IDENTIFICATION OF HANWOO (KOREAN NATIVE CATTLE) BEEF BY REAL-TIME PCR IN SOUTH KOREA

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Introduction

Korean consumers prefer beef from Korean native cattle (Hanwoo) to imported dairy cattle because they believe that juiciness and flavour of Hanwoo is better. Therefore, Hanwoo beef has been regarded as the most expensive and high quality beef in South Korea (Kim *et al.*, 2002). Real time-polymerase chain reaction (RT-PCR) is currently considered as the most sensitive method to detect low abundant DNAs in samples. Compared to conventional PCR, real-time PCR has a high reliability because of excluding false-positive results and can allow a simultaneous faster detection and quantification of target DNAs (Madani *et al.*, 2005). This study was carried out to identify the Hanwoo (Korean native cattle) beef by genotyping after DNA extraction of 295 commercial beef samples. Since Hanwoo, Holstein and imported cattle meat have different patterns in the MC1R gene associated with the coat colours of cattle (C-type, C/T-type or T-type), we could identify the genotype using real-time PCR (Kim *et al.*, 2002).

Materials and Methods

Beef samples were purchased from butcher shops in South Korea, designated 1 to 295 (Seoul; 1 to 100, *Jung-Bu* area; 101 to 170, *Young-Nam* area; 171-210, *Ho-Nam* area; 211-245, *Chung-Cheong* area; 246 to 295). Samples (10mg of tissue) were added to a 1.5ml tube containing 400 µl Lysis A buffer solution and 40µl Lysis B buffer solution with 5µl Proteinase K solution added to the lysate. The solution was then incubated at 65°C for 30 minutes, mixed with 300 µl Chloroform, and vortexed for 1 minute. After vortexing it was centrifuged at 12,000 rpm for 10 minutes and 300µl supernatant was poured into a clean 1.5ml micro tube containing 300µl DNA binding buffer and 300µl Isopropanol. Then it was centrifuged at 10,000 rpm for 1 minute and passed through the DNA binding column. 650µl 75% Ethanol was added into a DNA binding column, centrifuged at 10,000rpm for 1 minute and the Ethanol was removed. It was then centrifuged at 10,000 rpm for 3 minutes to dry. 100µl distilled water or TE buffer was added to the column tube and the sample stored at room temperature over 5 minutes, and centrifuged at 10,000 rpm for 3 minutes. The DNA extraction stored at -20°C.

The MC1R gene was amplified by PCR with a GeneAmp PCR System 9600(Perkin-Elmer, Cetus, USA). The PCR reaction contained 10µl of final solution consisting of: 10~100ng of template DNA, 2µl of primer mixture, 1µl of probe mixture, 5µl of 2X master mixture. The thermocycle program was as follows: 50°C for 3 minutes (initial denaturation), 94°C for 5 minutes; 40 cycles of 94°C for 30 seconds (denaturation), 60°C for 30 seconds (annealing) and a final extension step at 72°C for 30 seconds (extension). Real-time PCR reactions performed with the Real-Time PCR 7700 (ABI Prism™ 7700 Sequence Detector, Applied Biosystem, Singapole), PTC-200 (Peltier thermal cycler, MJ RESEARCH, USA) for SNP analysis (Figure 1).

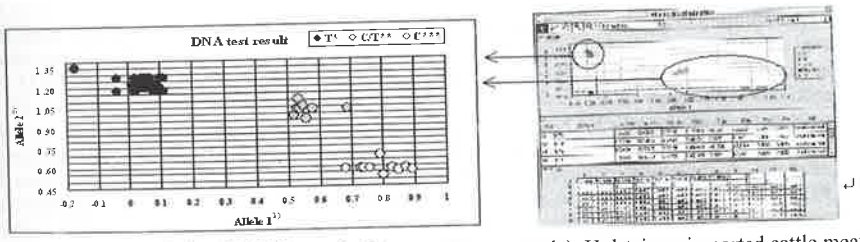

Figure 1: Real-time PCR analysis of MC1R gene in Hanwoo (Korean cattle), Holstein or imported cattle meat.
* T : MC1R gene of Hanwoo (Korean cattle) meat.
** C/T : MC1R gene possessed of Hanwoo (Korean cattle), Holstein and imported cattle meat.
*** C : MC1R of Holstein or imported cattle meat.
1) Control samples of Holstein and imported cattle meat.
2) Control samples of Hanwoo (Korean cattle) meat.

Results and Discussion

MC1R genotyping may be used to reveal Hanwoo (Korean cattle) meats that are purebred.

The result of real-time PCR assay for the proportions of Hanwoo beef were 84%, 85.7%, 95%, 91.4%, and 90% in Seoul, *Jung-Bu*, *Young-Nam*, *Ho-Nam* and *Chung-Cheong* area, respectively. Thus, beef samples in 295 butcher shops which are asserted to sell Hanwoo beef only, showed that 259 of 295 samples were Hanwoo beef gene type (T-type) and 36 of 295 samples were Holstein or imported dairy cattle gene type (C-type or C/T-type). In conclusion, the proportion of Hanwoo beef was 87.8% and the proportion of Holstein or imported dairy cattle meat was 12.2% (C-type; 9.8%, C/T-type; 2.4%).

Generally, most consumers could not differentiate imported meat from Hanwoo beef. Therefore, it will be necessary to make it mandatory for identification of Hanwoo beef and imported dairy cattle meat using MC1R genotyping based on Real-time PCR in butcher shops.

Conclusions

This study was carried out to identify the Hanwoo (Korean native cattle) beef by genotyping after DNA extraction of commercial beef of 295 samples. The proportion of Hanwoo beef was 87.8% and the proportion of Holstein or imported cattle meat was 12.2%.

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