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Availability of human homologous dietary microRNAs in cooked beef (#415)

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Introduction

Nutritional values of beef are usually associated to its major components such as fat, protein, vitamin and mineral content. However, the emerging role of food-derived compounds on nutritional epigenetics has revealed the importance of studying microRNAs (miRs) availability in foods. MiRs are a group of small non-coding RNA molecules that are able to control the expression of protein-coding genes in mammals during normal development and in response to disease. Once ingested, miRs have the potential to elicit biological effects via canonical binding to human messenger RNA targets leading to biological responses that may affect body homeostasis. In our study we investigated the survival of miRs during aging and after cooking to estimate their dietary availability.

Methods

Samples were collected at the University of Nevada, Reno USDA-inspected commercial harvest and processing facility (Wolf Pack Meats). Part of the m. *longissimus coli* was excised from two (n=2) angus cross bred steers 15 min *post-mortem*. While a small aliquot of tissue weighing approximately 1 gram was immediately flash-frozen in liquid nitrogen (-196°C) after the muscle was excised (day 0), the remaining sample was divided in three and aged for 7 (one subsample) and 14 days (two subsamples). After aged, tissues were excised from subsamples aged for 7 days (day 7) and 14 days (day 14). The third subsample was sous vide cooked for 1 hour at 71°C prior to tissue excision (14 CK). Tissues obtained from all subsamples were also flash-frozen until analysis could be made. Total RNA was extracted and isolated from samples via Triazol extraction and quantified via spectrophotometry on the NanoDrop 1000 (Thermo Scientific, USA). RNA was reverse transcribed to cDNA using mi Script II RT Kit (Quiagen, Germany) in a thermocycler for 60 minutes at 37°C, 5 minutes at 95°C, and 4°C. The expression of miRNAs (miR 17-5, 19a, 19b, 20a, 23a, 24, 206) was evaluated by real-time PCR (qPCR) for samples aged for 0, 7, 14 days, and for cooked samples aged for 14 days.

Results

Expression of miRs are presented in Figure 1. For miR 17-5 and 20a expression decreased about 26% and 19% 14 days post mortem. After cooked, a total of 32% and 39% of each miR was still present in beef. For miR 19a and 19b, a two-fold post mortem overexpression was observed 14 days post mortem. Survival amounts of both miRs after cooking were similar (40%). For MIRs 23a, 24, and 206, post mortem overexpression was 70%, 27% and 46% whereas survival amounts after cooking were 40%, 37%, and 56% respectively.

Conclusion

All homologous miRs evaluated in this study remained present in cooked tissue from 32 to 56% of the total previously identified on day 0. It was known that miRs are relatively stable and resistant when compared to RNAs. Low expression of miR 17-5 and 20a was possibly observed due to the decrease of cell activity post mortem. Overexpression of miRs 19 was possibly associated to apoptosis mechanisms and muscle tenderization. The miR 19a is a biomarker for calpastatin regulation regulating the CAST gene whereas miR 19b regulates the PDCD6 gene, associated to programmed cell death. The miR 23a participates in the regulation of muscle proteins mainly myosin (MYH1 and MYH2 genes) and programmed cell death (PDCD4 gene), as well as the miR 206 that regulates the PDCD10 gene. Independently to what post mortem mechanisms miRs are involved, they remain available after cooking. Further research will be necessary to evaluate their nutritional effects

Notes

Figure 1. MicroRNA expression [2 -ΔΔCt (GOI expression)] in beef (m. *longissimus coli*) aged for 0, 7, and 14 days (0, 7, and 14) and beef cooked after aged for 14 days (14 CK).

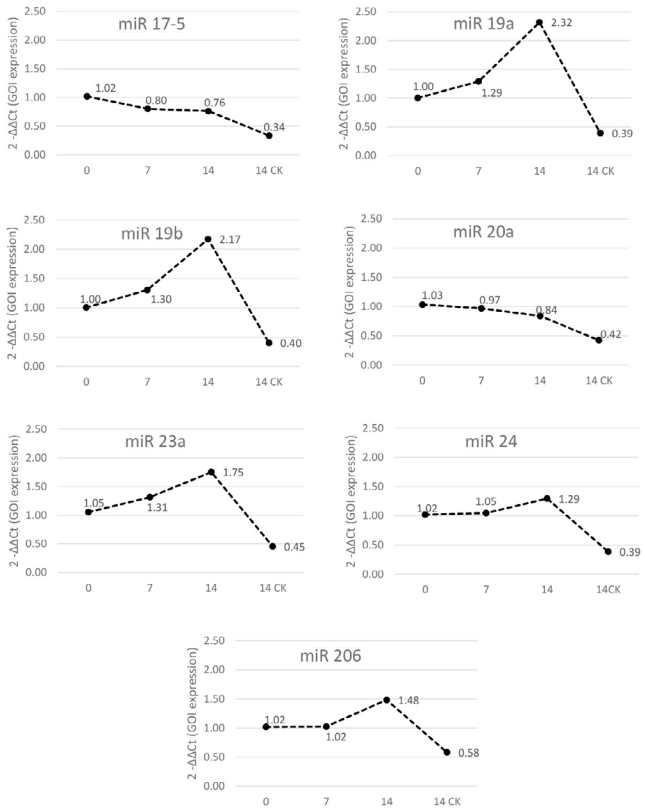

Figure 1

Notes