Effects of the Addition of Okara Flour on the Proximate and Amino Acid Compositions of Beef Sausage

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Abstract: Okara is the main by-product of soymilk industries with valuable components including soy fiber and soy protein. Okara has high potential market due to their high protein content. Increased in the utilization of plant protein is important to support the production of protein-rich foods that can replace animal protein which is known to be less healthy for human consumption. Hence this study aims to investigate the effects of the addition of okara flour on the proximate and amino acid compositions of beef sausage. A control beef sausage which contains 100% beef and 0% okara was compared with optimized beef sausage with added okara (89.59% beef and 10.41% okara) formulation. The optimized beef sausage with added okara was formulated according to that suggested by design expert software. The addition of okara flour in beef sausage had a significant effect on the proximate composition of the product. Arginine, glycine and serine were present in beef sausage with added okara but absent in the control beef sausage. Threonine, tyrosine and aspartic acid were present in Control Sausage but absent in Okara Sausage.

Keywords: proximate, sausage, okara, amino acid

Introduction

Okara is the residual left from ground soybean after extraction of the water extractable fraction used to produce bean curd (tofu) or soy milk. It is generally white or yellowish in color. It is part of the traditional cuisines of Japan, Korea and China and also been used in the vegetarian cuisines of Western nations. The large usages of soybean lead to the rise of the quantities of okara production in the worldwide as in China about 2 800 000 tons of okara are produced from the tofu production industry every year (Ahnet *et al.*, 2010). A significant disposal problem is made by the yearly huge amount of production of okara. Sausages are consumed worldwide because of their convenience. Okara has high protein content (40% on a dry weight basis) with good essential amino acid profile to be consumed and digestibility (Grizotto *et al.*, 2012; Ma *et al.*, 1997). Approximately one-third of the isoflavones present in the soybean remain in okara, suggesting that it is a good, low cost source of nutrients (Bowles and Demiate, 2006; Jackson *et al.*, 2001). Other components of soy present in okara include lignans, phytosterols, coumestans, saponins, and phytates (Turhan *et al.*, 2007). Despite the high nutritional and excellent functional properties (emulsification, foaming, and binding properties) of okara and its potential for application in food products, the most common use of this by-product is in the manufacture of animal feed (Pinto and Castro, 2008). Okara has been used to partially replace wheat flour, cassava starch, corn flour, and soy flour (Bowles and Demiate, 2006; Grizotto *et al.*, 2012; Aplevicz and Demiate, 2007). Recent studies have focused on applying okara flour in the production of bakery goods (Grizotto *et al.*, 2012; Larosa *et al.*, 2006), breakfast cereals (Santos *et al.*, 2004), tortillas (Waliszewski *et al.*, 2002), and French bread (Bowles and Demiate, 2006). Several studies investigated the usage of okara, including seasonings, spices and tempeh (Wang and Cavins 1989), extruded enriched cereal products (Rinaldi *et al.*, 2000), soy candy (Genta *et al.*, 2002) and tortillas

fortified with okara (Waliszewskiet *al.*, 2002), acid modification (Chan and Ma 1999), hydrolyzation to increase the digestibility with food processing enzymes (Kasai *et al.*, 2004), nitrogen source for the solid-state fermentation (Hsieh and Yang 2004), adding into beef patties (Turhanet *al.*, 2007) and fermented functional foods (Zhu *et al.*, 2010). Okara is also a valuable ingredient that can be used when formulating gluten free bakery products or trying to remove wheat allergens from such products as cookies and nutritional bars. Major weight gain was caused by saturated fatty acids. Meat nowadays is higher in fat because of the animals are raised in unnatural environments with no exercise or grazing capabilities. Saturated fat can cause potentially fatal fat deposits in the arteries. Meat is a very calorie-dense nutrient that can easily lead to weight gain if eaten regularly. This will cause high blood pressure and artery damage. Red meats are unhealthy and contain high amounts of saturated fats that will raise blood cholesterol and increase the risk of some dangerous diseases such as heart disease, diabetes and cancer. In addition, eating red meat has been known to increase the risk of developing colorectal, lung, stomach, pancreatic, esophageal, and endometrial cancers (Saricobanet *al.*, 2009).

Materials and Methods

Raw Materials

Okara paste was obtained from a local company in Gombak, Selangor, Malaysia. The minced beef, potato starch, white pepper, salt, sugar and shortening were purchased from Giant Supermarket Section 7, Shah Alam Selangor, Malaysia. Isolated soy protein (ISP), iced water and sodium tripolyphosphate (STTP) were obtained from the food processing laboratory of Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Shah Alam, Malaysia.

Preparation of Okara Flour

Okara paste was dried at 60°C in cabinet drier until constant weight around 5% moisture content was obtained and then milled using a grinding mill. By using a sieve shaker, the milled flour was passed through 120 mesh aperture size to obtain homogenized sized flour.

Ingredients

The ingredients for okara sausage are beef meat, okara flour, potato starch, white pepper, sodium tripolyphosphate (STTP), isolated soy protein (ISP), salt, sugar, iced water and shortening.

Preparation of Okara Sausage

Firstly, the beef meat was blend for 1 minute. Salt was added and continuously blend for another 1 minute. Then, sugar and STTP were added and the batters were blend for 4 minutes. Other ingredients (potato starch, ISP, white pepper, beef flavor, okara flour and iced water) were added and blend for 4 another minutes. The mixture was transferred into cellulose casing by using stuffer and tied into 3 inch long sausage. The sausages were cooked in a Combi oven at 55°C (20 minutes), 65°C (20 minutes), 75°C (20 minutes) and 80°C (15 minutes) continuously. The sausages were then sprayed with tap water for 5 minutes and then immersed in ice water. Finally, sausage casing were removed and sausages were vacuum packed and stored at -4°C prior to further analysis.

Proximate Composition

Proximate analysis was determined using AOAC (2000).

Acid Hydrolysis

The amino acid composition of the samples was analyzed by digesting the samples for 24 hours at 110°C in an oven with 5 ml 6 N HCl in sealed glass test tubes. The aliquot of the hydrolysate was taken and 0.4 ml AABA (alpha amino butyric acid (50 µmol ml⁻¹)) was added as the internal standard. Then 100 ml of deionized water was added to the aliquot. This aliquot was then filtered using Whatman filter paper No.1 followed by a syringe filter.

Derivatization of Amino Acids with 6-aminoquinolyl-N-hydroxysuccinimide Carbamate (AQC)

A clean syringe was used to deliver 10 µL filtrate of acid hydrolysis to the bottom of a 6 x 50 mm sample tube. A 70 µL of AccQ•Flour Borate Buffer (Reagent 1) was then added to the sample tube by using a micropipette. The sample tube was vortexed prior to adding 20 µL of reconstituted AccQ•Flour Reagent to the sample tube. After vortexed for several seconds, the sample tube was let to stand for 1 minute at room temperature.

Preparation of Internal Standard and Calibration Standard

The internal standard, alpha amino butyric acid (AABA) stock solution was used to prepare the calibration standard. To prepare a 50 µmol/mL internal standard stock solution, 0.258 g AABA was added to 50 mL 0.1N HCl. Prior to the preparation of the calibration standard with internal, 1 mL of 50 µmol/mL internal standard stock solution was transferred to a cleaned 20 mL volumetric flask. The solution was then made up to 20 mL with 0.1 N HCl until the final concentration of the internal standard solution was 2.5 µmol/mL. The calibration standard consisted of 1:1 (v/v) mixture of Pierce H amino acid standard (which contained 2.5 µmol/mL of each amino acid standard, except for 1.25 µmol/mL of cysteine) and a 2.5 µmol/mL of AABA. Typically, a calibration standard with an internal standard was prepared by combined 80 µL 2.5 µmol/mL AABA with 80 µL Pierce H and then was made up with 840 µL deionized water in 1000 µL cleaned vial. A volume of 10 µL calibration standard (contains of 2 nmol of each standard amino acid components) was transferred from the 1:1 (v/v) mixture of Pierce H amino acid standard, and 2.5 µmol/mL of AABA was placed in a derivatization tube and carried through the same derivatization procedure as mentioned earlier.

HPLC Analysis of Amino Acids

The Waters AccQ•Tag amino acid analysis method requires a fluorescence detector. The excitation wavelength was 285 nm, the emission wavelength was 354 nm, filter and gain set were 1.5 second and 10, respectively. Eluent A and Eluent B were AccQ•Tag concentrate and 60% acetonitrile:water, respectively. The column temperature was set at 37°C. The Column (Waters AccQ•Tag) was first conditioned with Eluent B at 1 mL/min flow rate for 5 minutes. This was followed by equilibrating the column in 100% AccQ•Tag Eluent A for 9 minutes at the same flow rate. Consistent period of the equilibration was kept for all the analysis. A blank was carried out before each analysis to determine baseline performance. The total time between injections to end of the analysis was 50 minutes.

Statistical Analysis

The data will be reported as an average of three replicates. Analysis of variance (ANOVA) of the three factors and interactions was applied to the different sets of data with significance level of 95% ($\alpha = 0.05$). The statistical analysis of the experiment data was conducted using SPSS software (Zaina *et al.*, 2013).

Results and Discussion

Table 1 shows the proximate analysis of control sausage and beef sausage with added okara. There were significant different at the 5% level in moisture content, crude fat content, crude fiber content, crude protein

content, ash content and carbohydrate content. The addition of okara flour into beef sausage cause decreased in moisture, fat and protein content while increased in crude fiber, ash and carbohydrate content.

Table 1: Proximate composition of control sausage and beef sausage with added okara

Proximate	Control Sausage	Okara Sausage
Moisture	69.90±0.59 ^a	68.27±0.32 ^b
Fat	12.85±0.23 ^a	10.39±0.15 ^b
Crude Fiber	2.29±0.06 ^b	3.25±0.05 ^a
Protein	10.35±0.19 ^a	8.13±0.05 ^b
Ash	3.28±0.03 ^b	6.02±0.10 ^a
Carbohydrate	1.32±0.70 ^b	3.93±0.12 ^a

Means within each row with difference superscript are significantly different at $p < 0.05$; Lower case letter indicate the difference in amino acid composition between control sausage and beef sausage with added okara.

Meat products substituted by plant based protein showed an increased in ash, fiber and protein while decrease in moisture and fat content (Grizzotto *et al.*, 2012). The decrease in fat content was due to the addition of vegetable-based protein such as soy, wheat and peanut (Brewer, 2012). Besides, fat is mainly from the meat where it is a major contributor for fat in sausage products as compared to the plant based part which is okara flour. Furthermore, the increase in fiber content may occur because of soybean is a vegetable-based fiber: mixture of amylopectins and cellulotics (Brewer, 2012). Based on study by Grizzotto *et al.* (2012), the lower protein content was due to the substitution of meat protein with non-meat protein, since meat protein is more complete than non-meat protein.

The methods used in this study only detected 17 amino acids which were arginine, lysine, valine, threonine, leucine, tyrosine, histidine, isoleucine, phenylalanine, methionine, cysteine, glycine, proline, alanine, glutamic acid, aspartic acid and serine. Upon acid hydrolysis, aspartic acids and glutamic acid formed from the hydrolysis of asparagines and glutamine respectively. Thus, the content of aspartic acid represents the total content of asparagines and aspartic acid, and the same applies to glutamic acid. In addition, tryptophan is destroyed upon acid hydrolysis (Zainal *et al.*, 2013). Table 2 shows the amino acids content of control sausage and okara sausage. The amino acids content was presented as gram amino acid/100 gram total amino acids. Arginine, glycine and serine were present in beef sausage with added okara while absent in Control Sausage. On the other hand, threonine, tyrosine and aspartic acid were present in Control Sausage while absent in beef sausage with added okara. There were significant different at the 5% level in valine, leucine, histidine, cysteine, alanine and glutamic acid while there were no significant difference at the 5% level in lysine, isoleucine, phenylalanine and proline. When comparing between Control Sausage and beef sausage with added okara, the amino acids content was slightly different. This is because the essential amino acid for Control Sausage comes from animal protein while the essential amino acid for Okara Sausage comes from the combination of animal and plant protein. The most abundant amino acid in Control Sausage (in decreasing order) were threonine, leucine, cysteine, glutamic acid, proline, aspartic acid, valine, alanine, tyrosine, lysine, phenylalanine, isoleucine, histidine and methionine. On the other side, the most abundant amino acids in Okara Sausage (in decreasing order) were leucine, proline, alanine, glutamic acid, lysine, phenylalanine, isoleucine, serine, methionine, cysteine, glycine, arginine, valine and histidine. Large amount of threonine was detected in control sausage but not detected in beef sausage with added okara. Threonine is needed to create glycine and serine. Threonine was breakdown to produce glycine and serine (James *et al.*, 2002). Okara has high quality protein, especially essential amino acids. It is well-documented that okara contains about 27% protein (dry basis) with good nutritional quality and a superior protein efficiency ratio, which shows a potential source of low cost vegetable protein for human consumption (Shuhong *et al.*, 2013). The essential amino acid profile of the okara protein isolate was comparable with the exception of the sulfur-containing amino acids (cysteine and methionine), valine and tyrosine which were lower in the okara protein isolate. Liu (1997) reported that the limiting amino acid in soy protein is a sulfur-containing

amino acid. However, soy protein is rich in lysine, and this amino acid is limited in cereals, which are generally rich in sulfur-containing amino acids (Rao *et al.*, 2002). Hence, the protein form in soy or okara is an ideal protein source for complementing cereal proteins.

Table 2: Amino acid composition of control sausage and beef sausage with added okara

Amino acid composition (g amino acid/ 100g total amino acid)		
Amino Acids	Control Sausage	Okara Sausage
Arginine	ND	0.231
Lysine	0.529 ^a	0.741 ^a
Valine	0.640 ^a	0.229 ^b
Threonine	63.775	ND
Leucine	17.589 ^b	21.467 ^a
Tyrosine	0.609	ND
Histidine	0.305 ^a	0.025 ^b
Isoleucine	0.348 ^a	0.635 ^a
Phenylalanine	0.472 ^a	0.659 ^a
Methionine	0.030 ^b	0.353 ^a
Cysteine	3.836 ^a	0.320 ^b
Glycine	ND	0.276
Proline	2.031 ^a	1.894 ^a
Alanine	0.638 ^b	1.141 ^a
Glutamic Acid	2.653 ^a	1.095 ^b
Aspartic Acid	1.315	ND
Serine	ND	0.506

Means within each row with difference superscript are significantly different at $p < 0.05$; Lower case letter indicate the difference in amino acid composition between control sausage and Okara Sausage.

Conclusion

The addition of okara flour in beef sausage caused decreased in moisture, fat and protein content while increased in crude fiber, ash and carbohydrate content. Beef sausage with added okara was found to be rich in leucine which is an essential amino acid for human being.

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