Color control of Japanese soy sauce (*shoyu*) using membrane technology

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ABSTRACT: The present study systemically decolorized soy sauce using a membrane process to analyze the separation mechanism. An ultrafiltration (UF) membrane (NTU-2120) exhibited only slight decolorization ability. A nanofiltration (NF) membrane with a lower molecular weight cut-off and produced by sulfonated polysulfone (NTR-7400 series) rather than polyvinyl alcohol/polyamide (NTR-7250) had higher decolorization ability. The NF membranes rejected total nitrogen by 17 to 24%, unsalted soluble solid content by 24 to 32%, reducing sugar by 25 to 43%, and amino acids by 10 to 25%. The NTR-7400 series membrane rejected lactic acid by 6 to 9%, and pyroglutamic acid by 11 to 21%; other quality indexes were maintained. In the NF membrane processes, higher rejection of acidic amino acids than neutral and base amino acids was observed. The separation performance was governed by the electrical effect as well as the sieve effect. Soy sauce color could be controlled by blending NF membrane-processed soy sauce with feed soy sauce. Color can be matched to preference in accordance with dishes by suitably blending NF membrane-processed soy sauce with feed soy sauce.

Keywords: Japanese soy sauce (*shoyu*), Membrane process, Color, Separation mechanism, Blending

1. Introduction

Developing food to accommodate consumer preference is very important for the development of gastronomic culture as well as production of vendible commodities. Color is a major factor in our acceptance of food, and many studies have confirmed that when the color of food does not match our expectations, our acceptance of that food is difficult (Frick, 2003).

Japanese soy sauce (*shoyu*) is a traditional seasoning of Japanese food and is currently used in cooking worldwide (Kobayashi, 2010). The main type of soy sauce, *koikuchi-shoyu* (common soy sauce), is an all-purpose seasoning characterized by a pleasant aroma, a strong flavor, and a deep reddish-brown color. It contains many umami taste components and thus is associated with palatable and pleasurable taste (Lioe et al., 2010).

Soy sauce production involves vigorous lactic and alcohol fermentation. The finished product is pasteurized at a rather high temperature (80°C). Half of the color is formed during the fermentation and aging of mash, and half is formed during pasteurization. Both are due primarily to heat-dependent browning, known as the Maillard browning reaction, between amino compounds and sugars (Yokotsuka, 1986). At the same time, various constituents that determine the soy sauce quality are formed during the fermentation based on the ingredients including soy bean, wheat and sodium chloride (NaCl). Good-quality soy sauce (*koikuchi-shoyu*) contains 1.3 to 1.8% (grams per volume) total nitrogen (TN), 1.5 to 5% reducing sugar (mainly glucose; RS), 1.5 to 2.5% ethanol, 1 to 1.5% polyalcohol (primarily glycerol), 1 to 2% organic acid, and 16 to 18% NaCl. In order for a soy sauce to have palatable taste, about half of its nitrogenous compounds must be free acids; in particular glutamic acid (Glu) is a very important component (Yokotsuka, 1986).

The authors study of general consumers' color preference for *Koikuchi-shoyu* (Miyagi, 2012) indicated that lighter-colored soy sauce was generally preferred over the conventional one, although the color preference varied with the dish. Establishing technology to control the color of soy sauce without changing its other sensory qualities has potential for developing new products for customer acceptance.

Adsorption and/or membrane processes have been proposed as simple decolorization processes of soy sauce. We investigated the systemic adsorption of soy sauce and reported that activated carbon, activated clay, and silica gel exhibited decolorizing ability, but diatomaceous earth and magnesium oxide did not (Miyagi et al., 2013). We also documented that activated carbon indicated the greatest decolorizing ability without changing its sensory qualities (Miyagi et al., 2013). However, disadvantages associated with adsorbent systems include leaching of free-form foreign powders into the feed, leaching of metals into the feed, treatment of used adsorbents, and cost.

In contrast, the membrane process is remarkably simple, offering many advantages over other separation and purification processes without the disadvantages of adsorbent systems. Hashiba (1973) and Sasaki et al. (1993) found it was possible to decolorize soy sauce using an ultrafiltration (UF) membrane. Ikeda et al. (1988) and Suzuki and Mori (1995) attempted to decolorize soy sauce using a nanofiltration (NF) membrane and reported that the NF membrane process had remarkable decolorization ability. Furukawa et al. (2006) developed method of concentrating soy sauce constituents using an NF membrane. Although membrane technology has been practically used for the soy sauce industry, the separation principle has not been sufficiently established. Advances in this technology will require clarifying the membrane separation mechanism and food development in line with consumer preferences using decolorized soy sauce.

The present study systematically investigated membrane separation for decolorization and changes in soy sauce (*koikuchi-shoyu*) quality (e.g., general nutritional components, amino acids, and organic acids) using various types of membranes and discussed its separation mechanism. In addition, control of soy sauce color was also attempted by blending feed and membrane-processed soy sauces.

2. Experimental

2.1. Materials

Soy sauce (*Koikuchi-shoyu*, *Jyokyu* (superior) grade) was purchased from Yamasa, Ltd. (Chiba, Japan). Five membrane types (NTU-2120, NTR-7250, NTR-7410, NTR-7430, and NTR-7450) were purchased from Nitto Denko, Ltd. (Shiga, Japan). The NTU-2120 membrane is classified as a UF membrane, and other membranes are classified as NF membranes. The materials and the approximate molecular weight cut-off (MWCO) values are listed in Table 1.

2.2. Membrane experiment

An apparatus with a magnetically stirred membrane cell (Model C-70B; Nitto Denko, Ltd.) used in the experiment was the same as that used in the previous report (Miyagi et al., 2011). In all the experiment runs, the temperature was 25°C, the operating pressure was 2 MPa, and the speed of the spin bar was 200 rpm. The initial charge of the feed sample was 100 g. The experiment run was continued until the permeate reached 50 g. Color was evaluated immediately after the samples were obtained. For other evaluations, the samples were poured into shield bottles, purged with nitrogen gas, and stored at 5°C until measurement.

2.3. Blend experiment

The NTR-7450 membrane had the highest decolorization ability in the membrane experiment, therefore we used the permeate in the blend experiment. Various colors of soy sauce were obtained by blending the NTR-7450 processed soy sauce with the feed. The feed/permeate blend ratios prepared were 4:1, 3:2, 2:3, 1:4, 1:9, and 1:19 (v/v). The color was determined immediately after blending.

2.4. Analyses

To estimate color, we determined the absorbance at 420 nm and the $L^*a^*b^*$ (CIELAB) color space in a 10 mm cuvette. The absorbance was recorded using a spectrophotometer (Model U-1500; Hitachi, Ltd., Tokyo, Japan), and the CIELAB color space was determined using a colorimeter (Model CT-210; Minolta, Ltd., Oosaka, Japan). When the absorbance exceeded 0.8, we measured the value after suitable dilution with pure water to be within 0.2 to 0.8, and recalculated according to the *Beer-Lambert* law.

As quality analyses, TN was determined by the *Kjeldahl* method, and NaCl was determined by the *Mohr* method. The unsalted soluble solid content (USSC) was obtained by subtracting the NaCl value from the reading of a sugar refractometer. Ethanol content was determined by gas chromatography with FID. RS was determined using the *Fehling-Lehmann-School* method. The pH was determined using the glass-electrode method. Amino acids were determined using an automatic amino acid analyzer (HPLC). Organic acids were determined using a bromothymol blue (BTB) post-column HPLC with a UV-VIS detector. All analyses were the same as in our previous report (Miyagi et al., 2013).

2.5. Calculation and data analysis

The performance of the membrane was expressed in terms of rejection (R) calculated as

$$R = 1 - (C_p / C_F) \qquad (1)$$

where C_F is the content of each component in the feed soy sauce and C_p is the content of each component in the processed soy sauce. The rejection of absorbance was also determined.

The blend ratio of the NTR-7450 membrane-processed soy sauce (BRP) was calculated as

$$BRP = VPS/(VPS + VFS) \qquad (2)$$

where VPS is the volume of the membrane-processed soy sauce and VFS is the volume of feed soy sauce.

All analyses except for the absorbance at 420 nm and pH were repeated three times, and the standard deviation was obtained. Statistically significant differences ($p<0.05$) between mean data were determined by the Tukey-Kramer post-hoc test using a useful add-in in Excel software (trade name Statcel2, OMS, Ltd., Saitama, Japan).

3. Results and discussion

3.1. Membrane experiment

3.1.1. Decolorization

Color properties, including absorbance at 420 nm, are listed in Table 2. The rejection of absorbance in order was NTR-7450 (96%), NTR-7430 (93%), NTR-7250 (89%), NTR-7410 (86%), and NTU-2120 (20%) processed soy sauces (Table 2), indicating the order of decolorization ability depending on membrane MWCO and materials (Table 1). Membranes with a lower MWCO and/or produced by sulfonated polysulfone (SPS) rather than polyvinyl alcohol/polyamide (PVA/PA) exhibited higher decolorization.

Motai and Inoue (1974) attempted to fractionate the color components of soy sauce using DEAE-cellulose, and obtained eight color components with molecular weights from 600 to 4270, therefore the lack of rejection of the color compounds for the UF membrane (NTU-2120; MWCO, 20,000), with higher decolorization ability for the NF membrane with a lower MWCO (e.g., NTR-7450; MWCO, 500), are reasonable.

The difference between membrane materials' color separation performances can be explained as follows. Lee et al. (1987) reported that melanoidin (main color compound) of soy sauce was electrofocused at a pH range of 2.5 to 3.5. Thus, melanoidin is negatively charged in the soy sauce because the pH is 4.6. Both membranes produced by SPS and PVA/PA are also negatively charged and therefore are likely to reject the color compounds because of electrical repulsion between the membrane and the color compounds. The zeta potentials are -80 to -90 mV for the SPS (NTR-7450) membrane and -5 to -10 mV for the PVA/PA (NTR-7250) membrane at pH 4.6. Therefore, the electrical effect that occurs between the color compounds and the SPS membrane may be more remarkable than the effect between the color compounds and the PVA/PA membrane.

3.1.2. Color estimation with CIELAB color space

In the CIELAB color space, L^* denotes lightness (brightness-darkness) with values from 100 (white) to 0 (black). Here, a^* and b^* indicate chromaticity corresponding to green (negative value) to red (positive value) for a^*, and blue (negative value) to yellow (positive value) for b^*. The L^*, a^*, and b^* values of feed and membrane-processed soy sauces are presented in Table 2.

The Δa^* and Δb^* values are calculated as the difference between color parameters of processed soy sauce and those of feed soy sauce. Figure 1 plots Δa^* against Δb^*. NTU-2120 processed soy sauce had some positive Δb^* value (increase in yellow) but no change in Δa^* value. In contrast, NTR-7450 processed soy sauce had a negative Δa^* value (increase in green) but no change in Δb^* value. NTR-7250, NTR-7410, and NTR-7430 processed soy sauces had a negative Δa^* value and a positive Δb^* value. The Δa^* and Δb^* values of NTR-7250 and NTR-7410 processed soy sauces were almost the same.

The metric chroma (C^*) is defined as follows (CIE, 2004).

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \qquad (3)$$

The ΔC^* value indicates the difference between the metric chroma of processed soy sauce and that of feed, and ΔL^* indicates the difference between the brightness of processed soy sauce and that of feed. Figure 2 plots ΔL^* against ΔC^* to show color differences in the processed soy sauce.

The ΔL^* values of all processed soy sauces were positive. However, the value of UF membrane-processed soy sauce was small (~8), while the values of NF membrane-processed soy sauce were large (62 to 76) (Fig. 2). The ΔC^* values of NTU-2120, NTR-7250, and NTR-7410 were positive in the range of 8 to 13, while those of NTR-7430 and NTR-7450 were negative in the range of -10 to -31.

3.1.3. General components and pH

TN is the basic constituent that determines soy sauce grade. NaCl makes the basic soy sauce taste, and NaCl with additional taste components (e.g., amino acids and organic acids) leads to the preferred taste (Nakadai, 2007). USSC indicates the total organic constituents (e.g., polyalcohols, amino acids, organic acids, and carbohydrates). Alcohol (mainly ethanol) is an important compound for soy sauce flavor, as well as an anti-mold agent (Nakadai, 2007). A main component of RS is glucose, in addition to small amounts of mannose, arabinose, galactose, and xylose (Yokotsuka, 1986). In general, the pH of Japanese soy sauce is between 4.6 and 4.9.

General quality indexes of feed and membrane-processed soy sauces are presented in Table 2. The UF membrane did not change any quality indexes (Table 2). The NF membrane did not change ethanol and pH, but it rejected TN by 17 to 24%, USSC by 24 to 32%, and RS by 25 to 43% (Table 2). The R values of TN and USSC were higher with lower membrane MWCO. RS was more likely to be rejected by the NTR-7400 series membrane than by NTR-7250. In contrast, the R values of NaCl indicated nominal negative values for the NF membrane. Similar results were observed in previous studies on membrane processing of soy sauce using the NTR-7400 series (negatively charged) membrane (Furukawa et al, 2006; Suzuki and Mori, 1995). Nominal negative rejection of monovalent salt such as NaCl was often observed in mixtures of salt and large charged organic molecules or mixed monovalent-multivalent solutions during NF membrane processing (Gilron et al., 2001; Tsuru et al., 1991). Perry and Linder (1989) interpreted the behavior by introducing the *Donnan* exclusion correction into the driving force term responsible for salt transport. Several transport models of charged membranes have indicated that separation performance depended upon feed concentration, membrane charge density, and permeate volume flux (Tsuru et al, 1991).

3.1.4. Free amino acids

Complex fermentation gives soy sauce a delicious taste, due primarily to the umami components. Free amino acids have an important influence on taste (Lioe et al., 2010). Acidic amino acids, especially glutamic acid (Glu), in the presence of NaCl are essential umami substances; also, other amino acids can contribute to umami taste through synergism (Lioe et al., 2010).

Amino acid contents of feed and membrane-processed soy sauce are presented in Table 3. The UF membrane did not reject any type of amino acid (Table 3). In the NF membrane, the R values of total amino acids were 10 to 13% for NTR-7250, NTR-7410, and NTR-7430, and 25% for NTR-7450 (Table 3). The R order for their membranes corresponded to the order of decolorization abilities (Table 2). For all types of amino acid, acidic amino acids were remarkably rejected by the NF membrane; specifically, NTR-7450 rejected them by 42 to 46% (Table 3). In contrast, the NF membrane did not reject Glycine (Gly), Methionine (Met), or Proline (Pro). And with the exception of NTR-7450, the NF membranes did not change the neutral amino acid contents of the hydroxyl group or the alkyl group (Table 3). Base amino acids were rejected by 7 to 28% with the NF membrane (Table 3).

On the whole, amino acids are likely to be rejected by membranes with decreased MWCO. However, there were some exceptions, especially for acidic amino acids. Considering the molecular weight of acidic amino acids (M_w, 133 to 147), the R values are too high compared to R values of neutral and/or base amino acids (M_w, 75 to 181). In addition, although NTR-7450 (500) has a greater MWCO than NTR-7250 (300), the R values of acidic amino acids of NTR-7450 (42 to 46%) were higher than those of NTR-7250 (24%). This can be explained as follows.

The pH of the present soy sauce is 4.6, while that of isoelectric points (IEP) is 2.8 to 3.2 for acidic amino acids, 5.5 to 6.3 for neutral amino acids, and 7.6 to 10.8 for base amino acids. Therefore, acidic amino acids are negatively charged in the soy sauce, neutral and base amino acids are positively charged in the soy sauce, and the membrane materials are negatively charged. In particular, the material of NTR-7450 (SPS) is more highly negatively charged (-80 to -90 mV) than the material of NTR-7250 (PVA/PA) (-5 to -10 mV) in the soy sauce. Consequently, electric interaction between the membrane material and amino acids affects the separation performance. The scheme is presented in Figure 3. Kimura and Tamano (1986) investigated separation of different amino acids using a UF membrane produced by SPS and concluded that their rejection can be changed by adjusting pH.

As indicated above, the electrical effect and the sieve effect are considered major factors of the present separation performance at constant pressure. Other parameters, such as back diffusion, may also affect the separation performance in the present system using the NF membrane.

3.1.5. Organic acids

Organic acid is important in evaluating the flavor and the physical and chemical properties of soy sauce (Uchida, 1988). It also establishes the pH, acidity, and buffer capacity of soy sauce, and helps inhibit microbial growth (Uchida, 1988). For example, lactic acid produces both sour taste and harmonic flavor, and leads to esterification with alcohol, producing ester (e.g., ethyl acetate), which plays a major role as a flavor component (Kadowaki, 1988).

Organic acid contents of feed and membrane-processed soy sauce are presented in Table 4. The NTU-2120 and NTR-7250 membrane processes did not reject any organic acids. The NTR-7400 series membrane processes passed acetic acid. However, the NTR-7400 series membrane rejected lactic acid by 6 to 9%, and the NTR-7450 membrane also rejected pyroglutamic acid by 21% even though the molecules of organic acids (90 to 130 Da) are much smaller than the MWCO of the NTR-7400 series membranes (500 to 2,000 Da). The phenomenon seems to be governed by the electric effect.

The acid dissociation constant (*pKa*) of lactic acid is 3.9, that of acetic acid is 4.8, and that of pyroglutamic acid is 3.3 (Bordwell pKa Table, 2013), while the pH of soy sauce is 4.6. This implies that lactic acid and pyroglutamic acid should be dissociated in the soy sauce. The membrane material (SPS) is negatively charged as mentioned before, and therefore there may be electric repulsion between the organic acids and the membrane material during the membrane process. In contrast, acetic acid should not be dissociated in soy sauce and therefore can pass through the membranes without repulsion.

3.2. Blend experiment

Color properties of blended soy sauces, including absorbance at 420 nm and CIELAB color space, are shown in Table 5. In the absorbance, the *R* values of the absorbance increased with increasing BRP (Table 5). In the CIELAB color space estimate, the L^* value increased with increasing BRP, while the opposite was observed for the a^* value (Table 5). The b^* values of the feed and the permeate had almost the same minimum value (31 to 33), and the maximum value (89) was observed when the BRP was 20% (Table 5).

Characteristic curves were observed in the plots of Δa^* against Δb^* and ΔC^* against ΔL^* as shown in Figures 4 and 5. In the plot of Δa^* against Δb^*, as BRP increased, the plot initially approached positive Δb^* (yellowness direction), then gradually reversed to negative Δa^* (greenness direction), and finally went back to $\Delta b^* = 0$ (Fig. 4). In the plot of ΔC^* against ΔL^*, a linear relation in the lightness direction was observed from 0 (feed) to 80% of BRP, and a steep inversion curve toward the paleness direction was observed with increasing BRP (Fig. 4). Similar plots were observed in the decolorization of soy sauce using adsorption with activated carbon, activated clay, and silica gel, as in our previous report (Miyagi et al., 2013).

Our previous color-preference study of soy sauce among general Japanese consumers (Chiba prefecture) indicated that color preference differed with dishes (Miyagi, 2012). For example, the average preference chromaticities of soy sauce were 13.4 (L^*= 22) for *Sashimi-shoyu*, 28.1 (L^*= 47) for *Ponzu-shoyu*, 21.2 (L^*= 35) for *Nimono-shoyu*, 15.5 (L^*= 26) for *Mentsuyu-shoyu* (*Tsukejiru*), and 23.1 (L^*= 39) for *Mentsuyu-shoyu* (*Kakejiru*) (Miyagi, 2012). By changing the blend ratio of the membrane-processed soy sauce to feed soy sauce, soy sauce with the preferred color for various dishes can be obtained.

4. Conclusions

We conducted a color control study of soy sauce using membrane and blend technologies. Our main findings are as follows.

・ Soy sauce was significantly decolorized using an NF membrane, but only slightly decolorized using a UF membrane. The decolorization performance of the NF membrane depended mainly on the MWCO and the electrical effect between the membrane material and the color compounds (melanoidin).

・ The UF membrane did not reduce any quality components. The NF membrane rejected TN, USSC, RS, and amino acids of soy sauce in the range of 5 to 43%. In particular, the NF membrane produced by SPS rejected lactic acid by 6 to 9%, and pyroglutamic acid by 11 to 21%, while maintaining other quality indexes.

・The NF membrane was likely to reject acidic amino acids more than neutral and base amino acids because of the electrical interaction between the membrane material and the amino acids.

・ The separation performance of organic acids was mainly governed by the electrical effect between the membrane material and the organic acids.

・ By changing the blend ratio, plots of Δa^* against Δb^* and ΔC^* against ΔL^*draw characteristic curves.

・ Color matched to preference in accordance with dishes can be obtained by suitably blending NF membrane-processed soy sauce with feed soy sauce.

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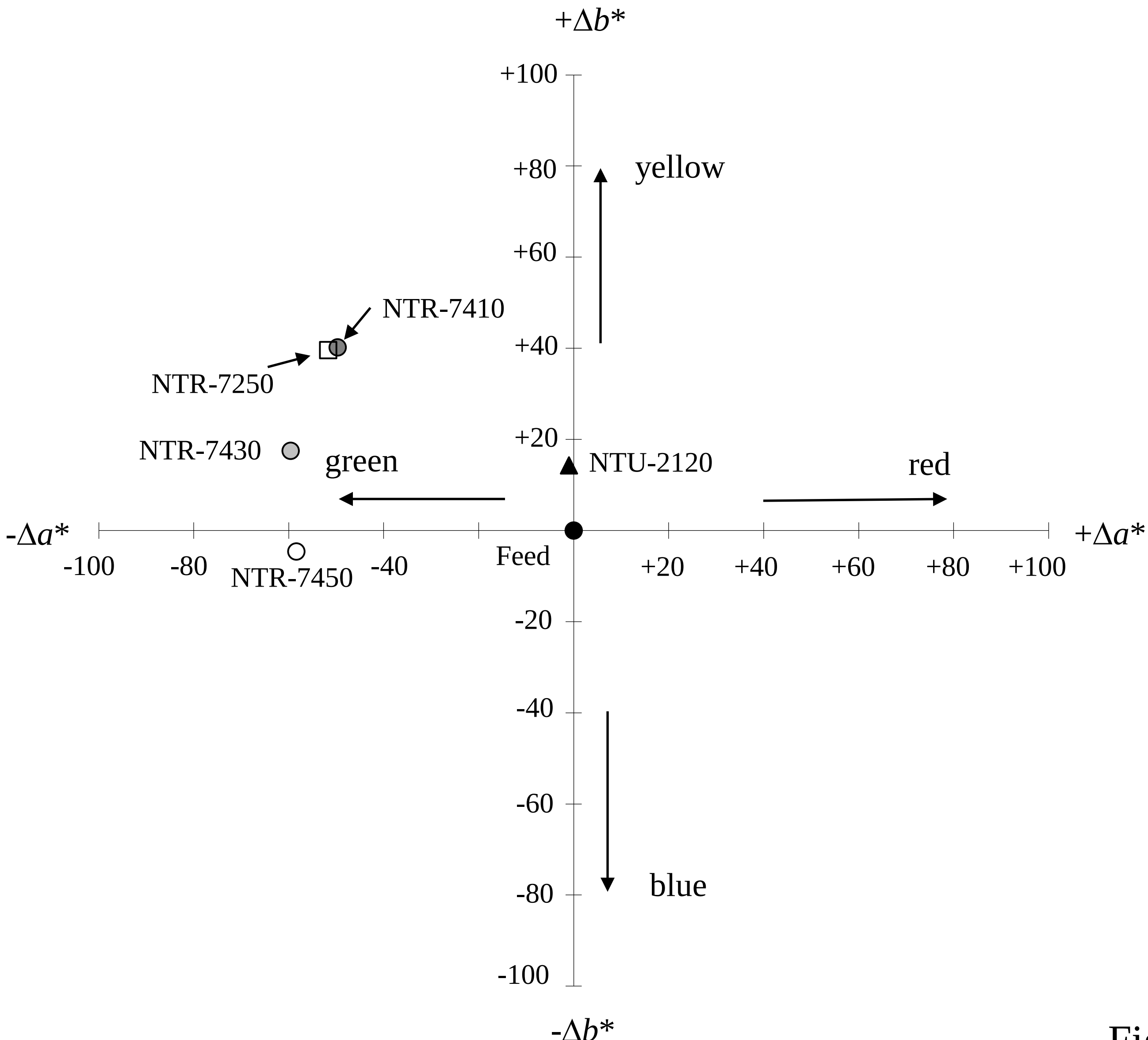

+Δb*
+100
+80
yellow
+60
NTR-7410
+40
NTR-7250
+20
NTR-7430
green
NTU-2120
red
-Δa*
+Δa*
Feed
-100
-80
NTR-7450
-40
+20
+40
+60
+80
+100
-20
-40
-60
-80
blue
-100
-Δb*

Fig. 1

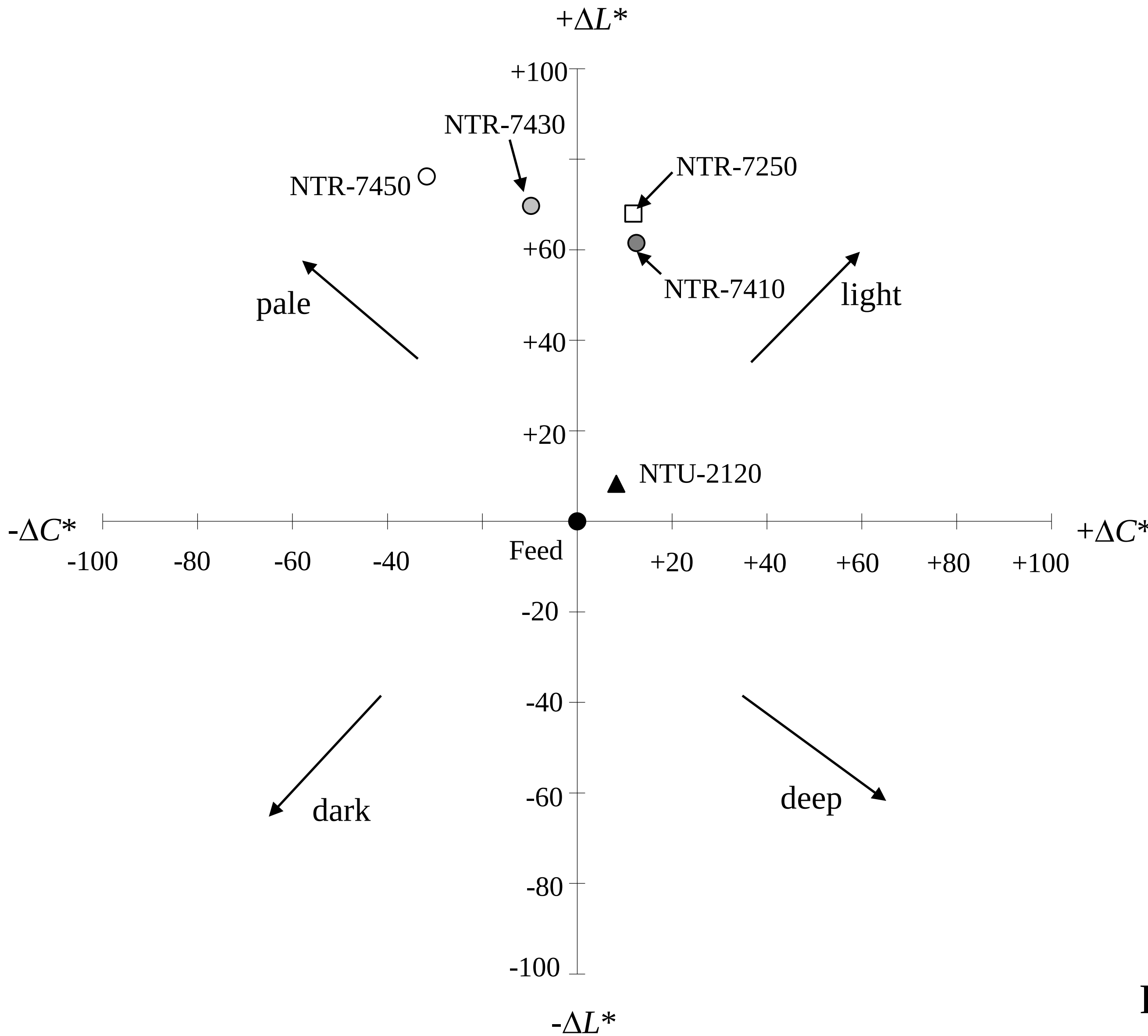

+ΔL*
+100
NTR-7430
NTR-7250
NTR-7450
+60
NTR-7410
pale
light
+40
+20
NTU-2120
-ΔC*
+ΔC*
Feed
-100
-80
-60
-40
+20
+40
+60
+80
+100
-20
-40
-60
dark
deep
-80
-100
-ΔL*

Fig. 2

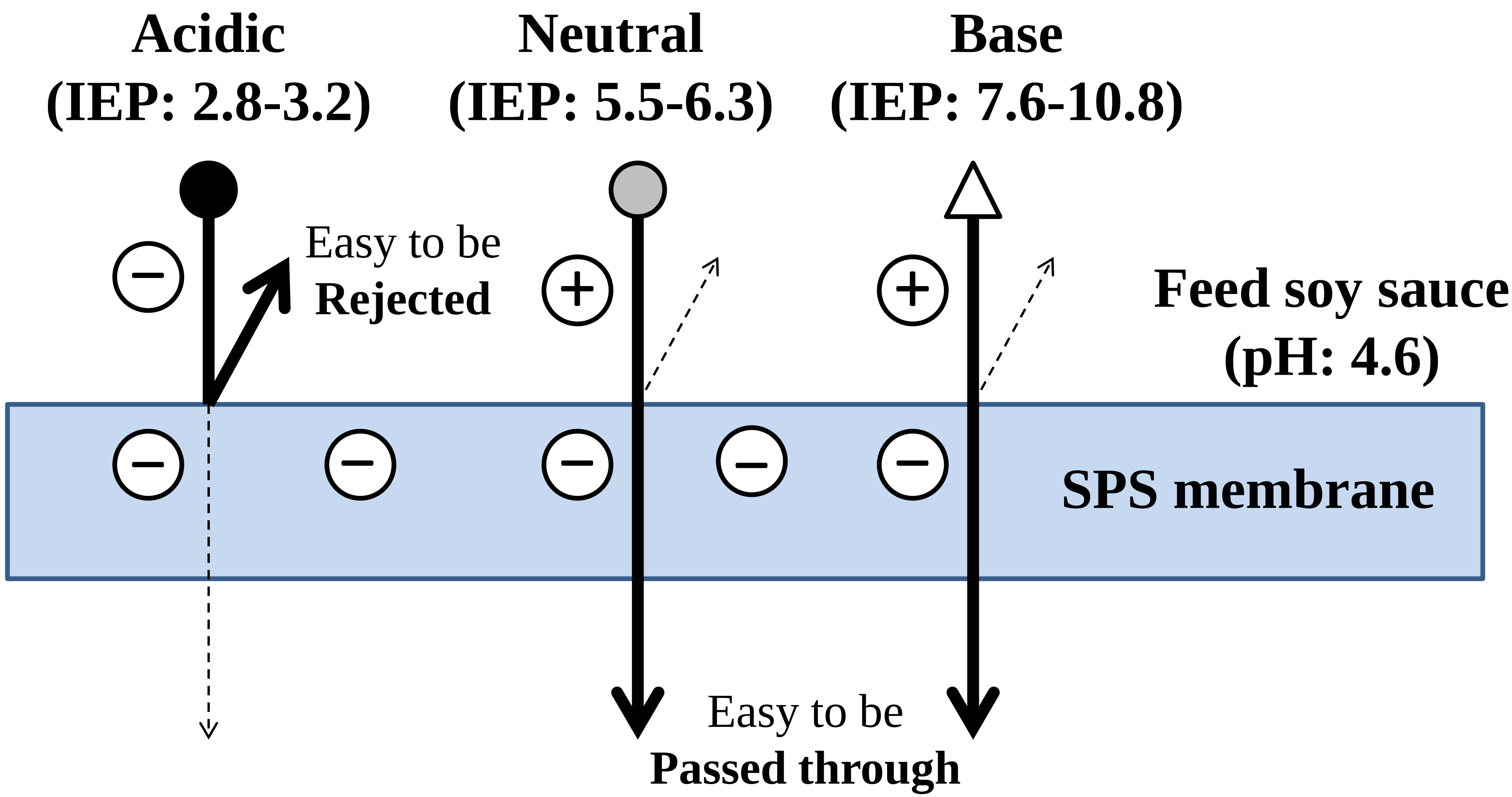
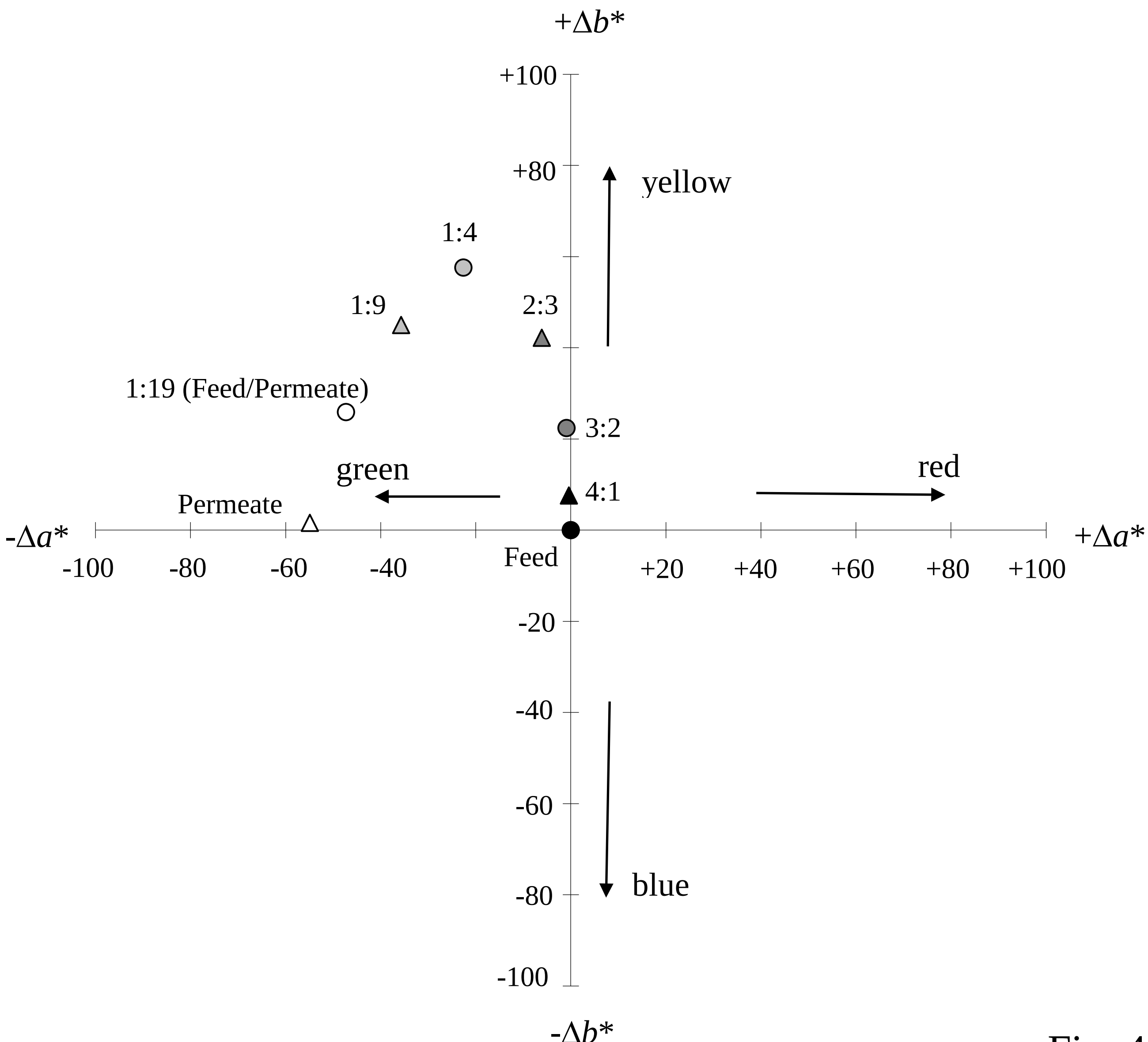
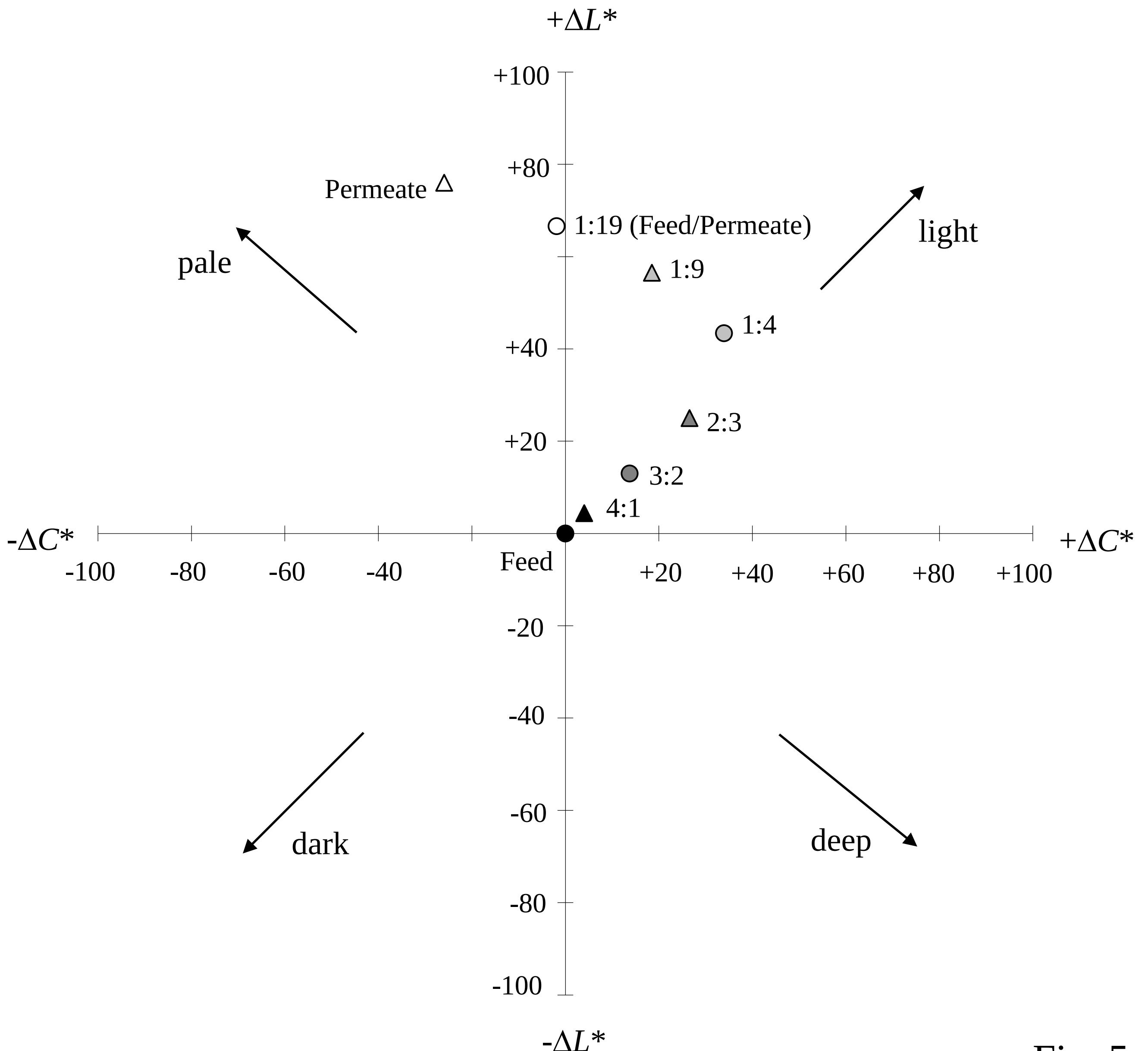

Acidic
(IEP: 2.8-3.2)
Neutral
(IEP: 5.5-6.3)
Base
(IEP: 7.6-10.8)
Easy to be
Rejected
Feed soy sauce
(pH: 4.6)
SPS membrane
Easy to be
Passed through

Fig. 3



+Δb*
+100
+80
yellow
1:4
1:9
2:3
1:19 (Feed/Permeate)
3:2
green
red
4:1
Permeate
-Δa*
+Δa*
Feed
-100
-80
-60
-40
+20
+40
+60
+80
+100
-20
-40
-60
-80
blue
-100
-Δb*

Fig. 4



+ΔL*
+100
+80
Permeate
1:19 (Feed/Permeate)
pale
light
1:9
1:4
+40
+20
2:3
3:2
4:1
-ΔC*
+ΔC*
-100
-80
-60
-40
Feed
+20
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-20
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-60
dark
deep
-80
-100
-ΔL*

Fig. 5

Figure captions

Fig. 1 Plots of Δa^* versus Δb^* for the membrane-processed soy sauces.

Fig. 2 Plots of ΔC^* versus ΔL^* for the membrane-processed soy sauces.

Fig. 3 Scheme of electrical effect on separation performance of amino acids using negatively charged nanofiltration (NF) membrane.

Fig. 4 Plots of Δa^* versus Δb^* with varying blend ratio (e.g.; 4:1, feed/permeate by NTR-7450 membrane (v/v)).

Fig. 5 Plots of ΔC^* versus ΔL^* with varying blend ratio (e.g.; 4:1, feed/permeate by NTR-7450 membrane (v/v)).

Table 1 Membrane properties used in studies.

	classification	material	MWCO [Da]
NTU-2120	UF	Polyolefin	20,000
NTR-7250	NF	Polyvinyl alcohol/Polyamide (PVA/PA)	~ 300
NTR-7410	NF	Sulfonated polysulfone (SPS)	1,000 ~ 2,000
NTR-7430	NF	Sulfonated polysulfone (SPS)	800 ~ 1,000
NTR-7450	NF	Sulfonated polysulfone (SPS)	~ 500

The abbreviations are: UF, ultrafiltration; NF, nanofiltration; MWCO, molecular weight cut off

Table 2 Color properties and general quality indexes of feed and membrane processed soy sauces.

	Absorbance (420 nm)	*R* [%]	CIELAB color space *L**	*a**	*b**	TN [g/L]	*R* [%]	NaCl [g/L]	*R* [%]	USSC [g/L]	*R* [%]	Ethanol [g/L]	*R* [%]	RS [g/L]	*R* [%]	pH
Feed	14.8		20.3±0.3 f	52.1±0.2 a	35.0±0.5 e	13.8±0.1 a		165±2 b		173±2 a		22.3±1.4 a		25.3±0.4 a		4.6
NTU-2120	11.8	20	28.6±0.0 e	51.1±0.0 b	49.3±0.1 d	13.6±0.0 a	1	170±3 ab	-3	162±2 b	6	20.2±1.7 a	9	24.7±1.0 a	2	4.6
NTR-7250	1.7	89	88.3±0.0 c	0.4±0.1 d	74.6±0.0 b	10.7±0.1 d	22	172±0 a	-4	128±1 d	27	22.1±0.9 a	1	18.9±0.1 b	25	4.6
NTR-7410	2.0	86	81.8±0.0 d	2.4±0.0 c	75.2±0.0 a	11.5±0.1 b	17	172±4 ab	-4	132±2 c	24	21.2±1.8 a	5	18.1±0.6 b	28	4.6
NTR-7430	1.1	93	90.0±0.0 b	-7.5±0.1 f	52.5±0.0 c	11.1±0.1 c	20	174±2 a	-5	129±1 cd	25	21.9±0.6 a	2	17.8±0.7 b	30	4.6
NTR-7450	0.6	96	96.5±0.0 a	-6.3±0.0 e	30.4±0.0 f	10.5±0.1 d	24	174±1 a	-5	117±1 e	32	22.4±1.9 a	-1	14.4±0.7 c	43	4.6

The abbreviations are: *R*, percentage rejection; TN, total nitrogen content; NaCl, sodium chloride content; USSC,unsalted soluble solid content; RS, reducing suger content.

The value in each cell is shown as 'mean±SD'.

Different letters in each column indicate significant differences based on Tukey-Kramer test（p < 0.05).

Table 3. Amino acid components of feed and membrane processed soy sauces.

	Acidic				Neutral							
					hydroxyl group				alkyl group			
	Glu [g/L]	*R* [%]	Asp [g/L]	*R* [%]	Thr [g/L]	*R* [%]	Ser [g/L]	*R* [%]	Gly [g/L]	*R* [%]	Ala [g/L]	*R* [%]
Feed	11.4±0.2 a		3.8±0.1 a		2.4±0.0 a		3.3±0.1 a		1.9±0.0 a		4.0±0.0 ab	
NTU-2120	11.5±0.1 a	-1	3.9±0.1 a	-3	2.4±0.0 a	0	3.3±0.0 a	0	1.9±0.0 a	0	4.0±0.1 a	0
NTR-7250	8.7±0.1 b	24	2.9±0.0 b	24	2.3±0.1 ab	4	3.2±0.1 a	3	1.9±0.0 a	0	3.9±0.1 ab	3
NTR-7410	9.0±0.2 b	21	3.1±0.1 b	18	2.3±0.0 ab	4	3.1±0.1 ab	6	1.9±0.0 a	0	3.9±0.1 ab	3
NTR-7430	8.7±0.6 b	24	3.0±0.2 b	21	2.2±0.1 ab	8	3.0±0.2 ab	9	1.8±0.1 a	5	3.8±0.2 ab	5
NTR-7450	6.2±0.7 c	46	2.2±0.3 c	42	2.0±0.2 b	17	2.7±0.3 b	18	1.7±0.2 a	11	3.5±0.4 b	13

	Neutral											
	alkyl group						aromatic group				including sulphur	
	Val [g/L]	*R* [%]	Ile [g/L]	*R* [%]	Leu [g/L]	*R* [%]	Tyr [g/L]	*R* [%]	Phe [g/L]	*R* [%]	Met [g/L]	*R* [%]
Feed	3.5±0.1 a		3.2±0.1 ab		5.0±0.1 a		0.7±0.0 a		3.2±0.2 ab		0.7±0.0 a	
NTU-2120	3.6±0.0 a	-3	3.3±0.1 a	-3	5.1±0.1 a	-2	0.7±0.0 a	0	3.3±0.1 a	-3	0.7±0.0 a	0
NTR-7250	3.2±0.1 ab	9	3.0±0.0 ab	6	4.6±0.0 ab	8	0.6±0.0 b	14	3.0±0.1 abc	6	0.7±0.0 a	0
NTR-7410	3.3±0.1 ab	6	3.0±0.1 ab	6	4.6±0.1 ab	8	0.6±0.0 b	14	3.0±0.1 abc	6	0.7±0.0 a	0
NTR-7430	3.2±0.2 ab	9	3.0±0.1 ab	6	4.6±0.2 ab	8	0.6±0.0 b	14	2.8±0.2 bc	13	0.7±0.1 a	0
NTR-7450	2.9±0.4 b	17	2.7±0.4 b	16	4.2±0.5 b	16	0.5±0.1 c	29	2.6±0.3 c	19	0.6±0.1 a	14

	Neutral		Base						Total	*R*
	imino group									
	Pro [g/L]	*R* [%]	His [g/L]	*R* [%]	Lys [g/L]	*R* [%]	Arg [g/L]	*R* [%]	[g/L]	[%]
Feed	3.2±0.1 a		1.0±0.0 a		3.6±0.1 a		2.7±0.1 a		53.5±0.6 a	
NTU-2120	3.3±0.3 a	-3	1.0±0.0 ab	0	3.6±0.0 a	0	2.7±0.1 a	0	54.2±1.0 a	-1
NTR-7250	2.9±0.2 a	9	0.8±0.0 bc	20	2.9±0.0 bc	19	2.2±0.1 b	19	46.3±0.3 b	13
NTR-7410	3.2±0.1 a	0	0.9±0.0 abc	10	3.1±0.1 b	14	2.5±0.1 ab	7	48.0±0.8 ab	10
NTR-7430	3.0±0.1 a	6	0.9±0.0 abc	10	3.0±0.2 b	17	2.4±0.2 ab	11	46.5±2.5 b	13
NTR-7450	2.9±0.4 a	9	0.8±0.1 c	20	2.6±0.3 c	28	2.1±0.2 b	22	40.0±4.9 c	25

The abbreviations are: Glu, glutamic acid; ASP, aspartic acid; Thr, threonine; Ser, serine; Gly, glycine; Ala, alanine; Val, valine; Ile, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Met, methionine; Pro, proline; His, histidine; Lys, lysine; Arg, arginine; For other abbreviation see as Table 2.

The value in each cell is shown as 'mean±SD'.

Different letters in each column for each process soy sauce indicate significant differences based on Tukey-Kramer test ($p < 0.05$).

Table 4. Organic acid components of feed and membrane processed soy sauces.

	Lactic acid [g/L]	*R* [%]	Acetic acid [g/L]	*R* [%]	Pyroglutamic acid [g/L]	*R* [%]
Feed	8.7±0.1 a		1.7±0.0 a		1.9±0.1 a	
NTU-2120	8.6±0.1 a	1	1.7±0.0 a	0	1.9±0.1 a	0
NTR-7250	8.6±0.2 ab	1	1.7±0.0 a	0	1.8±0.1 ab	5
NTR-7410	8.2±0.1 bc	6	1.7±0.1 a	0	1.7±0.1 ab	11
NTR-7430	8.2±0.2 bc	6	1.7±0.0 a	0	1.7±0.0 ab	11
NTR-7450	7.9±0.1 c	9	1.7±0.0 a	0	1.5±0.1 b	21

For abbreviations see as Table 2.

The value in each cell is shown as 'mean±SD'.

Different letters in each column for each process soy sauce indicate significant differences based on Tukey-Kramer test（p < 0.05).

Table 5 Color properties of blend soy sauces.

Feed:Permeate(v/v) (BRP [%])	Absorbance (420 nm)	*R* [%]	CIELAB color space *L**	*a**	*b**
Feed (0)	14.1		18.2±0.0	50.2±0.0	31.3±0.0
4:1 (20)	11.7	17	22.6±0.0	49.8±0.1	38.9±0.1
3:2 (40)	9.1	35	31.2±0.0	49.3±0.0	53.7±0.1
2:3 (60)	6.1	57	43.2±0.0	44.1±0.1	73.5±0.1
1:4 (80)	3.4	76	61.6±0.1	27.6±0.0	88.9±0.1
1:9 (90)	2.2	84	74.7±0.0	14.5±0.0	76.3±0.1
1:19 (95)	1.4	90	84.8±0.1	2.9±0.0	57.2±0.0
Permeate (100)	0.6	96	94.2±0.0	- 4.7±0.0	32.9±0.0

The abreviations are: BRP, blend retio of the NTR-7450 processed soy sauce, for other abbreviation see as Table 2.

The value in each cell is shown as 'mean±SD'.