Generation of the Desirable Aroma, the Conditioned Raw Beef Aroma, Induced by Storage of Meat in Air

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BACKGROUND

The taste of beef was reported not to be improved as remarkably as that of pork or chicken during conditioning because of the less increase in amino acids and peptides in beef than in other meats[1]. If this result is true, the changes in aroma rather than in taste seems to be much responsible for the improvement in flavor of beef during conditioning. Thus far, the aroma of cooked beef was reported to become stronger during conditioning[2], however only disappearance of a lactic acid-like odor and a blood-like odor in raw beef during conditioning has been known[3].

OBJECTIVES

The aim of this work is to reveal the changes in aroma of raw beef during postmortem storage under various conditions and the factors inducing these changes.

MATERIALS AND METHODS

Holstein cows or steers aged 20-76 months were slaughtered. The loins obtained from the dressed carcasses stored at 0-4°C for 2 or 4 days postmortem were used as beef samples. The treatments with antibacterial agents were performed by spraying beef with a 2000 ppm chloramphenicol or a 10 mM NaN_3 solution. Trained panelists sniffed various samples at room temperature to evaluate the aroma. Minced lean beef was homogenized with 2 vol. of water, and then the homogenate was centrifuged at 9,000 x *g* for 10 min. The obtained supernatant was filtered successively with gauze, filter paper and Sterifil™ -DGV (sterile vaccum filtration apparatus, 0.22μm pore size) to prepare sterile lean extract (L). The surface of the subcutaneous adipose tissue was burned and removed to prepare sterile adipose tissue (A). Minced adipose tissue was heated with the same volume of water at 100°C for 20min and then filtered with gauze. The fat at the upper layer was collected and autoclaved at 121°C for 15min, and then cooled to prepare sterile fat (F). About 5g of minced beef was mixed with 20ml of L and shaked at room temperature for 30min. The obtained beef suspension was diluted ten times with sterile saline solution (0.9%) and 0.1ml of the diluted suspension was spreaded on the standard medium (0.25% yeast extract / 0.5% peptone / 0.1% glucose / 1.5% agar, pH7.1). After incubation at 25°C for 48hr, 37, 15 and 37 colonies were isolated from the suspensions of beef H, I and J, respectively. LA or LF was inoculated with the various isolated bacteria. LA was 5ml of L floating about 1cm^3 A. LF was 5ml of L floating about 1cm^3 F. After incubation at 4°C for 11 - 12 days, the odors of these samples were evaluated. Identification of the isolated bacteria was perfomed according to the following criteria and tests described by Dainty *et al*[4] about bacteria proliferating on beef: gram stain, catalase production, cell morphology, motility, growth on the STAA medium (500μg/ml streptomycin / 50μg/ml thallous acetate / 50μg/ml actidione / 0.2% peptone / 1.5% glycerol / 0.1% K_2HPO_4 / 0.1% $MgSO_4 \cdot 7H_2O$ / 1.3% agar, pH7.0). Further identification was done according to the established criteria[5].

RESULTS AND DISCUSSIONS

The beef loins were divided into two portions and each portion was put into a polyethylene bag. One portion was stored at 0°C for 20days (0°C-stored beef), and the other was stored at –80°C for 18days, being followed by thawing at 4°C for 2days (frozen beef). Aroma evaluation of both raw samples with a paired preference test showed that the 0°C-stored beef was significantly preferable to the frozen beef (Table 1). On the quality of aroma, the frozen beef had a blood-like odor, and the 0°C-stored beef presented a sweet and milk-like aroma. Then, panelists ate samples heated at 200°C, and acessed preference of taste (with holding their noses) or taste and aroma (without holding their noses). As a result, there was no difference of taste between both samples, but the aroma and taste of the 0°C-stored beef was preferable to those of the frozen beef. Even after heating, the above-mentioned sweet aroma remained in the 0°C-stored beef. Therefore, one of the reason for the superior flavor of the 0°C-stored beef after heating was presumed to be the existence of this aroma. We propose to call this aroma the conditioned raw beef aroma (CRBA).

Seven panelists compared the aroma of the raw beef stored in 0°C-air for 25days with that stored in 0°C-vacuum, recognizing stronger CRBA in 0°C-air than in 0°C-vacuum. After the beef containing both leans and fats, leans alone or fats alone were stored in 0 °C-air for 24days, 6 panelists estimated the raw beef aroma. As a result, CRBA was produced most intensively in the beef containing both leans and fats. The in-air storage of beef sprayed with a chloramphenicol or a NaN_3 solution resulted in the depression of the generation of CRBA as shown in Table 2. Above-mentioned results suggested that some kinds of bacteria produced CRBA at the site containing both leans and fats in the presence of oxygen.

Thus, CRBA-producing bacteria were determined as follows. Thirty-seven, 15 and 37 isolates obtained from three individual beef H, I and J, respectively, were divided into several groups. Several isolates belonging to each group were mixed and inoculated into LA, and then incubated. Aroma evaluation after incubation showed that H(24-27) (the mixture of H24, H25, H26 and H27, which were isolates from beef H), I(5-8) and J(13-18) produced CRBA. Aroma evaluatin of LA or L incubated with each isolates contained in these mixtures were shown in Table 3. The LA inoculated with H25 possessed weak but apparent CRBA. H25 was streaked on the standard medium and incubated at 25°C for 48hr, resulting in the formation of white and yellow colonies, which appear clearly different each other. Picking up each colony and subsequent streak culture repeated twice separated a white colony (H25W) from an yellow colony (H25Y). The incubation of LF or L inoculated with each isolate at 4°C for 11days demonstrated that CRBA was generated only from the LF inoculated with H25W (Table 4).

The features of H25W were examined according to the criteria for grouping roughly bacteria proliferating on beef described by Dainty *et al*[4]. H25W was gram-positive and catalase-positive, and occurred as unbranched rods in short chains. It was nonmotile and grew on the STAA medium. These results suggested that H25W was *Brochothrix thermosphacta*. In addition to the features, H25W was facultatively anaerobic, Voges-Proskauer-positive and methyl red-positive and oxidase-negative. It did not utilize exogeneous

citrate and did not produce H_2S, indole and gas from glucose. It grew within the range of 4-30°C. These features and abilities of acid production from various sugars almost agreed with those of *Brochothrix thermosphacta* described by Sneath and Jones[5]. Furthermore, the LF was inoculated with *Brochothrix thermosphacta* ATCC11509 and incubated at 4°C for 11days, resulting in the generation of CRBA. From above-mentioned results, H25W producing CRBA was presumed to be *Brochothrix thermosphacta*.

CONCLUSIONS

The in-air storage of beef was found to generate the preferable raw beef aroma. The aroma was suggested to be produced by some kinds of bacteria at the site containing both leans and fats in the presence of oxygen. As a result of screening such bacteria, one of those was presumed to be *Brochothrix thermosphacta.*

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Table 1. Aroma of raw beef loins after storage at 0° or –80°C

Numbers of samples judged to be preferable		
0°C-stored beef †	Frozen beef ¶	Difference
22	2	***

Significant difference was indicated with *** ($P < 0.001$).
Aroma evaluation was perfomed by 8 panelists.
†: stored in 0°C-air for 20days after 4days postmortem.
¶: stored at –80°C for 18days after 4days postmortem and then thawed at 4°C for 2days.

Table 2. Effects of antibacterial agent treatments on the generation of CRBA of beef loins stored in air at 0°C for 24 days

Antibacterial agent	Strength of CRBA	Numbers of judgements
Chloramphenicol	Untreated > Treated	11
	Untreated = Treated	1
	Untreated < Treated	0
NaN3	Untreated > Treated	11
	Untreated = Treated	1
	Untreated < Treated	0

Aroma evaluation was performed by 6 panelists.
One panelist judged 2 pairs of samples.

Table 3. Odors of LA and L which were inoculated with isolates from beef and incubated at 4°C for 12days

Experiment[a]	Isolate	LA		L	
		Strength of CRBA[b]	Accompanying odors	Strength of CRBA[b]	Accompanying odors
I	Control	−	no odor	−	no odor, blood-like
	H24	±	body odor, fish meal-like	−	stale, fish meal-like
	H25	+	fish meal-like	−	dusty, fish meal-like
	H26	−	yohgurt-like, body odor	−	no odor
	H27	−	yohgurt-like	−	no odor, fish meal-like
II	Control	−	no odor, rancid	−	stale, dusty
	I5	−	no odor, rancid	−	stale, dusty
	I6	−	no odor, alcohol-like	−	no odor, dusty
	I7	−	acid odor, yohgurt-like	−	no odor, dusty
	I8	−	yohgurt-like, stale	−	stale, dusty, sweaty
III	Control	−	rancid	−	dusty, stale
	J13	−	putrid	−	putrid
	J14	−	yohgurt-like, body odor	−	stale
	J15	±	caramel-like	−	body odor, dusty
	J16	±	body odor, acid odor	−	dusty, stale
	J17	±	alcohol-like	−	dusty, stale
	J18	±	body odor	−	dusty, stale

a: Three, 4 and 5 panelists discussed to judge the strength of CRBA in experiments I, II and III, respectively.
b: Indication of the strength of CRBA: −, absent; ±, slight; +, weak; ++, medium; +++, strong.

Table 4. Odors of LF and L which were inoculated with H25W or H25Y and incubated at 4°C for 11 days

Isolate	LF		L	
	Strength of CRBA[a]	Accompanying odors	Strength of CRBA[a]	Accompanying odors
Control	−	no odor, fishy	−	no odor, stale
H25W	+	body odor	−	no odor, stale
H25Y	−	no odor	−	no odor, stale

Four panelists discussed to judge the strength of CRBA.
a: Indication of the strength of CRBA: −, absent; ±, slight; +, weak; ++, medium; +++, strong.