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Inhibition of advanced glycation endproducts in cooked beef patties by cereal bran addition

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ABSTRACT

Advanced glycation endproducts (AGEs) are formed in cooked meat products via Maillard reactions, which are seen as a contributor to chronic diseases such as diabetes and heart diseases. A number of reports have shown that natural antioxidants such as phenolic acids in grains, herbs, and spices can inhibit their formation. The objective of the study was to determine the inhibitory effects of selected wheat (Jagger, JA), triticale (Spring Triticale, ST; Thundercale, TH), and Rye (RY) bran on AGEs levels in cooked beef patties, as measured by N^{ε}-carboxymethyl lysine (CML) contents. The CML was detected in all the cooked samples, whereas the patties to which RY (42.0% inhibition), ST (27.5% inhibition), and TH (21.4% inhibition) brans were added significantly decreased CML formation. RY and ST, were more abundant in total phenolics content (TPC) and exhibit higher properties as free radical scavengers. Using Pearson's correlation and multiple linear regression analysis, the inhibition of CML in patties was correlated to the water-holding activity (WHC) of the samples, and the radical scavenging activity of the brans as measured by the 2,2-diphenylpicrylhydrazyl (DPPH) assay. These results suggest that addition of bran may be a potential method of decreasing the formation of AGE in cooked patties.

1. Introduction

Advanced glycation endproducts (AGEs) are formed from the Maillard reaction, which is a non-enzymatic reaction of proteins with reducing sugars (Ahmed, 2005). Well known AGEs in foods include N^{ε}-carboxymethyl lysine (CML), methylglyoxal (MGO), and pentosidine. The CML in food has been studied extensively and used as a marker since it is a relatively abundant AGE in food systems (Wu, Huang, Lin, & Yen, 2011). Formation and accumulation of AGEs are suspected to be involved in the pathogenesis of advanced aging and several diseases such as diabetes (Poulsen et al., 2013). The pathogenic effect of AGEs might modify the chemical and biological properties of molecules, as their binding capacity to cellular receptors in a wide range of tissues resulting in functional changes of DNA, proteins, and lipids (Ahmed, 2005). In cell culture studies, AGEs were found to induce cellular oxidative stress and cell activation, and excess consumption of dietary AGEs was also considered to increase inflammation and oxidative stress in some epidemiological studies (Goldberg et al., 2004; Uribarri et al., 2007). Moreover, a series of animal studies found that consumption of AGE-rich diets by mice was associated with kidney disorders and damage (Hofmann et al., 2002). These findings suggest that dietary AGEs may be considered a chronic risk factor for human health. It therefore is desirable to acquire information on the prevalence of dietary AGEs in food.

In view of previous research reports, limiting the formation of AGEs in foods depends on many factors, such as decreasing cooking temperature, cooking time, and increasing of water-holding capacity (WHC) of meat samples (Chen & Smith, 2015; Goldberg et al., 2004; Persson, Sjöholm, & Skog, 2003). However, a potential treatment may involve use of various natural antioxidants, which may scavenge the generated free radicals accompanied with the formation of AGEs though the Maillard reaction. Some reports have indicated that the phenolic extracts from spice (Ahmad & Ahmed, 2006), microalgae (Sun et al., 2010), buckwheat products (Szawara-Nowak, Koutsidis, Wiczkowski, & Zielinski, 2014), and wheat bran (Wang, Sun, Cao, & Tian, 2009) can inhibit AGE formation.

Adding cereal bran to meat products, which contains significant amounts of dietary fiber and antioxidants, may have a positive effect on health promotion (Liu, 2007; Reddy et al., 2000). Cereal is a

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good dietary source of natural antioxidants such as phenolic acids, which are concentrated more in the bran portion and include ferulic, vanillic, *p*-coumaric, and syringic acids (Mattila, Pihlava, & Hellstrom, 2005). Recently, the ability of certain phenolic acids such as vanillic acid, ferulic acid and *p*-coumaric has been shown to inhibit AGEs formation *in vitro* (Wu et al., 2011; Zhang, Tao, Wang, Chen, & Wang, 2015). Huang, Chuang, Wu, and Yen (2008) reported vanillic acid can scavenge reactive carbonyl species involved in the glycation process, thus decreasing AGE formation. During cooking, water is important for the transport of water-soluble precursors of Maillard reaction products (MRPs) from the center to the surface of meat (Persson et al., 2003). Some previous research has shown that additives, such as salt and phosphate having high WHC, can decrease the formation of some MRPs in meat systems (Persson, Sjöholm, & Skog, 2002). Thus, it is possible that the bran addition has an inhibitory effect on the formation of AGEs in meat products due to its WHC.

Although cereal bran has been investigated for their antioxidant activity, there is no information regarding their inhibitory effect of on the formation of AGEs in cooked meats. This study was conducted to determine the effects of selected wheat, triticale, and rye brans on AGEs levels in cooked beef patties as measured by CML contents. This study was also performed to determine if AGE inhibition is correlated with total phenolic content (TPC), antioxidant activity, or the WHC of samples.

2. Materials and methods

2.1. Materials

Fresh ground beef (10% fat) was purchased from a local supermarket. Cereal seed samples (wheat, rye, and triticale) were provided by a certified seed grower Vance Ehmke (Dighton, KS, USA). The samples included one wheat variety (Jagger 2010), two triticale varieties (Spring Triticale 2011, & Thundercale 2011), and one rye variety (Rye 2009). The N^{ε}-carboxymethyl lysine (CML) standard was purchased from NeoMPS (Strasbourg, France). High purity standards of phenolic acids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), boric acid, hydrochloric acid, Folin–Ciocalteu reagent, 2-mercaptoethanol, sodium chloride, sodium borohydride, anhydrous magnesium sulfate, and sodium tetraborate decahydrate were purchased from Sigma Aldrich (St. Louis, MO, USA). In addition, solvent and chemicals such as acetonitrile (HPLC grade), chloroform (HPLC grade), methanol (HPLC grade), *ortho*-phthalaldehyde (OPA) reagent, ACS grade acetone, petroleum ether, hexanes, sodium carbonate, sodium hydroxide, ethanol, ethyl acetate, ethyl ether, and methanol were obtained from Fisher Scientific (Fairlawn, NJ, USA).

2.2. Bran sample preparation

Before the milling of the cereal seed samples, moisture content was tested with a Single Kernel Characterization System (SKCS) (Perten Instruments, Hägersten, Sweden). The moisture content was adjusted to 15%, and the samples were equilibrated in the glass bottles for 24 h at room temperature. A Quadrumat Junior mill system (Brabender, Duisburg, Germany) was used to mill the tempered seeds. The bran fractions were collected and sieved with a Ro-Tap sieve shaker (W.S. Tyler, Mentor, Ohio) over a 0.4 mm particle size screen for 3 min. The samples were collected, flushed with nitrogen, stored in glass bottles, and refrigerated at 4 °C.

2.3. Beef patty sample preparation

The selected wheat, triticale, and rye bran was added and homogenized with 100 g of fresh ground beef at a level of 5%, a concentration that does not cause noticeable change in flavors (Talukder & Sharma, 2010). Control samples contained no bran. In order to ensure uniformity, a petri dish (10 cm × 1 cm) was utilized to form patties. Each patty was refrigerated overnight at 4 °C, and cooked in a frying pan at a surface temperature of 204 °C (400 °F), with a controller (Bernant, Barrington, USA). The patties were cooked until their internal temperature reached 71 °C (160 °F), which is recommended by the U.S. Department of Agriculture – Food Safety and Inspection Service (USDA-FSIS) (1998). After cooling for 30 min at room temperature, approximately 2 mm of the top of the patty samples was excised by a meat slicer (Cabela grade slicer, 1/3 hp, Sidney, NE, USA), and then ground and homogenized by a processor (KitchenAid, model KFP 750) and refrigerated at 4 °C. The determination of CML contents in the samples was performed the next day. Each sample was analyzed in triplicate.

2.4. Determination of CML

The CML of beef patty samples was extracted according to Drusch, Faist, and Erbersdobler (1999) except that chloroform/methanol (2:1, v/v) solution was used as the defatting solvent (Chen & Smith, 2015). Each sample (0.20 g) was defatted using 20 mL chloroform/methanol (2:1, v/v) solution followed by centrifugation (10,600*g* at 4 °C) for 10 min (Model 21000R Centrifuge, Fisher Scientific, Pittsburgh, PA, USA). Reducing reagent of 4 mL sodium borate buffer (0.2 M, pH 9.4) and 2 mL sodium borohydride (1 M in 0.1 M NaOH) were added to the dried sample for 4 h at room temperature. Subsequently, hydrochloric acid was mixed to a final concentration of 6 M HCl, and the sample was hydrolyzed for 20 h at 110 °C. The final CML extracts were concentrated until dry with a rotary evaporator and dissolved in 10 mL of sodium borate buffer (0.2 M, pH 9.4), followed by a final membrane filtration (nylon, 0.45 mL). The extract (50 μL) was reacted with 200 μL of OPA derivatization reagent for 5 min prior to HPLC analysis.

According to the method of Peng et al. (2010), the CML was analyzed with a HP1090A Series II HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled with a HP 1046A fluorescence detector programmed to excitation/emission wavelengths of 340 nm and 455 nm. A reversed-phase TSK gel ODS-80 TM column (25 cm × 4.6 mm, 5 μm, 80 Å, Tosohass, Montgomeryville, PA, USA) was utilized to separate CML with the mobile phases: (solvent A) acetate buffer (pH 6.7, 20 mM)/acetonitrile (90:10, v/v), and (solvent B) acetonitrile. The flow rate was 1.0 mL/min and the injection volume was 20 μL. The CML separation was achieved with a linear gradient program that started with 5% B and changed to 70% B within 5 min, and kept at 70% B for 17 min. The gradient was set back to 95% B in 1 min followed by a post run of 15 min for equilibration. The identity of CML was confirmed by comparing retention times between samples and standards in the fluorescence spectra, and levels were determined by the peak areas of their corresponding derivatives.

2.5. Determination of WHC of beef patty samples

WHC of samples was determined by the method of Wardlaw, Mccaskil, and Acton (1973). A meat sample of 15 g was mixed with 22.5 mL of 0.6 M sodium chloride solution, and then stirred for 1 min and refrigerated at 4 °C for 15 min. The slurry was stirred again and centrifuged (12,000*g*) for 15 min (Fisher Scientific, Model 21000R Centrifuge, Pittsburgh, PA, USA). The supernatant was decanted and the volume recorded. The amount of solution retained by meat was reported as the WHC in mL per 100 g sample.