Changes in Chemical and Sensory Properties of *Migaki-Nishin* (Dried Herring Fillet) during Drying

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ABSTRACT: *Migaki-nishin* is a Japanese term that refers to dried herring fillets. It is widely consumed in Japan due to its characteristic flavor enhancing properties. This study was conducted to investigate the changes in chemical and sensory properties of *migaki-nishin* during drying. Chemical analyses showed that extractive nitrogen and amount of peptides increased significantly ($P < 0.05$) up to the eighth day of drying and then slightly decreased by the tenth day. Glutamic acid, alanine, glycine, and histidine were the most abundant free amino acids and the largest increase was found in samples dried for 10 days. A decrease in Hunter's L^* value (lightness) and increase in b^* value (yellowness) as well as browning intensity suggested that nonenzymatic browning occurred in *migaki-nishin* during drying. Fluorescence spectrophotometric determination also revealed that Maillard reactions progressed throughout the drying period. In addition, available lysine content and free amino groups decreased significantly ($P < 0.05$) as drying progressed. Sensory evaluation showed that addition of water-soluble extracts to Japanese noodle soup (*mentsuyu*) linearly enhanced the flavor characteristics such as thickness, mouthfulness and continuity with the increased length of drying time. These results suggest that during the drying period, proteolysis as well as Maillard reaction products increased markedly, which might contribute to the characteristic taste and flavor of *migaki-nishin*.

Keywords: Herring, seafood processing, peptides, Maillard reaction, taste

Introduction

Migaki-nishin is a Japanese term that refers to dried herring (*Clupea pallasii*) fillets. It remarkably enhances the characteristic taste and flavor of savory dishes like noodles by enhancing flavor characters, such as thickness, mouthfulness, and continuity. These flavor characters are often called *koku* (a Japanese term). To the best of our knowledge, Japan is the only *migaki-nishin* producing country, and processing of herring fillet is carried out based on experience. Processors are usually selling their products after 10 d drying of herring fillet. There is a need for reliable data on the processing of *migaki-nishin* to help decide the proper drying time and quality of the final product or the stage at which optimum taste and flavor are developed.

The flavor of fish and shellfish principally originates from extractive components (Konosu and Yamaguchi 1982). It is also well known that amino acids and peptides contribute to the taste of a wide variety of foods. Some amino acids and peptides have been shown to be important in either intensifying or masking the flavor of certain foods (Maga 1994).

In the case of dried seafood products nonenzymatic browning is an important factor enhancing flavor, nutrition, and possibly safety. Nonenzymatic browning takes place during processing and storage. Nonenzymatic browning is a result of reactions between products of lipid oxidation such as carbonyl compounds and amino compounds (Koizumi and others 1959; Takiguchi 1992). Nonenzymatic browning is more prominent in fatty fish than lean fish muscle because fatty fish contains high amount of polyunsaturated fatty acids, which are highly susceptible to oxidation. Furthermore, the high content of highly reactive free amino acids such as taurine, lysine, and methionine in species like Atlantic short finned squid greatly contribute to Maillard browning reaction during the processing and storage (Haard and Arcilla 1985), and this reaction is of considerable importance in the dried fish products (Haard 1995).

However, to the best of our knowledge, no study has so far been reported concerning biochemical changes that are responsible for the characteristic taste and flavor of *migaki-nishin* during drying. Therefore, the present study was aimed to evaluate the changes in chemical and sensory properties of *migaki-nishin* during drying.

Materials and Methods

Migaki-nishin sample

Migaki-nishin was obtained from Iwasaki Suisan Ltd., Hakodate, Japan. Herring (*Clupea pallasii*) was captured at the coast of Kamchatka Peninsula, Russia, in October 2006. It was kept frozen for approximately 2 to 3 mo until it was processed. Upon arrival in the factory, herring was thawed, gutted, washed, and then filleted for drying. Herring fillets were dried using huge electric fans. Room temperature and relative humidity were maintained at approximately 14 °C and 45%, respectively. During drying, samples were randomly taken for analysis at days 2, 4, 6, 8, and 10. Samples were minced to uniformity and used for analyses.

Preparation of water-soluble extracts (WSE)

Water-soluble extracts (WSE) were prepared following the method described by Hondo and Mochizuki (1968). Distilled water (80 mL) was added to 20 g of minced *migaki-nishin* sample and then homogenized. The resulting slurry was heated at 100 °C for 10 min. After being cooled, the slurry was filtered, using filter paper (Advantec Nr 1, Toyo Roshi Kaisha Ltd., Tokyo, Japan). The resulting filtrate was made up to 100 mL and was used as WSE. The WSE were freeze-dried and stored at –50 °C until used.

Chemical analyses

Proximate composition (moisture, crude protein, and ash) was determined based on the AOAC (1995) methods. Total lipid was extracted according to the method of Bligh and Dyer (1959). Extractive nitrogen content of WSE was determined by the Dumas method (AOAC 1995; Schmitter and Rihs 1989), and an automatic nitrogen analysis system (Thermo Electron Corp., Waltham, Mass., U.S.A). Free and total amino acid compositions of WSE were determined before and after hydrolyzing with 6N HCl at 115 °C for 24 h and then analyzed with an amino acid analyzer (JEOL JLC-500/V, Nihon Denshi Datem Co. Ltd., Tokyo, Japan). Amounts of peptides were calculated by subtraction of the free amino acid concentration from the total amino acid concentration in the hydrolyzed WSE. The WSE were analyzed on HPLC (Hitachi 655A, Hitachi, Ltd., Tokyo, Japan) with a gel filtration column (TSK-GEL G2000SW, Tosoh Corp., Tokyo, Japan) and it was filtered through 0.45 μm filters prior to injection. The mobile phase consisted of an aqueous solution of 45% acetonitrile (Wako Pure Chemical Industries Ltd., Osaka, Japan) containing 0.1% trifluoroacetic acid. Flow rate was 0.8 mL/min. UV absorbance at 214 nm was monitored to visualize chromatographic profile. To analyze the coloration of WSE, the HPLC was connected with fluorescence spectrophotometer (Hitachi F-2500, Hitachi High-Technologies Corporation, Tokyo, Japan). Detection wavelengths at λ ex 350 nm and λ em 440 nm were used to determine Maillard reaction products (Yeboah and others 1999). Aprotinin (MW 6500 Da), oxidized insulin chain B (MW 3495 Da), glycylglycylglycine (MW 189 Da) and glycine (MW 75 Da) were used to prepare calibration curve and to determine molecular weight.

Color measurement

Changes in color of the *migaki-nishin* samples were measured by Hunter's *L**, *a** and *b** values using a color difference meter (Nippon Denshoku Kogyo Co. Ltd., Tokyo, Japan). The color variations

of each sample were compensated for by recording the average of 3 readings taken on the surface of the sample.

Browning intensity

Approximately 5 g of sample were homogenized in 30 mL of cold 7% trichloroacetic acid for 2 min, centrifuged at 4000 × *g* for 20 min, and then filtered. This procedure was repeated 3 times. The supernatants were then combined and made up to 100 mL. The absorbance at 420 nm (A_{420}) was measured to express the browning intensity (A_{420}/g) of the *migaki-nishin* samples.

Available lysine content

Available lysine content was determined following the method described by Carpenter (1960). An accurately weighed sample (containing 30–50 mg of nitrogen) was derivatized with $NaHCO_3$ and ethanolic 1-fluoro-2,4-dinitrobenzene (FDNB) solution (2.5% v/v), and then hydrolyzed with 8.1 N HCl at 120 °C for 20 h. Absorbance of the reaction product of *ε*-DNP-lysine was measured spectrophotometrically at 435 nm. Available lysine content was expressed as g/16 g of N.

Determination of free amino groups

Free amino groups were determined by the method of Benjakul and Morrissey (1997). One gram of sample was accurately weighed and homogenized with 29 mL of 1.0% SDS at 8000 rpm for 1 min. The mixture was incubated at 85°C for 15 min and then centrifuged at 10,000 × *g* at room temperature for 10 min. The reaction mixture containing 125 μL of supernatant and 2 mL of 0.2125 M phosphate buffer pH 8.2 was mixed with 1 mL of 0.01% (w/v) 2,4,6-trinitrobenzenesulfonic acid (TNBS) solution and incubated at 50 °C for 30 min in the dark. To terminate the reaction, 2 mL of 0.1 M sodium sulfite

were added. The absorbance was measured at 420 nm. Free amino groups were calculated as micromoles of leucine per gram dry matter.

Sensory evaluation

Sensory evaluation was carried out by adding WSE to *mentsuyu* (a Japanese noodle soup) following the method of Ueda and others (1997) with some modifications. *Mentsuyu* was prepared according to the method of Shah and others (2009). It was diluted with 6 volumes of distilled water and used as a control solution. The WSE were dissolved in *mentsuyu* at a concentration of 0.10% (w/v) and then warmed to 60 °C in a water bath. About 50 mL of sample and control solutions were served in opaque disposable plastic cups at the same time. Panel members were instructed to put an adequate volume in the mouth, and then to expectorate. The panelists were asked to judge the intensities of the test samples using a scale of 1–7, where 3 points was given to the control solution. Scoring was done on the basis of saltiness, umami, thickness, mouthfulness and continuity. Sensory evaluation was performed in the separated sensory booths. The panel was composed of 5 trained assessors (3 males and 2 females; ages between 26 and 37 y) from the Food Creation Center, Kyowa Hakko Food Specialties Co., Ltd. Ibaraki, Japan. All the panel members had extensive experience in tasting and agreed on the intensities of saltiness, umami, thickness, mouthfulness, and continuity in *mentsuyu*.

Statistical analysis

For each measurement, analyses were repeated 3 times, data were pooled, and the mean and standard deviation were determined. Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to identify differences among the means at $P < 0.05$ using

STATGRAPHICS *Plus* version 2.1 (StatPoint, Inc., Virginia, USA). We used a linear regression model to analyze the relationship between sensory attributes and drying time.

Results and Discussion

Changes in proximate composition

Changes in proximate composition (percent fresh matter basis) of *migaki-nishin* are shown in Table 1. The moisture contents of *migaki-nishin* ranged from 31.37% to 59.61%. The highest crude protein content was observed in the 10th day of drying *migaki-nishin.* Total lipid content increased significantly ($P < 0.05$) from 16.50% to 27.20% by the 8th day of drying while it decreased slightly to 26.80% by the 10th day of drying. Aro and others (2005) also reported that the lipid content of pickled herring decreased significantly ($P < 0.05$) from 13.8% to 11.5% during 12 mo of storage. The ash content increased significantly ($P < 0.05$) from 1.38% to 2.95% during the drying period. Decrease of moisture content with corresponding increase in crude protein, total lipid, and ash content can be explained by increased dehydration of *migaki-nishin* during the drying period.

Changes in chemical components

Changes in extractive nitrogen content of *migaki-nishin* during drying are shown in Figure 1. Extractive nitrogen mainly comprises of water-soluble peptides, free amino acids, and nucleotides. The extractive nitrogen content of WSE increased significantly ($P < 0.05$) from 13.61% to 14.61% by the 8th day of drying. It then decreased slightly to 14.32% by the 10th day of drying. This result suggests that some free amino acids especially lysine or other nucleotides might be interacted with lipid oxidation products such as carbonyl compounds. Changes in free amino acid composition of *migaki-nishin* during drying are presented in Table 2. Total amount of free amino acids significantly

increased ($P < 0.05$) from 711.53 to 1211.36 mg/100 g dry matter, probably as a result of proteolysis. Glycine, alanine, histidine, and lysine were the most abundant free amino acids, although most of the individual amino acid contents fluctuated during the drying period. These free amino acids were also found to be predominant in dried sardine (Takiguchi 1999) and dried skipjack (Fuke and others 1989). The concentrations of glutamic acid, alanine, valine, and leucine significantly ($P < 0.05$) increased while lysine decreased during drying. Notably concentrations of glutamic acid (desirable for umami) as well as glycine and alanine (desirable for their sweet taste) were higher in samples dried for 10 d than in samples dried for 2 d. It was postulated that the delicious taste of *migaki-nishin* is due to these amino acids. Glutamic acid and glycine are recognized as taste active amino acids in abalone, sea urchin, snow crab, scallop and short-necked clam irrespective of their amounts (Fuke and Konosu 1991). Yamaguchi and Kimizuka (1979) also reported that addition of glutamic acid to synthetic extracts not only increases umami but also improves overall attributes by imparting continuity, complexity, fullness, and mildness.

As shown in Figure 1, amount of peptides increased significantly ($P < 0.05$) from 2.52 to 3.70 g/100 g dry matter during the first 8 d of drying period then slightly decreased by the 10th day (3.59 g/100 g dry matter). However, there was no significant difference between the amount of peptides in the 8th and 10th days dried sample. This result suggests that proteolysis occurred in *migaki-nishin* up to 8 d of drying period and then it was terminated. The importance of peptides in the sensory perception of food has been recognized for long time. Itou and others (2006) reported that peptide content increased remarkably in *narezushi* (a fermented mackerel product) during processing and this was thought to be responsible for the umami taste of *narezushi*. Moreover, Ishii and others (1995) reported that amount of peptides increased during low temperature heating of beef, enhancing preferable taste of meat. Gel filtration chromatograms of WSE in *migaki-nishin* during drying are

shown in Figure 2. It can be observed from the chromatograms that concentration of peak 3 was increased gradually up to 8 d WSE then slightly decreased in the WSE of 10 d. Average molecular weight of this peak was 1.3 KDa. This result suggests that protein hydrolysis was progressed up to 8 d drying period and then it was terminated.

Changes in coloration and browning intensity

Changes in color components and browning intensity of *migaki-nishin* during drying are presented in Figure 3. During drying, the *L** value (lightness) decreased significantly ($P < 0.05$) from 36.60 to 28.41 within 4 d of drying and slowly thereafter. However, no significant change in the *L** value (lightness) was observed in the later stages of drying period ($P > 0.05$). Hunter's *a** value (redness) was mostly stable while *b** value (yellowness) gradually increased ($P < 0.05$) throughout the drying period. Changes in *L**- and *b**- values might be attributed to non-enzymatic browning reaction, which progressed continuously during drying of *migaki-nishin*. Takiguchi (1999) also reported that Hunter's *a**- and *b**- values increased gradually in the pulverized *niboshi* (boiled and dried anchovy) throughout the storage period at 25°C; while these values increased rapidly in the frozen sample after thawing.

The increase in absorbance at 420 nm was used as an indicator of browning development in the final stage of the browning reaction (Ajandouz and others 2001). The browning intensity of *migaki-nishin* muscle increased significantly ($P < 0.05$) throughout the drying period (Figure 3). As *migaki-nishin* contains very small amounts of carbohydrate, secondary lipid oxidation products such as aldehyde or other carbonyl compounds might react with free amino acids during drying.

Coloration of WSE was also analyzed using a fluorescence spectrophotometer. From the chromatogram, Maillard reaction products with molecular weights ranging from 12.5 kDa to 31 kDa and 0.3 kDa to 4 kDa were observed (Figure 4). Concentration of Maillard reaction products especially

low molecular weight compounds that eluted at around 14 to 20 min was increased gradually throughout the drying period. This observation agrees with that of Ogasawara and others (2006) who analyzed a water-soluble fraction of *miso* (soybean paste) ripened for 20 mo. They observed apparent peptide products of Maillard reactions (1–5 kDa).

Changes in available lysine and free amino groups

Available lysine content in *migaki-nishin* ranged between 5.84 and 7.09 g/16 g of N, decreasing significantly ($P < 0.05$) as drying progressed (Figure 5). Decreases in available lysine content might be the result of Maillard reaction, during which ε-amino group of lysine and carbonyl compounds produced by lipid oxidation form complex intermediate compounds by interacting with each other during drying. In an earlier study, it was reported that lipid oxidation takes place in *migaki-nishin* during drying (Shah and others 2009). It has been reported that lysine is one of the amino acids with the highest browning rate in a simulated model system of dried squid (Tsai and others 1991). Furthermore, Nakamura and others (1976) reported the reaction of lysine with some lipid oxidation products resulting in brown pigments. Takiguchi (1992) also reported that as lipid oxidation proceeded in *niboshi*, the contents of methionine, histidine, and lysine decreased and a brown discoloration developed during storage.

Free amino groups decreased significantly ($P < 0.05$) from 295.40 to 210.17 μM leucine/g dry matter as drying progressed (Figure 5), which suggested that nonenzymatic browning might be due to the interactions between fatty acid decomposition products and the amines in protein. Loss of free amino groups of *migaki-nishin* also correlated with an increase of Hunter's b^* value. Nonenzymatic browning is recognized as a consequence of peroxidizing lipids in the presence of protein (Gardner 1979). Most investigators theorize that nonenzymatic browning in muscle foods during lipid oxidation

starts with the condensation of aldehydes with amines via Schiff base reaction pathways (Pokorny 1981; Kikugawa and others 1984).

Changes in sensory properties

The relationships between drying time and perceived taste or flavor intensity of water-soluble extracts in *mentsuyu* are shown in Figure 6. The WSE had no taste but acquired a faint aroma in distilled water at a concentration of 0.10% (data not shown). The addition of WSE to *mentsuyu* did not show any significant influence on saltiness intensity ($P > 0.05$) with the increase of drying time. However, addition of WSE to *mentsuyu* showed a positive significant relationship ($P < 0.05$) between the umami intensity and drying time. Furthermore, addition of WSE to *mentsuyu* showed a positive significant ($P < 0.05$) relationship between drying time and flavor characteristics such as thickness, mouthfulness, and continuity. These results suggest that the flavor enhancement of WSE in *mentsuyu* is linearly dependent on the drying time of *migaki-nishin.* Ogasawara and others (2006) reported that peptide products of Maillard reactions contain key substances that give the characteristic flavor (mouthfulness and continuity) of *miso* ripened for extended periods. It has also been reported that pyrazines and some peptides generated together in certain foods during the process of boiling or aging for a long period that brought *koku* (Ogasawara 2003). The multivalent taste-modulating Maillard reaction product alapyridaine in beef broth enhances sweetness and umami character (Ottinger and Hofmann 2003). Glutathione reportedly has a characteristic flavor that includes continuity, mouthfulness, and thickness in an umami solution (Ueda and others 1997).

Conclusion

Drying of herring fillets causes changes such as increase of extractive nitrogen, free amino acids, and peptides while at the same time decreasing available lysine and free amino groups as drying progresses. Significant increase of browning intensity and Maillard reaction products suggest that Maillard reaction progressed throughout the drying period. Thus, it can be concluded that during the drying period, proteolysis as well as Maillard reaction products markedly increased, which might have a contribution to the characteristic taste and flavor of *migaki-nishin*.

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Figure Captions

Figure 1 – Changes in extractive nitrogen and peptides of *migaki-nishin* during drying. The values represent means of triplicates ± SD. [abc] Means with different letters in each line indicate significant differences ($P < 0.05$).

Figure 2 – Gel filtration chromatograms of water-soluble extracts from *migaki-nishin* during drying.

Figure 3 – Changes in color components and browning intensity of *migaki-nishin* during drying. The values represent means of triplicates ± SD. [abc] Means with different letters in each line indicate significant differences ($P < 0.05$).

Figure 4 – Gel filtration chromatograms of water-soluble extracts in *migaki-nishin* during drying.

Figure 5 – Changes in available lysine and free amino groups of *migaki-nishin* during drying. The values represent means of triplicates ± SD. [abc] Means with different letters in each line indicate significant differences ($P < 0.05$).

Figure 6 – Relationships between drying time and perceived taste or flavor intensity of water-soluble extracts in *mentsuyu*. Taste or flavor intensity was scored on a 7-point scale where 3 points were assigned to the control (*mentsuyu*). Bars represent mean ± SD (n = 5).

Table 1 – Changes in proximate composition[a] (% fresh matter basis) of *migaki-nishin* during drying.

Drying time (days)	Moisture	Crude protein	Total lipid	Ash
2	59.61 ± 1.54^{b}	21.64 ± 0.60^{f}	16.50 ±0.67^{e}	1.38 ± 0.19^{e}
4	45.41 ± 1.31^{c}	29.46 ± 1.16^{e}	21.75 ± 1.17^{d}	2.01 ± 0.05^{d}
6	39.33 ± 3.77^{d}	32.86 ± 0.69^{d}	24.50 ± 0.89^{c}	2.41 ± 0.13^{c}
8	34.01 ± 3.58^{e}	35.47 ± 0.65^{c}	27.20 ± 0.99^{b}	2.49 ± 0.05^{c}
10	31.37 ± 1.23^{e}	37.36 ± 0.35^{b}	26.80 ± 0.44^{b}	2.95 ± 0.11^{b}

[a] Each value is expressed as mean ± SD (n =3). Means with different superscripts within a column are significantly different ($P < 0.05$).

Table 2 – Changes in free amino acid composition[A] (mg/100 g dry matter) of *migaki-nishin* during drying.

Amino acid	Drying time (days)				
	2	4	6	8	10
Aspartic acid	5.88 ± 1.30^{b}	11.04 ± 1.33^{a}	4.13 ± 1.07^{b}	4.54 ± 1.60^{b}	4.76 ± 1.86^{b}
Threonine	19.86 ± 1.09^{bc}	21.85 ± 0.89^{b}	26.97 ± 0.24^{a}	18.43 ± 2.90^{c}	18.57 ± 2.40^{bc}
Serine	19.95 ± 1.82^{c}	38.07 ± 1.50^{a}	25.50 ± 0.60^{b}	15.84 ± 2.96^{d}	16.26 ± 3.26^{cd}
Glutamic acid	12.22 ± 0.43^{d}	30.89 ± 1.30^{b}	20.33 ± 1.90^{c}	24.14 ± 4.26^{c}	64.50 ± 1.37^{a}
Glycine	179.41 ± 5.85^{d}	236.74 ± 7.74^{b}	185.68 ± 5.27^{d}	253.10 ± 9.91^{a}	211.98 ± 3.78^{c}
Alanine	149.72 ± 7.16^{d}	281.96 ± 6.97^{b}	217.33 ± 12.88^{c}	287.33 ± 21.28^{b}	448.26 ± 18.65^{a}
Valine	15.00 ± 0.69^{d}	17.12 ± 0.23^{c}	18.18 ± 0.85^{c}	23.05 ±1.42^{b}	45.92 ± 1.66^{a}
Cystine	ND	ND	ND	ND	ND
Methionine	7.87 ± 0.68^{c}	9.72 ± 0.87^{c}	9.57 ± 0.37^{c}	12.52 ± 1.69^{b}	16.18 ± 1.29^{a}
Isoleucine	7.20 ± 0.28^{c}	8.68 ± 0.34^{bc}	8.00 ± 0.90^{c}	10.10 ± 1.14^{b}	23.31 ± 1.51^{a}
Leucine	15.62 ± 0.47^{d}	17.92 ± 0.29^{c}	16.49 ± 0.79^{cd}	21.96 ± 1.61^{b}	47.73 ± 1.74^{a}
Tyrosine	4.91 ± 0.48^{c}	6.44 ± 1.03^{b}	6.38 ± 0.97^{b}	7.45 ± 0.53^{b}	18.69 ± 0.20^{a}
Phenylalanine	5.27 ± 0.40^{c}	7.01 ± 0.70^{bc}	7.75 ± 0.53^{b}	8.54 ± 0.93^{b}	26.49 ± 2.60^{a}
Histidine	61.53 ± 0.49^{b}	43.22 ± 2.83^{c}	55.07 ± 7.06^{b}	62.49 ± 4.85^{b}	73.90 ± 7.18^{a}
Lysine	187.77 ± 7.32^{b}	133.00 ± 7.61^{d}	235.62 ± 2.62^{a}	103.13 ± 2.90^{e}	146.55 ± 3.59^{c}
Arginine	9.39 ± 0.68^{b}	9.89 ± 1.31^{ab}	10.49 ± 0.96^{ab}	6.24 ± 1.92^{c}	11.98 ± 1.28^{a}
Hydroxyproline	ND	ND	ND	ND	ND
Proline	9.93 ± 0.64^{d}	14.73 ± 1.88^{c}	16.95 ± 0.70^{bc}	17.69 ± 1.23^{b}	36.28 ± 2.04^{a}
Total	711.51 ± 18.23^{c}	888.28 ± 22.78^{b}	864.44 ± 11.81^{b}	876.56 ± 17.68^{b}	1211.36 ± 2.74^{a}

[A] Each value is expressed as mean ± SD (n = 3). Means with different superscripts within a row are significantly different ($P < 0.05$).

ND = not detected.

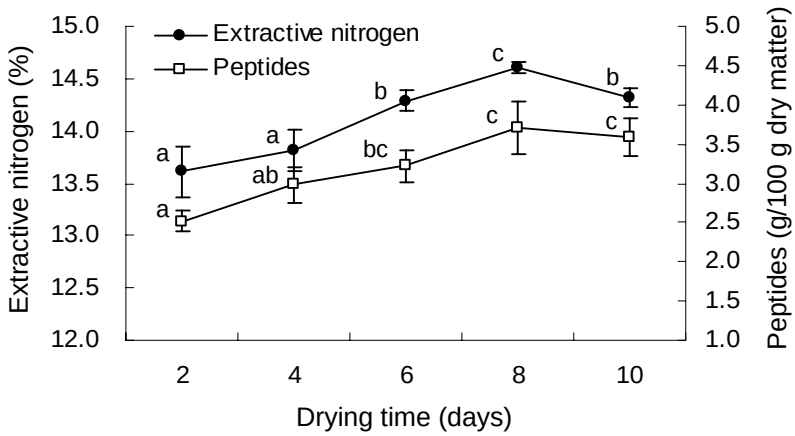

Figure 1 (Shah and others)

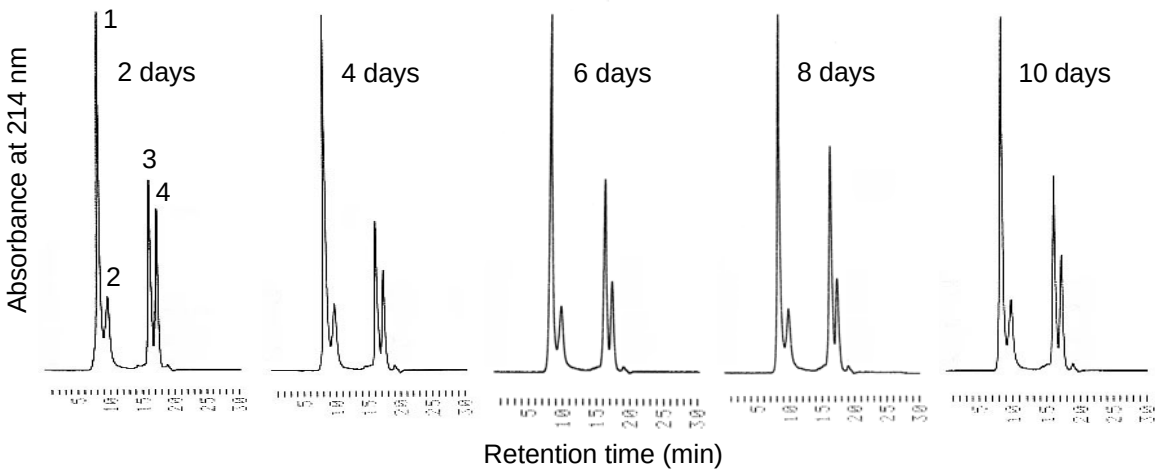

Figure 2 (Shah and others)

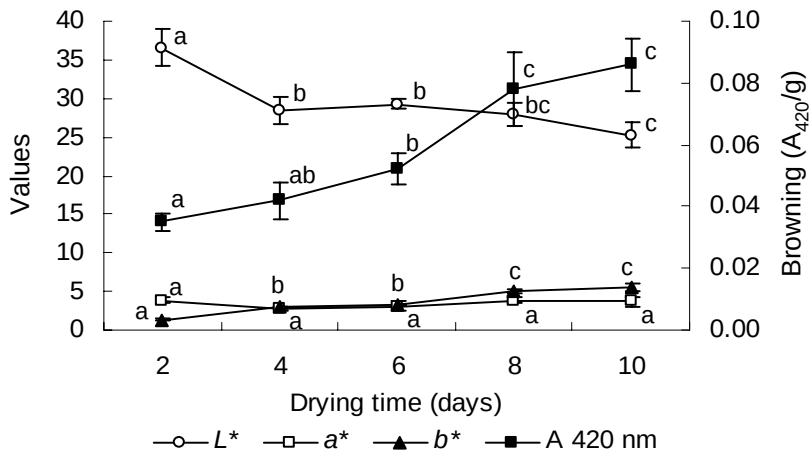

Figure 3 (Shah and others)

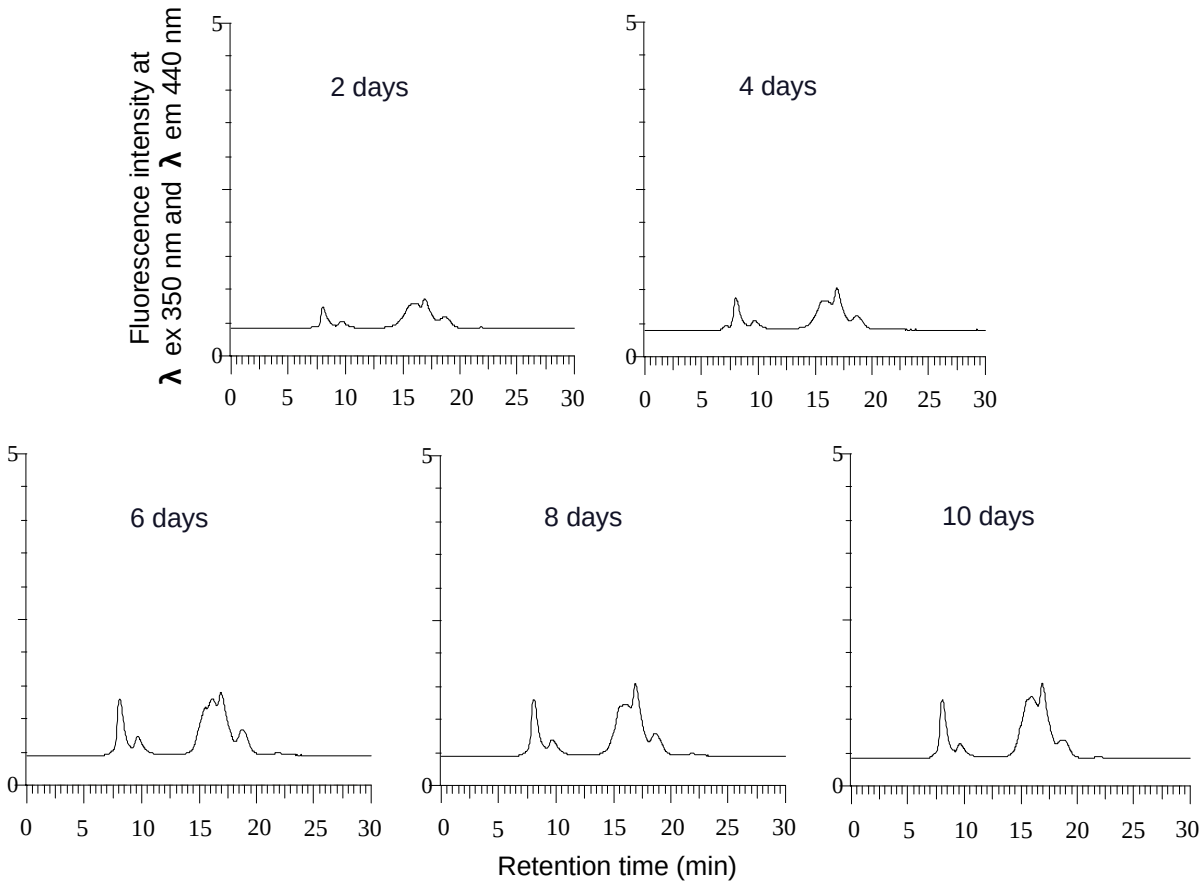

Figure 4 (Shah and others)

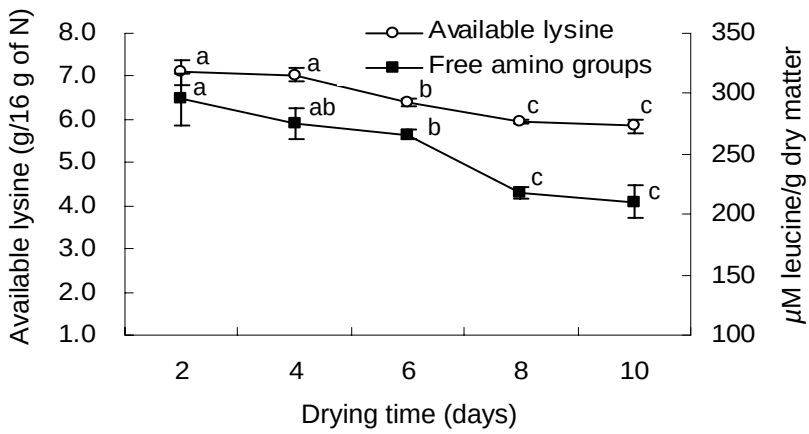

Figure 5 (Shah and others)

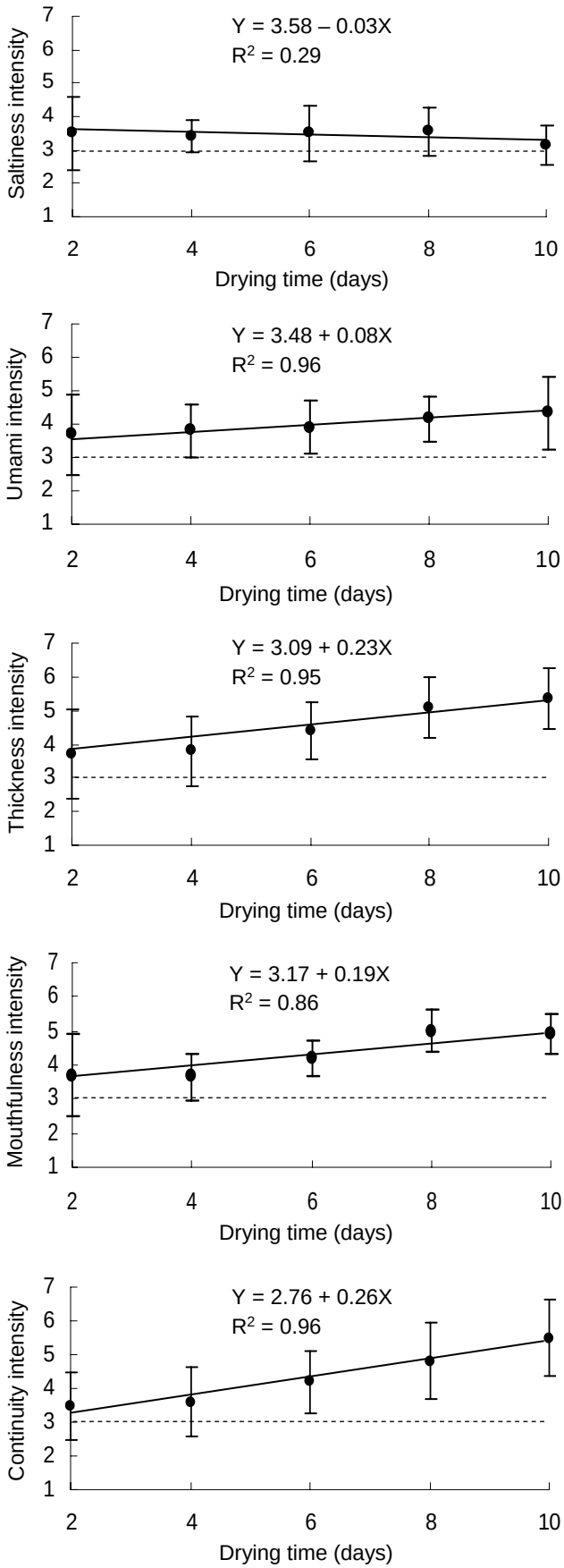

Figure 6 (Shah and others)