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# Influence of Degree of Doneness on the Alpha-Gal Content of Striploins and its Relationship with Red Meat Allergy

*S.R. Hene, J.R. Kress, J.T. Looper, E.S. Beyer, T.G. O'Quinn, and M.D. Chao*

## Abstract

Alpha-Gal Syndrome (AGS) is an acquired sensitivity to galactose-alpha-1, 3-galactose (α-Gal) after exposure to a bite from the Lone Star Tick (*Amblyomma americanum*). Affected individuals can experience a range of symptoms from mild itching to potentially fatal anaphylaxis after consuming products containing α-Gal from mammalian tissues. However, little research has been done to examine the α-Gal content of different products nor how it relates to the severity of a reaction. Thus, the objective of this study was to establish the α-Gal content of striploin steaks cooked to varying degrees of doneness to evaluate if heat treatment reduces the α-Gal content of red meat. Ten beef striploins were collected, cut into steaks, and cooked to either medium rare (MR; 130°F), medium (MED; 140°F), or well done (WD; 160°F). Whole muscle proteins from each sample were extracted and measured for α-Gal content. Notably, α-Gal content increased as the degree of doneness increased, with WD steaks having the highest concentration and raw steaks having the lowest α-Gal concentration ($P < 0.01$). The results of this study indicated that heating is not a viable method to decrease the α-Gal content of red meat products.

## Introduction

Alpha-Gal Syndrome (AGS), commonly known as red meat allergy or mammalian meat allergy, is an immunoglobulin mediated hypersensitivity to galactose-alpha-1, 3-galactose (α-Gal), an oligosaccharide found in all mammalian tissues except humans and Old World monkeys. Following a tick bite, the body produces a specific α-Gal IgE antibody and binds to the α-Gal epitope presented on glycoproteins and glycoproteins of the mammalian product, subsequently eliciting an allergic response. Patients that have been sensitized to α-Gal can experience a wide spectrum of responses from mild symptoms like itching and gastrointestinal discomfort to a severe and potentially fatal anaphylactic reaction. In many ways, AGS differs from typical food allergies in many ways, and as a result, the variation from patient to patient makes the diagnosis and management of AGS more difficult.

Despite the implication of red meat to AGS, there is no research that investigated quantifying the amount of α-Gal in different meat products and the impact of different processing methods on α-Gal content in these products. Knowing the baseline α-Gal content in various products is essential for proper diagnosis and the construction of

appropriate management plans for patients. Thus, our objective was to determine the α-Gal content of striploin steaks cooked to varying degrees of doneness to understand the effect of heating as a potential intervention to reduce the α-Gal antigen in beef.

## Experimental Procedures

Ten beef striploins were collected from a Midwest beef processing plant and transported under refrigeration to the Kansas State University Meat Laboratory (n = 10). Striploins were cut into four steaks each and either left raw or cooked to medium rare (MR; 130°F), medium (MED; 140°F), or well done (WD; 160°F). Whole muscle proteins were extracted, and protein concentration was adjusted. Proteins were separated by gel electrophoresis, transferred to a polyvinylidene difluoride membrane, and tested by immunoblot against a primary anti-α-Gal antibody. Each gel contained a reference sample of α-Gal conjugated human serum albumin (HSA) with a known α-Gal content of 59.2 pmol. The concentration of α-Gal was determined as a ratio of the lane densities of the sample and the HSA reference multiplied by the known α-Gal content of the HSA reference. The α-Gal concentration is expressed as pmol/μg of protein of the striploin.

## Results and Discussion

A representative image of the western blot is depicted in Figure 1. Striploins that were cooked to WD had the greatest α-Gal concentration of 16.32 pmol/μg ($P < 0.01$; Figure 2). Steaks cooked to MR and MED did not differ from each other and had the second greatest α-Gal concentration of 13.86 pmol/μg and 13.57 pmol/μg, respectively ($P < 0.01$). Finally, steaks that were left raw had the lowest α-Gal concentration at 10.30 pmol/μg ($P < 0.01$).

Our results are consistent with previous research, which also found that cooking pork and beef meat extracts by roasting or boiling increased the binding of anti-α-Gal antibodies as compared to raw samples. During the cooking process, proteins undergo considerable modification that leads to tissue shrinkage and hardening. Muscle fiber shrinkage during cooking results in the loss of water and water-soluble proteins in the form of purge, further concentrating the α-Gal content in WD steaks on a per protein basis. Furthermore, the linearization of proteins during denaturation from heating may expose more α-Gal epitopes, allowing for more efficient binding of IgE antibody and may elicit a greater immune response.

## Implications

The increase in α-Gal concentration in beef after cooking suggested another unique characteristic of AGS as compared to other traditional food protein allergies—heat stability of the α-Gal epitope. While there are other potential methods of α-Gal epitope modifications that should be explored, cooking beef to a higher degree of doneness does not seem to be a viable solution to make meat consumption safe for AGS patients. Further research is needed to evaluate the efficacy of other interventions to improve the care and management of AGS patients.

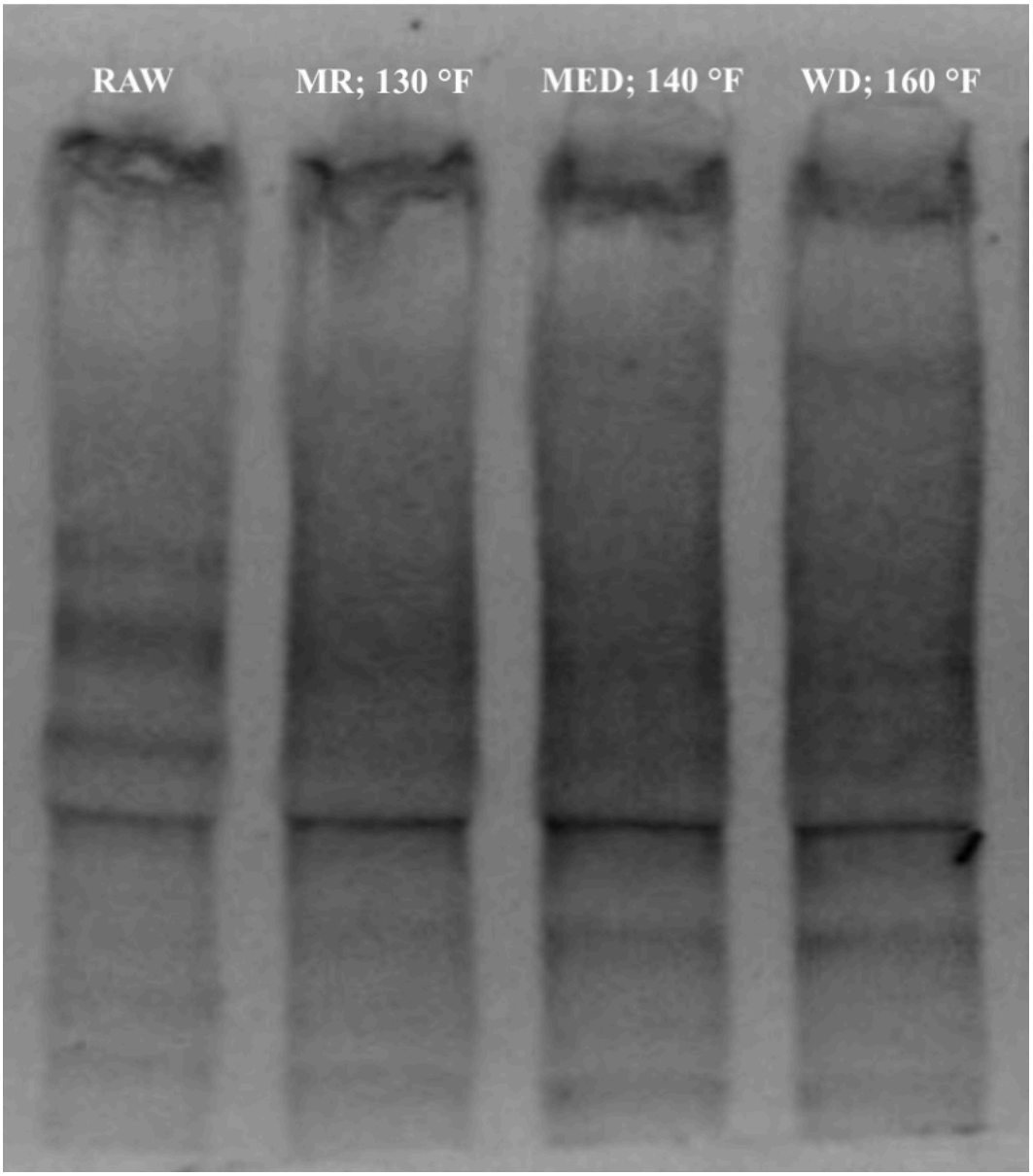


**Figure 1. Representative western blot of α-Gal content of striploins steaks cooked to varying degrees of doneness.**

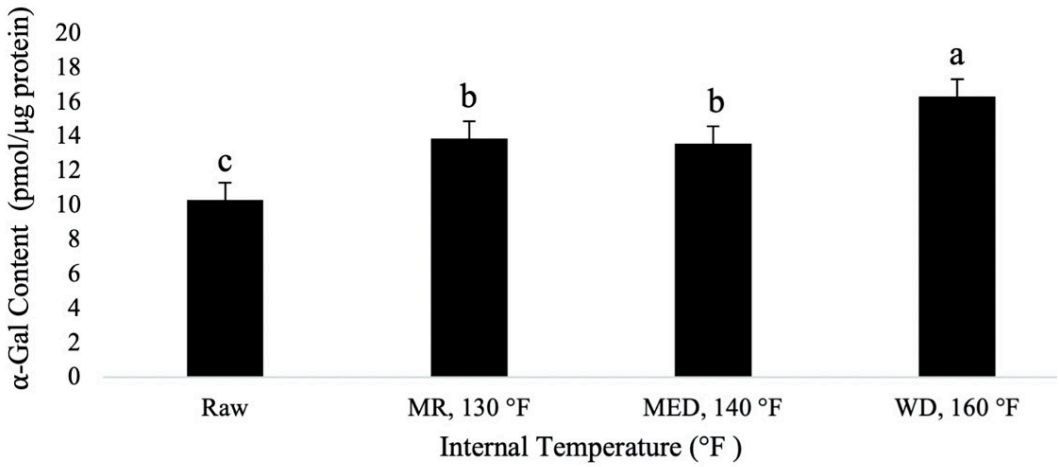


**Figure 2. α-Gal content of striploins at varying degrees of doneness.**