Sensory aroma characteristics of alcalase hydrolyzed rice bran protein concentrate as affected by spray drying and sugar addition

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Abstract The sensory aroma characteristics of alcalase hydrolyzed rice bran protein concentrate as affected by spray drying and sugar addition were investigated. Rice bran protein concentrate (RBPC) was hydrolyzed by alcalase. Sucrose, glucose or fructose was added to the liquid rice bran protein hydrolysate (LRBPH) and subsequently spray dried. The sensory aroma intensities of the hydrolysates were evaluated. Results showed that after spray drying, the rice bran protein concentrate powder (RBPC-P) had higher sweet and cocoa-like aroma intensities than RBPC ($p \leq 0.05$) and hydrolyzed rice bran protein powder (HRBPP) had higher milk powder-like aroma intensities than LRBPH ($p \leq 0.05$). The sweet, cocoa-like and milk powder-like aroma intensities in hydrolyzed rice bran protein powder with fructose addition (HRBPP-F) were significantly higher ($p \leq 0.05$) than those of hydrolyzed rice bran protein powder with sucrose or glucose addition (HRBPP-S or HRBPP-G). HRBPP-F had the highest overall aroma liking score. These results also indicate that spray drying and sugar addition could improve the sensory aroma characteristics of alcalase hydrolyzed RBPC.

Keywords Alcalase hydrolysis · Protein hydrolysate · Rice bran protein concentrate · Sensory aroma characteristic · Spray drying · Sugar addition

Introduction

Rice bran is a by-product obtained from the outer rice layers when white rice is produced. It is used in animal feed or rice bran oil. Rice bran is an excellent source of protein, minerals,

fatty acids, and dietary fiber content (Adebiyi et al. 2007). Defatted rice bran has protein content of 15–20 % (Pan et al. 2005; Chandi and Sogi 2007) and is considered as a source of hypoallergenic ingredients (Agboola et al. 2005; Chandi and Sogi 2007; Jarunrattanasri et al. 2007; Tang et al. 2003). Therefore, rice bran has a potential to be used as a novel protein source. However, rice bran protein concentrate (RBPC) has a low consumer acceptance because of its undesirable flavor (Adebiyi et al. 2007; Jarunrattanasri et al. 2007).

Free amino acids and peptides from rice bran protein can generate specific flavor compounds from the Maillard reaction (Boekel 2006) and they are a nitrogen source of the Maillard reaction (Nimlos et al. 2003). Therefore, enzymatically hydrolyzed vegetable protein is a new alternative to break down protein into peptides or free amino acids; this method cuts peptide bonds with limited or specific position by controlling the hydrolysis conditions (Adler-Nissen 1986).

The Maillard reaction is important in producing flavor compounds. There are in excess of 3,500 volatile compounds that have been attributed to Maillard reactions with numerous of these compounds having very low sensory thresholds, making them important to food aroma (Reineccius 2006). The aroma generation during cooking and thermal processing such as sweet, bakery aroma, roasted cocoa, nut, chocolate, malt, popcorn and bread are aromas produced from the Maillard reaction (Cerny 2008). Boekel (2006) reported that the Maillard-derived flavor compounds contribute roasted, cereal-like, sweet, burnt, cereal, malt and coffee aromas. The parameters that influence the overall flavors and aromas in a Maillard reaction are the temperature, moisture content and type of sugar (Schieberle and Hofmann 1997). Moreover, high temperature processes increase the chemical reaction rates and accelerate the total loss of water from sugar molecules in the Maillard reaction.

Drying is one of the main techniques for preserving food products (Bonazzi and Dumoulin 2011). The high temperature

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of spray drying can induce the Maillard reaction which generate thermally processed aromas. Muresan et al. (2000) reported that volatile intensities of block-milk increased with heat treatment and drying processes and many volatile compounds are formed due to the Maillard reaction. Anese et al. (1999) also found that in a high temperature drying process (110 °C), furosine, an early Maillard reaction compound was remarkably increased; however, its concentration did not change during a lower temperature drying process (50 °C). Moreover, heat processing produced the largest effect to volatile profiles of oats. High levels of toasted cereal and yeasty aroma were detected in kiln-dried oats (Sides et al. 2001). Chin et al. (2008) also reported that high heat treatment during spray drying induced the formation of volatile compounds which resulted in roasted, sweet and caramel-like aromas.

Sugars are an important flavor precursor in the Maillard reaction. In general, pentose sugars are more reactive than hexose sugars. Ketoses (fructose) are more reactive than aldoses (glucose) because of a high proportion of open chain forms in the Maillard reaction (Laroque et al. 2008). Moreover, caramelization is more likely to occur from ketoses than from aldoses (Ajandouz et al. 2001). Caramelization is a complex series of chemical reactions driven by the direct heating of sugars that promote flavor generation (Bonazzi and Dumoulin 2011). Wong et al. (2008) reported that the aroma produced in glucose and amino acids during the Maillard reactions can produce a pleasant caramel-like aroma, a burnt caramel aroma, savory, meaty and soy sauce-like flavors.

Among the parameters affecting the Maillard reaction, drying and sugar addition are important factors influencing aroma formation. They may be capable of improving the sensory aroma characteristics and consumer acceptance of alcalase hydrolyzed RBPC. Therefore, the sensory aroma characteristics of alcalase hydrolyzed RBPC as affected by spray drying and sugar addition were investigated in this study.

Materials and methods

Chemicals

Rice bran from jasmine rice (*Oryza sativa*, Variety Khao Dawk Mali 105) was obtained from Patum Rice Mill and Granary Company, Ltd. (Bangkok, Thailand). Alcalase 2.4L FG was received from Novo Co. (Novo Nordisk, Bagsvaerd, Denmark). Reference samples used for sensory analysis were a malt drink (AB Food & Beverage (Thailand) Ltd., Samutprakarn, Thailand), a chocolate malt beverage and milk powder (Nestle (Thai) Ltd., Bangkok, Thailand), and a seasoning powder (Unilever Thai Trading Ltd., Bangkok

Thailand). All chemical reagents were analytical grade. NaOH and HCl were purchased from Merck (Darmstadt, Germany). Sucrose, glucose and fructose were purchased from Ajax Finechem (NSW, Australia).

Preparation of defatted rice bran

Rice bran was defatted twice using hexane with a rice bran to hexane ratio of 1:3 w/v, stirred at 800 rpm, then centrifuged 10,000×g at 25 °C for 30 min, followed by air drying overnight in a fume hood. It was then ground and passed through a 0.5 mm sieve (Wang et al. 1999).

Preparation of RBPC

Alkali protein extraction procedures followed by isoelectric precipitation were then used to prepare RBPC from defatted rice bran. Defatted rice bran was mixed with distilled water at a ratio of 1:4 w/v and adjusted to pH 9.5 with 1.0 mol/L NaOH, followed by centrifugation at 10,000×g at 25 °C for 30 min. The supernatant was adjusted to pH 4.5 with 1.0 mol/L HCl, then centrifuged at 15,000×g at 25 °C for 30 min (Gnanasambandam and Hettiarachchy 1995). The precipitate was RBPC. Then, the aroma characteristics of RBPC were evaluated by quantitative descriptive analysis.

Preparation of liquid rice bran protein hydrolysate (LRBPH)

RBPC was dispersed in distilled water in ratio of 1:3 w/v, then adjusted to pH 8.5 with 1.0 mol/L NaOH. The 5 % protein suspension was hydrolyzed by alcalase at a concentration of 1.25 AU/g rice bran protein at 50 °C for 4 h with constant shaking. The LRBPH was rapidly inactivated by heating in boiling water for 15 min (modified from Humiski and Aluko 2007). Then, the aroma characteristics of LRBPH were evaluated by quantitative descriptive analysis.

Preparation of rice bran protein concentrate powder (RBPC-P) and its hydrolysate powder

The pH values of RBPC dispersed in distilled water at a ratio of 1:3 w/v, and LRBPH were separately adjusted to pH 7.0 with 1.0 mol/L NaOH or 1.0 mol/L HCl before heating to 95 °C for 10 min. Then the suspensions of RBPC and LRBPH were spray dried using a two nozzle system and co-current air flow. The nozzle diameter was 1 mm with inlet and outlet temperatures of 180 °C and 100 °C, respectively (Basic model, GEA Niro A/S, Thailand). The resulting products were RBPC-P and rice bran protein hydrolysate powder (HRBPP). Then, the aroma characteristics of RBPC-P and HRBPP were evaluated by quantitative descriptive analysis. Color measurement and proximate analysis of RBPC-P and HRBPP were investigated.

Preparation of hydrolysate powder with sugar addition

LRBPH was adjusted to pH 7.0 with 1.0 mol/L HCl. Then, 18.83 g of sucrose, 9.91 g of glucose or fructose were added to 550 ml of LRBPH to obtain sugar concentration of 0.1 mol/L, followed by heating at 95 °C for 10 min. After that the LRBPH, with added sugar was spray dried (Basic model, GEA Niro A/S, Thailand; inlet 180 °C, outlet 100 °C). The resulting products were rice bran protein hydrolysate powder with added sucrose (HRBPP-S), added glucose (HRBPP-G) and added fructose (HRBPP-F), respectively. Then, the aroma characteristics of hydrolysate powders with added sucrose, glucose or fructose were evaluated by quantitative descriptive analysis. Color measurement and proximate analysis of HRBPP-S, HRBPP-G and HRBPP-F were investigated.

Sensory evaluation

Panel selection

Aroma characteristics of the alcalase hydrolysates were evaluated by descriptive sensory analysis using a trained panel. The panelists were graduate students from the Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University. The sensory capabilities of all panelists (4 males and 12 females, between the ages of 23 and 30) were screened for sensitivity and consistence performance before training.

Term generation, development of definitions and references for the attributes

A descriptive analysis “brainstorming” session was conducted. The panelists were asked to sniff samples then discuss the definitions and references of samples. Reference materials were provided to assist with terminology and standardization.

Scale design and training

After discussion and agreement among the panelists on specific odor descriptors, the panels were trained to identify and evaluate aroma intensities compared to reference standards (Table 1) before testing. Panelists received training in descriptive sensory analysis for more than 30 h and had experience in sensory profiling of various food samples. The training was continued until results from individual panelists were consistent.

Sample testing

Samples consisted of hydrolysate powder (2.0 g) or liquid hydrolysate (10 mL) in 60 mL glass vials which were covered with aluminum foil to prevent any visual bias. All samples were coded with 3-digit numbers and were evaluated in triplicate at room temperature (ca. 25 °C). The analysis took place daily at approximately 2–3 p.m. Aroma intensities were rated on 15-cm linear scales anchored on the left with “none” or “0” and on the right with “very” or “15”. The aroma intensity rating of the standards were used as references for rating the aroma intensities of the samples (Modified from Kaewka et al. 2009; Theerakulkait et al. 1995).

Overall aroma liking score of hydrolysates was evaluated using a 9-point hedonic scale with 30 untrained panelists (1 = dislike extremely, 9 = like extremely) (Lawless and Heymann 1998).

Proximate analysis

The percent crude protein, crude fat, ash, moisture and carbohydrate of RBPC-P and the hydrolysate powder were evaluated according to AOAC procedures (AOAC 1990) and the procedure described by Jarunrattanasri et al. (2007).

Color measurement

L^* , a^* and b^* values of the RBPC-P and the hydrolysate powders were measured using a spectrophotometer in a reflectance mode (CM-3500d Minolta, Minolta Corp., New Jersey, USA). The powder was contained in a glass petri-dish (45 mm diameter) during measurement according to the procedure described by Kaewka et al. (2009). Color difference between samples (ΔE^*) was calculated from $((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$; where ΔL^* , Δa^* and Δb^* was the difference in the L^* , a^* and b^* values of the sample, respectively (modified method of Girelli et al. 2004).

Statistical analysis

A randomized complete block design was used in all experiments. The block corresponded to each of the sixteen panelists in each replication (Theerakulkait et al. 1995). Data on descriptive sensory aroma profiles of RBPC and LRBPH, data on overall aroma liking scores of RBPC-P, HRBPP, HRBPP-S, HRBPP-G and HRBPP-F before drying were determined using of analysis of variance (ANOVA) and least significant difference procedures. Differences were reported as significant at $p=0.05$, using a standard statistical software package. Three samples

Table 1 Term, references, ratings and definition used in sensory descriptive analysis

Term	References	Rating ^a	Definition
Sweet	Ovaltine white malt (8 g) ^b	10.61	The characteristic note of “sweet” associated with cereal beverage, a little burnt sugar, honey
Cocoa-like	Milo (8 g) ^b	11.82	The characteristic note of “cocoa-like” associated with caramel-like, chocolate beverage, dirty dog
Milk powder-like	Canation milk powder (8 g) ^b	10.56	The characteristic note of “milk powder-like” associated with vanilla, creamy, milk ice cream
Rice bran-like	Rice bran (8 g) ^b	8.56	The characteristic note of “rice bran-like” associated with sour, feed food, rancid
Salty	Seasoning powder “Knorr” (8 g) ^b	13.95	The characteristic note of “salty” associated with chicken soup, potato chip

^a Aroma intensity value were rated on 15 cm universal scales, with intensity rating of 0 to 15 corresponding to “none” and “very”, respectively

^b Weight of sample in glass vial

were used per replication and each set of experiments was replicated twice as appropriate.

Results and discussion

Effect of spray drying on aroma characteristics of RBPC and its hydrolysate in liquid and powder forms

The descriptive aroma analysis results of RBPC, RBPC-P, LRBPH and HRBPP are shown in Fig. 1. The analysis revealed that aroma intensities of sweet and cocoa-like aroma in LRBPH were significantly ($p \leq 0.05$) higher than those of RBPC, while its rice bran-like aroma intensity note was significantly ($p \leq 0.05$) lower. After spray drying, the aroma intensities of sweet and cocoa-like aroma in RBPC-P were significantly ($p \leq 0.05$) higher than for RBPC. In addition, the aroma intensities of milk powder-like aroma in HRBPP were significantly ($p \leq 0.05$) higher than LRBPH. The HRBPP also had higher ($p \leq 0.05$) sweet aroma intensity and lower ($p \leq$

Fig. 1 Descriptive sensory aroma profiles of rice bran protein concentrate (RBPC, □) in powder form (RBPC-P, ▨) or as liquid rice bran protein hydrolysate (LRBPH, ▤) and in powder form (HRBPP, ■). Data are given as mean ± standard deviation ($n=16$). The different letters are significantly different at 0.05 significance level

0.05) rice bran-like odor intensities than the RBPC-P. This might suggest that spray drying had an effect on aroma generation in alcalase hydrolyzed RBPC. The smaller peptides and free amino acids presented in LRBPH when spray dried could affect the aroma in alcalase hydrolyzed RBPC. This is especially true because the Maillard reaction is a crucial reaction generating aromas in heated foods. High temperature increases reaction rates and changes the pattern of the Maillard reaction (Saeki 1997). Ajandouz et al. (2008) reported that higher temperature increased the rate of the Maillard reaction. Klensporf and Jeleń (2008) proposed that the most significant effect on the desired sensory attributes of oat flakes was formed during the drying process. Moreover, Boekel (2006) reported that there were many aroma characteristics generated during the Maillard reaction, for example, sweet, caramel-like and cracker-like aromas. In addition, Kaewka et al. (2009) also found that spray-dried acid hydrolyzed rice bran protein powders had lower rice bran-like odor intensities and received a higher overall liking score compared to the liquid hydrolysate in which cracker-like aroma was not detected. Jarunrattanasri et al. (2005) reported that hydrolyzed vegetable protein from rice bran protein had predominant free amino acids such as glutamic acid, arginine, aspartic acid, alanine, leucine and glycine. These amino acids generated malty, dark chocolate-like notes and soy sauce-like character of hydrolyzed rice bran protein. Besides, proline and valine were detected in acid hydrolyzed rice bran protein. Therefore, the increment of sweet, cocoa-like, milk powder-like aromas and the reduction of rice bran-like odor in HRBPP might be due to the effect of Maillard reaction which occurred from free amino acids presented in LRBPH and high temperature during spray drying process.

Effect of sugar addition on aroma characteristics of hydrolysate powders

In our previous study, the effect of sugar type and concentration on aroma characteristics of rice bran protein powder were

evaluated (data not shown). The desirable aroma characteristics such as nut or almond-like, cereal beverage-like and sweet note, which were not found in the sample without sugar addition, were detected in rice bran protein powder with 0.1 mol/L sugar addition. Therefore, sugar addition at 0.1 mol/L was chosen for this study. The aroma intensities of hydrolysate powders with sucrose, glucose or fructose added to LRBPH before drying are illustrated in Table 2. Aroma intensities of a rice bran-like note was significantly ($p \leq 0.05$) decreased in HRBPP-G. In addition, the aroma intensities of a salty note in HRBPP was significantly ($p \leq 0.05$) higher than HRBPP-S and HRBPP-G. Moreover, after sucrose or glucose was added before drying, the aroma intensities of cocoa-like and salty in HRBPP-G were higher than in HRBPP-S, while the rice bran-like aroma in HRBPP-G was significantly lower ($p \leq 0.05$) than in HRBPP-S. Additionally, HRBPP-F had significantly ($p \leq 0.05$) higher sweet, cocoa-like and milk powder-like aroma intensities when compared to HRBPP, HRBPP-S and HRBPP-G. This result may be because fructose is a ketose reducing sugar which is more reactive than aldose in reducing sugar (glucose) or non-reducing sugar (sucrose). The open-chain structure of fructose results in more reactivity in the Maillard reaction than other sugars. This agrees with the results of Laroque et al. (2008) who reported that fructose reacted with free amino groups and disappeared rapidly during the Maillard reaction. In addition, a desirable aroma from sugar addition may be generated by caramelization or sugar degradation during high temperature exposure. Lauridsen et al. (2006) reported the sensory evaluation of cooked pork with added sugar had increased levels of caramel and grilled odors. The addition of glucose and ribose resulted in consistently high levels of Maillard-derived volatiles. Ribose and fructose occur as furanoses. They have the same chemical structure that includes five membered rings in cyclic sugars consisting of four carbon atoms and one oxygen atom. Therefore, ribose and fructose are known for their high reactivity with protein via the Maillard reaction (Friedman 1996). Moreover, Lee et al. (2012) revealed that volatile components,

especially 2, 5-dimethylpyrazine, contributed the chocolate-like or roasted nut flavor of enzymatic hydrolyzed wheat gluten. Their concentration increased by nine fold in enzymatic hydrolyzed wheat gluten after thermal reactions with fructose over those with glucose. Lee et al. (2010) also reported that thermal reaction of glutathione with fructose produced a larger amount of the aroma-active compounds which provided for popcorn-like, caramel and bread-like aromas than those reacted with glucose. Sugars are the main precursors of dicarbonyls which is important for different types of volatile compounds such as pyrazines, furans, furanones, pyranones from the Maillard reaction (Reineccius 1990). These volatile compounds provide for roasted, baked cereal, sweet, caramel-like, and cracker-like aromas (Boekel 2006). Therefore, the aroma characteristics in HRBPP-F could have originated from the Maillard reaction via fructose addition and heat treatment during spray drying.

Overall aroma liking score, color values and chemical composition of hydrolysate powder

Descriptive analysis revealed that the powder form of alcalase hydrolyzed RBPC after spray drying and sugar addition has the potential to be used as an ingredient in cocoa flavored products. After spray drying and sugar addition, the desirable aroma intensities of sweet, cocoa-like or milk powder-like aroma increased, while undesirable aromas such as rice bran-like odor and salty notes decreased. The overall aroma liking score of the hydrolysate powder is shown in Table 3. Alcalase hydrolysis significantly increased the overall aroma liking score and this was more pronounced when fructose was added compared to RBPC-P. The highest aroma liking score was for HRBPP-F (7 = Like moderately). Moreover, HRBPP-F showed the lowest L^* value, whereas a^* and b^* were the highest compared to HRBPP, HRBPP-S and HRBPP-G (Table 4). In addition, the color difference value of HRBPP-F was the most different from RBPC-P followed by HRBPP-G, HRBPP and HRBPP-S, in descending order. From color values, HRBPP-F showed more dark, red and yellow than

Table 2 Descriptive sensory aroma profiles of hydrolyzed rice bran protein powder (HRBPP), HRBPP with sucrose added (HRBPP-S), HRBPP with glucose added (HRBPP-G) or HRBPP with fructose added (HRBPP-F) before drying

Samples	Aroma intensity*				
	Sweet	Cocoa-like	Milk powder-like	Rice bran-like	Salty
HRBPP	5.03±0.72b	2.04±0.40b	2.27±0.76b	6.19±1.16a	1.18±0.61a
HRBPP-S	4.88±0.84b	1.28±0.36c	1.51±0.15b	5.18±0.52a	0.37±0.12b
HRBPP-G	5.80±0.52b	1.89±0.17bc	1.05±0.26b	2.85±0.22b	0.46±0.04b
HRBPP-F	7.57±1.26a	3.38±0.69a	3.19±1.31a	4.80±1.91a	0.99±0.57ab

* Score in a range of 0 (none) to 15 (very high intensity)

^{a-c} Means (± standard deviation, $n=16$) within the same column with different letters are significantly different at 0.05 significance level

Table 3 Overall aroma liking score of rice bran protein concentrate powder (RBPC-P), rice bran protein hydrolysate powder (HRBPP), and HRBPP with sucrose added (HRBPP-S), HRBPP with glucose added (HRBPP-G), or HRBPP with fructose added (HRBPP-F) before drying

Samples	Overall aroma liking score
RBPC-P	5.29±1.18d
HRBPP	6.39±1.39bc
HRBPP-S	6.46±1.56b
HRBPP-G	5.76±1.53cd
HRBPP-F	7.18±1.23a

^{a-d} Means (± standard deviation, *n*=30) with the same letter are not significantly different at 0.05 significance level

other samples. According to Hwang et al. (2011), fructose and an amino acid mixture had higher browning intensity than glucose and the same amino acid mixture. The difference might be due to the open chain of fructose, which has a higher concentration of the open chain form and results in more browning (Boekel 2001). In addition, it was found that when considering glucose addition there were darker, redder and more yellow colors than in HRBPP. This suggests that the main precursor of the Maillard reaction was reducing sugar. Moreover, the Maillard reaction produces melanoidins which is a brown product (Silvan et al. 2006). The chemical composition of the hydrolysate powders is shown in Table 4. The main chemical components of hydrolysate powders were proteins and carbohydrates, which are the aroma precursors of the Maillard reaction. Fat content in the hydrolysate powder proved that lipid oxidation could take place in the hydrolysate powder. Protein content was high in RBPC-P and HRBPP. Sugar addition was an important factor in the change of

chemical compositions and it resulted in significantly higher carbohydrate contents in HRBPP-S, HRBPP-G and HRBPP-F than RBPC-P and HRBPP. Furthermore, glucose and fructose addition increased moisture content significantly. Result shows that glucose and fructose are hygroscopic compounds which have the property of moisture adsorption (Donnelly et al. 1973). Moreover, lower glass transition temperature of glucose (34.88 °C) and fructose (16.85 °C) than sucrose (57.85 °C), could affect to the moisture content of the hydrolysate powders (Magon and Pytda 2009). The higher ash content observed in hydrolysate powder might be due to salt generation during pH adjustment.

Conclusions

Spray drying and sugar addition affected the aroma characteristics of alcalase hydrolyzed rice bran protein concentrate. After spray drying, sweet and cocoa-like aromas in rice bran protein concentrate powder and milk powder-like aroma in hydrolyzed rice bran protein powder increased. Sugar addition, especially when fructose was added before drying (HRBPP-F), could promote sweet, cocoa-like and milk powder-like aromas. HRBPP-F showed the highest overall aroma liking score. The hydrolysate with sucrose and glucose added before drying had reduced rice bran-like odor and salty notes in the hydrolysate powder. However, sugar addition increased dark, red and yellow color of the hydrolysate powder. Thus, these methods are effective ways to improve the sensory aroma of protein concentrate from rice bran so that it can be used as an innovative food flavor ingredients.

Table 4 Quality analysis of rice bran protein concentrate powder (RBPC-P), Hydrolyzed rice bran protein powder (HRBPP), HRBPP with sucrose added (HRBPP-S), HRBPP with glucose added (HRBPP-G) or HRBPP with fructose added (HRBPP-F) before drying

Quality analysis	Type of hydrolysate powder				
	RBPC-P	HRBPP	HRBPP-S	HRBPP-G	HRBPP-F
Color values					
L*	77.76±0.23a	63.94±0.12c	71.85±0.20b	62.39±0.28d	61.94±0.12e
a*	2.57±0.09d	4.28±0.03b	3.11±0.07c	4.33±0.08b	5.22±0.05a
b*	14.18±0.14e	15.55±0.13d	16.07±0.11c	16.27±0.13b	20.97±0.01a
ΔE*		13.99	6.23	15.61	17.42
Chemical compositions (% dry basis)					
Protein	56.48±0.06a	54.44±0.11b	35.13±0.12e	42.16±0.03d	42.71±0.03c
Fat	5.64±0.06a	6.31±0.36a	2.78±0.37b	6.93±0.70a	6.87±0.85a
Ash	3.55±0.13c	4.42±0.13a	3.63±0.09b	4.40±0.52a	4.31±0.10ab
Carbohydrate	34.33±0.12c	34.79±0.36c	58.43±0.42a	46.52±0.21b	46.11±0.78b
Moisture	8.66±0.05c	7.78±0.06c	8.17±0.26c	17.77±0.02a	16.37±0.88b

^{a-c} Means (± standard deviation, *n*=3) within the same row with different letters are significantly different at 0.05 significance level

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