02^{a}{}_{A}$	$5.63 \pm 0.05^{a}{}_{A}$	5.92 ± 0.04^{a}	$4.57 \pm 0.04^{b}{}_{AB}$	4.56 ± 0.18^{b}	*
9	$6.6 \pm 0.1^{a}{}_{A}$	$5.89 \pm 0.06^{a}{}_{A}$	5.34 ± 0.18^{bb}	$5.22 \pm 0.19^{bb}{}_{A}$	4.78 ± 0.14^{b}	*
P	**	*	–	*	–	

Mean values within the same row with different capital letters are different.
Mean values within the same column with different superscript small letters are different; * $P < 0.05$, ** $P < 0.01$, $-P > 0.05$.
Nd, means not determined.

TABLE 3.
THE COUNTS OF TOTAL MOULDS AND YEASTS IN BEEF MEAT TREATED WITH VARIOUS CONCENTRATIONS OF ONION EXTRACTS AT REFRIGERATION CONDITION

Days	Control	5%	10%	20%	50%	*P*
0	$3.2 \pm 0.12_{C}$	Nd	Nd	Nd	Nd	
1	$3.63 \pm 0.03_{C}$	$3.73 \pm 0.05_{B}$	$3.68 \pm 0.02_{B}$	$3.43 \pm 0.07_{B}$	$3.33 \pm 0.19_{B}$	–
3	$4.44 \pm 0.06_{C}$	$5.3 \pm 0.14_{A}$	$4.33 \pm 0.04_{B}$	$4.44 \pm 0.12_{AB}$	$4.27 \pm 0.19_{AB}$	–
6	$5.62 \pm 0.08^{ab}{}_{B}$	$6.18_{A} \pm 0.54^{a}{}_{A}$	$5.76 \pm 0.03^{ab}{}_{AB}$	$5.76 \pm 0.17^{ab}{}_{A}$	$4.44 \pm 0.07^{b}{}_{AB}$	*
9	$7.47 \pm 0.14^{a}{}_{A}$	$6.30 \pm 0.12^{ab}{}_{A}$	$5.72 \pm 0.08^{b}{}_{A}$	$5.13 \pm 0.06^{b}{}_{A}$	$5.34 \pm 0.07^{b}{}_{A}$	*
P	**	**	*	*	*	

Mean values within the same row with different capital letters are different.
Mean values within the same column with different superscript small letters are different; * $P < 0.05$, ** $P < 0.01$, $-P > 0.05$.
Nd, means not determined.

Tables 1, 2 and 3, onion extract significantly affected *E. coli*, yeast and mould counts ($P < 0.05$, $P < 0.01$) of stored beef fillet but also affected other microorganism groups in some concentrations and in some days.

The initial mesophilic aerobic bacteria counts of control samples were 4.67 log cfu/g but increased up to 8.33 log cfu/g at the end of the storage. The increasing counts of mesophilic aerobic bacteria in the 50% concentration storage had lower concentrations than the others and reached to only 6.77 log cfu/g. Except for the 50% concentration, differences between the storage days were found significant ($P < 0.01$, $P < 0.05$) for other concentration treatments. After the ninth day of storage at 4C, the meat samples that were not exposed to onion extract (control group) and samples treated with 5% onion extract show characteristics of unacceptable meat properties with putrid odor and viscous appearance. Onion extract showed inhibition of aerobic mesophilic bacteria, which was stated in some previous studies (Griffiths *et al.* 2002; Ghahfarokhi- Sharm *et al.* 2006). Researches on the flavonoid content of onions (*Allium cepa* L.) have indicated that the main flavonoids quercetin, quercetin-4′-glucoside, quercetin-3,4′-diglucoside, quercetin-7,4′-diglucoside and isohamnetin glycoside have strong antimicrobial effects against microorganisms (Fossen *et al.* 1998).

The presence of coliform bacteria in meat is necessary to control. That is, coliforms showed poor sanitation and hygienic conditions. In general, coliform counts decreased in the refrigeration storage. In our conditions, the initial count of coliforms 3.43 log cfu/g is a high value compared with 2 log cfu/g that is typical for fresh meat processed in good hygiene standards (Table 1). Hinton *et al.* (1998) found the coliform bacteria in minced beef meat in the range of 1.39–2.79 log cfu/g. The effects of concentrations and storage time on coliforms were not significant ($P > 0.05$). Only differences in control group were found significant ($P < 0.05$) at day 0. Goktan and Tuncel (1988) stated that counts of coliform did not increase and they kept their numbers more or less constant during the refrigeration. The number reached 5.62 log cfu/g after 9 days in control groups.

The initial counts of *E. coli* in untreated samples were found to be 2.22 log cfu/g (Table 2). The effects of concentrations and storage time on *E. coli* were found to be important significantly ($P < 0.05$). Differences resulted from the first day for 5, 20 and 50% concentrations and on the third day for 10% treatment. The *E. coli* number reached log 3.38 log/cfu for control groups. In some researches, it was reported that fresh onion juice has inhibitory effects against *E. coli* (Kivanc and Kunduhoglu 1997; Kyung and Lee 2001).

In meat spoilage, *Pseudomonas* spp. are important microorganisms and they represent hygienic characteristics of beef carcasses. On the sixth and ninth days, *Pseudomonas* spp. counts between the concentration treatments

were found to be important ($P < 0.05$) in research. Except for 10 and 50%, differences for other treatment groups were important significantly ($P < 0.01$ and $P < 0.05$). In this study, *Pseudomonas* spp. number of control samples was 3.2 log cfu/g and then reached to 6.6 log cfu/g after the ninth day (Table 1). Sun and Ockerman (2004) reported 4 log cfu/g *Pseudomonas* spp. for beef meat.

Yeast and mould counts show insufficient hygienic conditions and some contaminations from environments during production. In this study, yeast and mold counts in meat samples were 3.2 log cfu/g (Table 3). On the first and third days, differences between the numbers were not found significant ($P > 0.05$), but for the sixth and ninth days, differences were found important ($P < 0.05$). Yeast-mould count differences for all concentrations were found significantly important ($P < 0.01$ and $P < 0.05$). Ghahfarokhi-Sharm *et al.* (2006), Satya *et al.* (2005) and Yin and Tsao (1999) found strong inhibition activities of onion extract against some yeast-moulds in their studies.

Fresh meat is a good medium for microbial growth, which leads to accelerated spoilage if not stored properly. Caring hygienic conditions during process of beef meat cause a drop of microbial load much effectively. During the slaughtering and processing, meat may be contaminated with some pathogenic and spoilage microorganisms which causes rapid spoilage, loss of valuable protein and can also affect human health. Therefore, it is very important to reduce some initial microbial load aside from this, can also be used as decontaminants of meat. Onion and onion extracts are used widely all over the world for preparation of meat meals for seasoning and/or aromatic purposes; aside from this property, onion can be used preserving of fresh beef meat.

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