Alteration of Porcine Serum Albumin Levels in Pork Meat by Marination in Kiwi or Pineapple Juice and Subsequent Pan Broiling

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Abstract

This study was conducted to evaluate the changes in porcine serum albumin (PSA), a major allergen, which occur when raw pork ham is marinated with kiwi or pineapple juice, and/or when the ham is pan broiled at 300°C for 4 min after marination. In this study, raw pork ham was soaked for 4 h or 8 h in marinades containing commercial marinating sauce only, commercial marinating sauce and 7% kiwi juice, or commercial marinating sauce and 7% pineapple juice. When the meat was marinated and then pan-broiled, pork ham meat protein was significantly denatured and hydrolyzed, and the level of PSA in the meat was significantly reduced. The PSA contents of pork broiled without marination, pork that had been marinated in commercial marinating sauce alone, pork that had been marinated in commercial marinating sauce with kiwi juice, and pork that had been marinated in commercial marinating sauce with pineapple juice, were 95.4, 43.3, 14.3, and 5.4 ng/mL, respectively (p<0.05). Marinating with pineapple juice was more effective than marinating with kiwi juice; and marination for 8 h was more effective than marinating for 4 h. These results indicate that the level of PSA in pork ham is effectively reduced, when the meat is first marinated in sauces that contain kiwi or pineapple extracts for 8 h, rather than 4 h, and then cooked. Further study is needed to determine whether marinated pork meat reduces allergenicity in vivo, as well.

Key words: porcine serum albumin, marinating, allergen, sandwich ELISA, pork ham

Introduction

The term food allergy is used to describe adverse immune responses to foods (Sicherer and Sampson, 2010). Food allergy rates vary by age, local diet, and various other factors. Food-induced allergic reactions are responsible for a variety of symptoms and disorders involving the skin and gastrointestinal and respiratory tracts and can be attributed to IgE-mediated, non-IgE-mediated, and mixed-type mechanisms. Recently, scientists have reported IgG antibody in food allergy patients and Fc receptor of IgG4 antibody on the surface of mast cell (Chen et al., 2008; Lee et al., 1993). Since then scientists also focus on IgG4 antibody to explain food allergy which is non-IgE mediated (Cho et al., 2001; Lee et al., 1993). Food allergens have a number of common characteristics: they are water-soluble glycoproteins, 10 to 70 kDa in size, and relatively resistant to acid, heat, and proteases (Sicherer and Sampson, 2010).

Pork is the most popular meat in Korea, and the level of pork meat consumption has increased in recent years. Pork meat consumption per capita in 2005 was 17.8 kg and rose to 19.2 kg in 2012 (Korea Meat Trade Association, 2014). However, a survey of urban preschool children revealed that pork was one of the ten foods most often associated with allergy, as the incidence of pork allergy among the children was 1.9% (Chung et al., 2001). Although the incidence seems low, the consequence is severe and results in anaphylaxis shock. Moreover, pork has been classified as a major food allergen (Kim et al., 2011) and, as per Article 10 of the Korea Food Sanitation Act, must be indicated as such on labels for food products.

The most well-known allergens from meat are serum albumin (66 kDa), gamma-globulin (60 kDa), and actin...
(42 kDa), as well as several additional proteins of various sizes (14, 18, 20, 45, and more than 60 kDa). These allergens are found in not only beef but also pork and lamb and can cause cross-reactivity among foods (Fiocchi et al., 2000). Purified porcine serum albumin (PSA) or extracts from unprocessed meats that contain PSA have been used not only to evaluate allergenic potential but also to study the feasibility of reducing allergenicity by means of heat processing (Kim et al., 2011).

Current management of food allergies consists of educating the patient to avoid ingesting the responsible allergen and to initiate therapy (e.g., by injecting epinephrine to treat anaphylaxis) in case of unintended ingestion (Sicherer and Sampson, 2010). However, heating is one of the most efficient methods to reduce allergenicity. Fisher (1982) reported the case of a patient who presented with anaphylactic symptoms after ingesting rare-cooked beef but who was able to tolerate well-cooked beef. Although treatment of meat with digestive enzymes has been reported to reduce the allergenicity of serum albumin from different species (Kim et al., 2011), there has been no specific study examining the effects of fruit enzymes and pan broiling at 300°C on altering the allergen PSA. Therefore, the aim of this study was to use SDS-PAGE, immunoblotting, and sandwich ELISA to determine the effect of pan broiling and/or marinating with kiwi or pineapple juice on the level of PSA in pork meat.

Materials and Methods

Marination and pan broiling

Raw pork ham, commercial marinating sauce, kiwis, and pineapples were purchased from a local market. Raw pork ham was soaked for 4 h or 8 h in a marinade containing 27% commercial sauce only, commercial sauce with 10% kiwi juice, or a commercial sauce with 10% pineapple juice (Table 1). Then, it was either analyzed immediately or subjected to pan broiling on a hot plate for 4 min at 300°C prior to analysis (Fig. 1). In addition, pork that was pan broiled without prior marination was also examined.

Porcine serum albumin (PSA) extraction

PSA was extracted from the pork using the method described in Wang et al. (2002), with a slight modification. Twenty grams of meat was added to 40 mL of 0.01 M phosphate-buffered saline (PBS, pH 7.3). Next, the meat was homogenized at 10,000 rpm for 30-60 sec and then centrifuged at 16,000 g for 30 min. The supernatant was filtered using Whatman No. 1 filter paper and then used for further experiments.

Commercial PSA and antibodies

Commercial PSA and a horseradish-peroxidase (HRP)-conjugated rabbit anti-goat-IgG antibody were purchased from Sigma Chemical Co. (USA). A goat anti-PSA IgG antibody was obtained from Bethyl Laboratories Inc. (USA).

SDS-PAGE

Proteins from pork meat extracts were separated according to their molecular weights on 12% separating gels and 5% stacking gels. Prior to loading, the samples were analyzed for protein content using a BCA protein assay kit (B9643, Sigma). Coomassie Brilliant Blue R 250 (Sigma) was used for visualization (Laemmli, 1970). Electrophoresis was performed using a miniPAGE (page Run, AE-6531; ATTO, Japan) system at 100 V for 3 h. The sizes of the protein bands were identified using a standard Prosi prestained protein marker (GenDEPOT, USA).

Immunoblotting assay

After SDS-PAGE, proteins were transferred to methanol-activated polyvinylidene difluoride (PVDF) membranes and then to immobilization paper (Shleicher & Schuell, USA) for 30 min at 200 mA using an electrophoresis power supply (Northeastern Science Company, USA) and a Mini Trans-Blot Transfer Cell (Bio-Rad Laboratories). The PVDF membranes were blocked with 3% gelatin in TBS-T (20 mM Tris, 137 mM NaCl, pH 7.6, with 0.1% Table 1. Manufacturing compositions of pork meat marinated with commercial sauce alone or with kiwi or pineapple juice

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<th>CON</th>
<th>RPM</th>
<th>RPMK</th>
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<tr>
<td>Raw pork ham</td>
<td>100</td>
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<td>Commercial marinating sauce</td>
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<td>27</td>
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<tr>
<td>Kiwi juice</td>
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<td>10</td>
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<td>Pineapple juice</td>
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<td>10</td>
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<tr>
<td>Total</td>
<td>100</td>
<td>127</td>
<td>137</td>
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CON, without marinating; RPM, raw pork ham marinated with commercial sauce; RPMK, raw pork ham marinated with commercial sauce and kiwi juice; RPMP, raw pork ham marinated with commercial sauce and pineapple juice.