Meat Science 124 (2017) 48–53

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Influence of pre- and post-slaughter factors on the reduced glutathione content of beef muscles

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ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 22 August 2016
Accepted 18 October 2016
Available online 19 October 2016

Keywords:
Reduced glutathione
Pre-slaughter
Post-slaughter
Beef muscle

ABSTRACT

The aim of this experiment was to assess the effect of certain factors (muscle anatomy, paternal breed, diet, age at slaughter, castration, process of meat aging and grilling) on the content of reduced glutathione (GSH) in beef. The research material included selected beef muscles acquired from steers and bulls obtained by crossing Polish Holstein-Friesian cows with meat breed bulls (Limousin, Charolais, Hereford). An analysis of ante-mortem factors such as the castration, slaughter age, and fattening of the animals showed no significant effect on the content of GSH ($\alpha = 0{,}05$). On the other hand, the paternal breed of animals was observed to have a significant effect on GSH content. In the study, GSH content significantly increased during meat aging. In contrast, grilling caused a loss approximately 40% of GSH content. Based on the study, it can be concluded that the distribution of GSH in anatomical beef muscles is uneven.

1. Introduction

Reduced glutathione (γ-glutamyl-cysteinyl-glycine) is an important bioactive compound contained in bovine meat. This compound is the main low-molecular sulfur compound which is commonly found in all eukaryotic cells, plants, and animals. It is among the most important antioxidants, along with ascorbate, albumin, cysteine, urinary creatinine, flavonoids, phytic acid, melanin, alpha-tocopherol (vitamin E), beta-carotene, bilirubin, biliverdin, and coenzyme Q (Prior & Cao, 1999). The main place of glutathione synthesis is in the liver, where blood containing GSH is supplied to other tissues. The cells that are capable of taking GSH bear the enzyme γ-glutamyl transpeptidase on their surface. Measurements of the activity of this enzyme show that GSH is concentrated in the kidneys, brain, red and white blood cells, lungs, heart, intestines, and hardworking muscles. This increases protection against oxidative stress (Griffith, Novogrodsky, & Meister, 1979; Halliwell, 1999; Itoh, Ishii, Wakabayashi, & Yamamoto, 1999; Lash & Jones, 1984). Although GSH is present in foods in small amounts and is a small fraction of the overall amount of sulfur containing amino acids, its consumption is associated with measurable increases of its concentration in various tissues (Valencia, Marin, & Hardy, 2001). While the effect of increasing glutathione levels under the influence of diet is short-term, it has a very important role in the proper functioning of the body and the prevention of many diseases (Valencia et al., 2001).

The concentration of GSH in food products is diverse and depends not only on their type, but also on the method of preparation and preservation of the product. Frozen food contains a similar amount of glutathione as fresh food, but other forms of preparation and preservation of food result in a significant or even complete loss of GSH (Valencia et al., 2001). Fats, oils, dairy products, eggs, and most cereals and drinks have a low amount of glutathione. In contrast, fresh vegetables, fruit, and cooked meat are rich in this compound. Furthermore, the amount of GSH in food is also influenced by the presence of GSH precursors, especially cysteine and methionine (Bukowska, 2004).

The content of GSH in meat is determined by many factors, i.e., breed, environmental conditions (including feeding methods), physical activity, age at slaughter, sex, physiological state, castration, and many other factors that are still unidentified (Lawrie, 2006; Pisula & Pośpiech, 2011). The impact of most of these factors on bioactive compounds such as GSH is not fully understood (Driskell et al., 2011; Florek, Litwińczuk, Kędzierska-Matysek, Grodzicki, & Skałecki, 2007; Gerber, Scheeder, & Wenk, 2009; Leheska et al., 2008; Leonhardt & Wenk, 1997; Lombardi-Boccia, Lanzi, & Aguzzi, 2005; Reykdal, Rabieh, Steingrimsdottir, & Gunnlaugsdottir, 2011). In modern literature, there is no data on GSH losses in bovine meat resulting from culinary processes.

The aim of this study was to evaluate the effect of selected factors, such as muscle anatomy, paternal breed, diet, age at slaughter, castration, and the processes of aging and grilling meat, on the content of GSH.

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http://dx.doi.org/10.1016/j.meatsci.2016.10.010
0309-1740/

2. Materials and methods

2.1. Animals, samples, and experimental designs

The animals were grown in the same farm and were fed according to the guidelines for intensive and semi-intensive fattening under strictly controlled conditions. In the intensive and semi-intensive fattening the animals were fed ad libitum with grass silage and the addition of a mixture of concentrate (post-extraction rapeseed meal, middlings triticale and mineral supplement). Participation of concentrate at doses was calculated based on energy density dose recommended in the system of evaluation and nutrition INRA (Dobrowolska, 1993), according to the models provided for animals of beef breeds or animals obtained by crossing. Intake of concentrate depended on the level of fattening animals - animals belted in intensively they received a greater quantity of concentrated compared to animals fed semi-intensively. In the semi-intensive fattening of animals was carried out the slaughter mass of up to 550 kg and to the age of 18 months (average daily gain of animals: 1000 g/day). In the intensive fattening of animals was carried out the slaughter mass of up to 600 kg and to the age of 18 months (average daily gain of animals: 1300 g/day). Composition of the initial mix intended for intensively fed bulls weighing from 250 kg to 450 kg was as follows: 58.5% - triticale, 39% - extracted rapeseed meal, 2.5% - mineral-vitamin premix. In the case of semi-intensively fed animals this mixture consisted of 31.5% triticale, 66% rapeseed extraction meal, 2.5% mineral-vitamin premix. Mixture intended for intensively fed bulls weighing >450 kg consisted of 74.9% triticale, 22.6% rapeseed extraction meal and 2.5% mineral-vitamin premix. In the case of semi-intensively fed animals this mixture consisted of 64.5% triticale, 33.0% rapeseed extracted meal and 2.5% mineral-vitamin premix.

The animals were transported to a local slaughterhouse. After rigor mortis (48 h, 2 ± 1 °C), the muscles were removed from each carcass. The muscles were then subjected to "wet" aging process, through vacuum packing into barrier polyethylene bags and refrigerated storage (2 ± 1 °C) for 7, 14, and 21 days. After each aging period, samples were frozen (−22 ± 1 °C) using Küppersbusch "blast-freezer" and stored at −18 °C until their analysis. The thawing process was conducted at 2 ± 1 °C for 24 h. After the defrosting process, the muscles were removed from their packages and 2.54 cm thick steaks were sliced.

The first experiment concerned the assessment of the distribution and content of GSH in raw beef meat. The second experiment concerned the assessment of the impact of ante-mortem factors such as paternal breed, fattening, castrating and slaughter age of the animals on the content of GSH in raw beef meat, while the third experiment concerned the assessment of the impact of post-mortem factors (aging, grilling) on GSH content in bovine meat.

In order to assess the content of GSH in the muscles in **Experiment 1**, twelve muscles were collected (*semitendinosus*, *pectineus*, *gracilis*, *sartorius*, *psoas major*, *biceps femoris*, *adductor femoris*, *tensor fasciae latae*, *longissimus thoracis*, *gluteus medius*, *semimembranosus*, *longissimus lumborum*) from four carcasses of 18-month-old bulls. The research material was obtained from animals derived by crossing commodity dairy cattle of the Holstein-Frisian breed with bulls of the Limousin meat breed. The animals were fed according to the semi-intensive system (described below).

In **Experiment 2**, the effect of the ante-mortem factors was studied on the example of two muscles after 14 days of aging (*gluteus medius*, *longissimus lumborum*). The culinary elements were obtained from back side of the carcasses. The research material was obtained from eight animals – 18-month-old steers and bulls derived by crossing commodity dairy cattle of the Holstein-Frisian breed with bulls of the Charolais meat breed. The animals were obtained by the same group of breeders, were grown in the same farm and were fed according to the guidelines for intensive and semi-intensive fattening under strictly controlled conditions.

The impact of the aging and grilling processes on GSH content (**Experiment 3**) was studied in six bovine muscles (*semitendinosus*, *psoas major*, *longissimus thoracis*, *gluteus medius*, *semimembranosus*, *longissimus lumborum*) derived from four carcasses of 18-month-old bulls. The research material was obtained from animals derived by crossing commodity dairy cattle of the Holstein-Frisian breed with bulls of the Limousin meat breed. The animals were fed according to the semi-intensive system. The process of aging was performed (after vacuum packing the beef muscles) at 2 ± 1 °C in three periods of time: 7, 14, and 21 days after slaughter. In order to carry out a controlled grilling process, the beef muscles were cut into steaks with a thickness of 2.5 cm after 14 days of aging. The steaks were then heat-treated using an electric grill with a grooved top cover (S-165 K ELEKTROGERÄTE GmbH 59757 Arnsberg, Germany) having a temperature of 190 °C at the top and 210 °C at the bottom. The process was conducted until the temperature at the geometric center of the product reached 70 °C (thermocouple thermometer: NiCr-NiAl, type TP-151 with EMT-50-K CZAKI, THERMO PRODUCT, Poland). Then, the steaks were subjected to a six-minute relaxation process at 60 °C.

2.2. Determination of reduced glutathione content

Glutathione content was measured by the spectrophotometric method using the SIGMA company's test: Glutathione Assay Kit, Catalog Number CS0260 (Akerboom & Sies, 1981). The principle of the method was based on the continuous reduction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) to 5-thio-2-nitrobenzoic acid (TNB), the content of which was proportional to the concentration of GSH in the sample. The yellow product (TNB) was measured spectrophotometrically (Helios Gamma, Thermo Scientific, AnchemComp.,Warsaw, Poland). In order to determine the content of GSH, 300 mg of freeze-dried meat tissue was weighed in a centrifuge tube (10 ml volume), then 3 ml of 2.5% trichloroacetic acid solution was added to the tube. The sample was then centrifuged (8944× *g* for 10 min, temp. 8 °C; centrifuge MPW-380R, Med. Instruments, Warsaw, Poland). The supernatant was filtered by the qualitative filter (medium, Chemland, catalog number 10.000102.110, Stargard, Poland) and 20 ml of the filtrate was collected in a glass cuvette and added to 300 ml of the reaction mixture (a mixture of analytical buffer - 100 mM potassium phosphate buffer, pH 7.0, with 1 mM EDTA - and a solution of DTNB at a concentration of 1.5 mg/ml in a volume ratio of 35:1). The prepared sample was incubated for 5 min at room temperature and then the absorbance was measured at a wavelength of $\lambda = 412$ nm. The resulting indication of GSH for a sample of beef was the arithmetic average of six independent measurements. A six-point calibration curve made for standard of GSH was used for the quantitative calculations. Repeatability, expressed as the relative standard deviation, did not exceed 5.5%.

2.3. Statistical analysis of the results

Statistical analysis of the results was performed using STATISTICA 10 software (**Experiment 2**) and STATGRAPHISC Plus 5.1 software (**Experiments 1 and 3**). To assess the effect of factors such as paternal breed, diet, age at slaughter, and hormonal status on the content of GSH in beef, a t-Student test and factorial analysis of variance was used in a completely random arrangement. To assess the effect of factors such as muscle anatomy and the processes of meat aging and grilling on the content of GSH, a factorial analysis of variance was used in a completely randomized block design to eliminate the impact of an uncontrolled factor—the interindividual variability of the animals. In order to study the differences between groups, a Duncan test ($\alpha = 0.05$) was used.

In order to find the factors most differentiating the content of GSH in bovine meat, Classification and Regression Tree (C&RT) models were used.