ELSEVIER

Food Chemistry 65 (1999) 239–243

Food Chemistry

Progressive increase in minimum proton exchange rate with maturation of liquor

Shoichi Okouchi*, Yoshimasa Ishihara, Shigeo Ikeda, Hisashi Uedaira

Department of Materials Chemistry, College of Engineering, Hosei University, Kajino, Koganei, Tokyo 184, Japan

Received 16 February 1998; accepted 7 October 1998

Abstract

Proton exchange rate measurement was carried out by ^{17}O-NMR spectroscopy using mother liquors of malt (aged for 0 to 15 years) and grain (aged for 0 to 8 years) whiskies, and of shochu (aged for 1 to 21 years) and awamori(aged for 0 to 10 years) of Japanese traditional spirituous liquor, and commercially available whiskies (aged for 5 to 21 years) and brandies (aged for 5 to 45 years). Through the examination of proton exchange rate based on ^{17}O-NMR half width - pH curve plots, it has been found that the minimum proton exchange rate increases as the period of maturation of liquor becomes longer. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Proton exchange; NMR, ^{17}O; Maturation; Liquor; Whisky; Brandy; Shochu; Awamori

1. Introduction

Unlike immature liquor, mature liquor has a favourable effect on vital functions of subjects as well as good smell and taste as evidenced in our previous paper (Haseba et al., 1993). In an experiment on mice, the latter resulted in earlier sobering and less reduction in rectal temperature and longer time to death due to acute alcoholism. We presumed that maturation alleviates alcoholic toxicity of liquor.

By leaving distilled alcohol in a storage cask for a long period, it is transformed into 'spirituous liquor', not just aqueous alcohol that is irritant and undrinkable. Based on the speculation that reactions of eluates from cask wood and constituents of liquor might account for this transformation, microanalyses (Schoeneman et al., 1971; Baldwin & Andreasen, 1974) have been made to identify trace components. Nevertheless, 'spirituous liquor' cannot be produced just by mixing identified components with aqueous ethanol. For elucidation of liquor maturation, it is therefore necessary to turn to a basic investigation of solution structure of ethanol and water which are approx. 99% of the components of 'spirituous liquor'.

In studies of maturation, contradictory speculations have so far been reported about the relationship between maturation and molecular cluster size in solution, i.e. the cluster size decreases with maturation (Tamaki et al., 1986; Aishima et al., 1988, 1991; Matsushita et al., 1990) or contrarily it increases with maturation (Akaboshi & Ohkuma, 1984; Saita, 1993; Nishi & Saita, 1994). Matsushita et al. (1990) reported the result that ^{17}O-NMR half width of water contained in mature liquor was narrowed by maturation. Based on this result, they suggested that maturation reduced the degree of water molecular clustering and made molecular motion faster. On the contrary, Akaboshi and Ohkuma (1984) reported the result of ^{1}H-NMR measurement at 241K, indicating that the half width increased as the maturation period of commercial liquor was longer. On the basis of this result, they presumed that the degree of molecular clustering in aqueous solution increased with progress of maturation. Also, Nishi and Saita (1994), based on an analysis in which liquor samples were atomized in vacuum, expanded adiabatically and analyzed by mass spectrometry (Nishi et al., 1988), pointed out that the degree of molecular clustering increased with maturation.

In our previous paper (Okouchi et al., 1994a,b,c, 1995) we revealed that ^{17}O-NMR half width represents as a function of proton exchange rate and rotational motion of water molecules, and that the half width of water and aqueous ethanol solution is widened appreciably in the vicinity of neutrality (a difference between

---

* Corresponding author.

PII: S0308-8146(98)00235-0

the maximum and minimum half widths is as large as approx. 100 Hz) due to possible changes in proton exchange rate attributable to alteration in pH. The proton exchange rate represents how fast protons of water molecules are exchanged with one another or those of hydroxyls of alcohol molecules, successively. Then, we pointed out a fallacy in the presumption that a value of ^{17}O- and ^{1}H-NMR half widths directly represents the degree of molecular clustering.

Through our present measurement of maximum half width values at different pH levels, we found that variation up to approx. 20 Hz (10%) occurs depending on the period of maturation. Since a value of T_1 (spin-lattice relaxation time) is constant under a condition that the concentration of ethanol remains the same (Okouchi et al., 1994b), variation in maximum half width with the degree of maturation must be caused by possible changes in proton exchange rate (Meiboom, 1961; Halle & Karlstrom, 1983; Dratsaoglu et al., 1990; Okouchi et al., 1994a). Therefore, we attempt to explain the maturation mechanisms in terms of proton exchange rate. If the proton exchange rate is proved to change with maturation, it will become possible to unravel solution structure modifications caused by maturation.

2. Materials and methods

Samples of mature liquors used were mother liquors of malt (aged for 0 to 15 years) and grain (aged for 0 to 8 years) whiskies, and of shochu (aged for 1 to 22 years) and awamori (aged for 0 to 10 years), and commercially available scotch whiskies (aged for 5 to 21 years) and brandies (aged for 5 to 45 years). Shochu and awamori are Japanese traditional spirituous liquor made from grain. The ages of maturity of commercial liquors were estimated from their grades using the method proposed by Ohtsuka et al. (1979). Aliquots having different pH values of these samples and aqueous ethanol solutions were prepared with each 0.5 mol/dm^3 of hydrochloric acid and sodium hydroxide.

The half width, Hw, and spin-lattice relaxation time, T_1, of the naturally abundant ^{17}O of water molecules in the aliquots were measured by ^{17}O-NMR using a JEOL JNM-EX270 spectrometer operating at 36.63 MHz at 298 K. The ethanol concentration was measured with a Perkin-Elmer Head Space Analyzer 8500 and an Anton Paar Digital Density Meter, DMA60/602.

3. Results and discussion

The proton exchange processes in aqueous alcohol (Luz et al., 1959) can be expressed by Eqs. (1–7):

$$\mathrm{H_3O^+ + H_2O \overset{k_1}{\rightleftarrows} H_2O + H_3O^+} \tag{1}$$

$$\mathrm{OH^- + H_2O \overset{k_2}{\rightleftarrows} H_2O + OH^-} \tag{2}$$

$$\mathrm{C_2H_5OH + H_3O^+ \underset{k'_3}{\overset{k_3}{\rightleftarrows}} C_2H_5OH_2^+ + H_2O} \tag{3}$$

$$\mathrm{C_2H_5OH + OH^- \underset{k'_4}{\overset{k_4}{\rightleftarrows}} C_2H_5O^- + H_2O} \tag{4}$$

$$\mathrm{C_2H_5OH_2^+ + C_2H_5OH \overset{k_5}{\rightleftarrows} C_2H_5OH + C_2H_5OH_2^+} \tag{5}$$

$$\mathrm{C_2H_5O^- + C_2H_5OH \overset{k_6}{\rightleftarrows} C_2H_5OH + C_2H_5O^-} \tag{6}$$

$$\mathrm{C_2H_5OH + H^*OH \overset{k_7}{\rightleftarrows} C_2H_5OH^* + HOH} \tag{7}$$

where k_1 to k_7, k'_3 and k'_4 are the rate constants of proton exchange. Eqs. (1) and (2) represent the water–water proton exchange processes, Eqs. (3) and (4) express the ethanol–water proton exchange processes, Eqs. (5) and (6) show the ethanol–ethanol proton exchange processes, and Eq. (7) indicates the nonionic ethanol–water proton exchange process.

In case of water, the relationship between the half width Hw of ^{17}O-NMR signal and the proton exchange rate R (Halle et al., 1983; Okouchi et al., 1994a) can be defined by Eqs. (8) and (9).

$$\mathrm{Hw} = 4\pi J^2/R + 1/\pi T_1 \tag{8}$$

$$R = -(1/[\mathrm{H_2O}])\mathrm{d}[\mathrm{H_2O}]/\mathrm{d}t = k_1[\mathrm{H_3O^+}] + k_2[\mathrm{OH^-}] \tag{9}$$

where J is the coupling constant between proton and ^{17}O. The Hw–pH curve of water has a hill shape, i.e. the half width is narrow and constant on the acidic and basic sides, and it is maximized in the vicinity of neutrality (Halle & Karlstrom, 1983; Dratsaoglu et al., 1990; Okouchi et al., 1994b).

On the other hand, the proton exchange rate R for ^{17}O-NMR in aqueous alcohol can be given by Eq. (10).

$$R = \mathrm{k_a}[\mathrm{H_3O^+}] + \mathrm{k_b}[\mathrm{OH^-}] + k_7[\mathrm{C_2H_5OH}] \tag{10}$$

where $\mathrm{k_a} = k_1 - \mathrm{K}_3 k'_3/[\mathrm{H_2O}] + k_3[\mathrm{C_2H_5OH}]/[\mathrm{H_2O}]$ and $\mathrm{k_b} = k_2 - k'_4[\mathrm{C_2H_5OH}]/\mathrm{K}_4[\mathrm{H_2O}] + k_4[\mathrm{C_2H_5OH}]/[\mathrm{H_2O}]$ with the equilibrium constant $\mathrm{K}_3 = [\mathrm{C_2H_5OH_2{}^+}][\mathrm{H_2O}]/[\mathrm{H_3O^+}]$ and $\mathrm{K}_4 = [\mathrm{C_2H_5OH}][\mathrm{OH^-}]/[\mathrm{C_2H_5O^-}][\mathrm{H_2O}]$, respectively.

Some studies have reported that the rate constants k_1 and k_2 of proton exchange in water were on the order of 10^9–10^{10} [dm$^3\cdot$mol$^{-1}\cdot$s^{-1}] at 298K (Meiboom, 1961; Halle & Karlstrom, 1983; Dratsaoglu et al., 1990; Okouchi et al., 1994a). As to other rate constants of proton exchange, Luz et al. (1959) reported that constants k_3, k_4 and k_6 at 293 K were in the order of 10^6 [$dm^3 \cdot mol^{-1} \cdot s^{-1}$], k_5 was in the order of 10^8 [$dm^3 \cdot mol^{-1} \cdot s^{-1}$], and k_7 was approx. 0.8 [$dm^3 \cdot mol^{-1} \cdot s^{-1}$]. The equilibrium constants K_3 and K_4 were 0.059 (Guss & Kolthoff, 1940) and 1.3 (Caldin & Long, 1954), respectively. By taking these constants into account, the third term on the right side of Eq. (10) can be neglected in the case of the alcohol concentrations used in this work. Hence, the proton exchange rate can be expressed by Eq. (11), which is identical to that for water.

$$R = k_a[H_3O^+] + k_b[OH^-] = k_a[H_3O^+] + k_bK_w/[H_3O^+] \tag{11}$$

where K_w is the ionic product of water. The half width Hw of aqueous alcohol is expressed as a function of pH by substituting Eq. (11) in Eq. (8). Since the coupling constant J and T_1 are independent of pH, differentiating Eq. (8) by $[H_3O^+]$ for zero gives Eqs. (12) to (14) to a pH value (pH_{max}) at maximum half width (Hw_{max}) as shown below.

$$\begin{aligned}\Delta Hw_{max} &= Hw_{max} - 1/\pi T_1 = 4\pi J^2/R_{min} \\ &= 2\pi J^2/(k_ak_bK_w)^{0.5}\end{aligned} \tag{12}$$

$$k_a = 2\pi J^2/\Delta Hw_{max} \times 10^{pH\,max} \tag{13}$$

$$k_b = 2\pi J^2/\Delta Hw_{max} \times 10^{14-pH\,max} \tag{14}$$

$$pH_{max} = \log(k_a/k_bK_w)^{0.5} \tag{15}$$

where R_{min} is the minimum proton exchange rate. It is therefore possible to determine a value of minimum proton exchange rate, R_{min}, using ΔHw_{max} on the Hw–pH curve plotted with respect to experimentally altered pH values of liquor.

Fig. 1 demonstrates an example of the Hw–pH curve of mature liquor, which is very similar to that of water, and ΔHw_{max} can be determined as indicated in Fig. 1. This similarity of mature liquor to water can be interpreted as being due to the approximate equality in hydrogen bonding strength between ethanol and water (Nishi et al., 1988).

Fig. 2 shows ΔHw_{max} data for malt and grain whiskies, shochu and awamori, commercial liquors of scotch whiskey and brandy, and aqueous ethanol samples at various concentrations. At the same concentration level, ΔHw_{max} of any mature whiskey and brandy samples was smaller than that of its comparable aqueous ethanol samples.

Also, to examine an effect of dissolved trace components on ΔHw_{max}, we prepared aqueous ethanol samples each containing acetic acid (0.08%) as acids, ethylacetate (0.15%) as esters, acetaldehyde (0.02%) as aldehydes and fusel oil (0.3%) in the same manner as that taken by Akaboshi and Ohkuma (1984), and carried out ΔHw_{max} measurement of each sample. The parenthesized concentration values are almost equal to the maximum concentrations of these components contained in commercial whiskey and brandy. Acetic acid reduces its

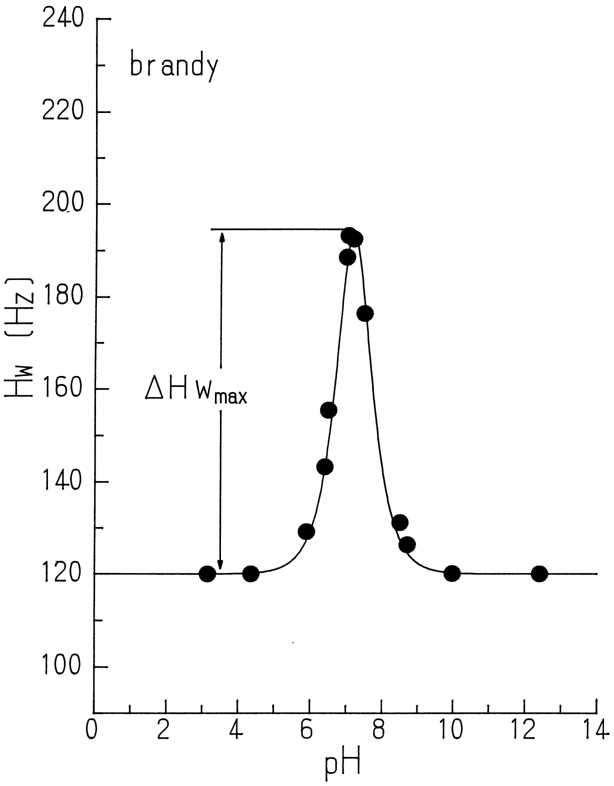

Fig. 1. Plots of Hw–pH curve for mature liquor (commercial brandy) at 298 K.

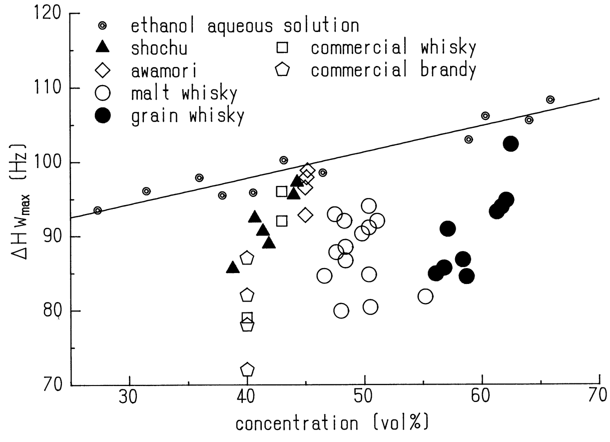

Fig. 2. Relationship between ΔHw_{max} and alcohol concentration for mature liquors and aqueous ethanol solution at 298 K.

ΔHw_{max} in proportion to concentration. But, at the maximum concentration level of acetic acid in commercial mature liquor, this ΔHw_{max} reduction effect of acetic acid was far smaller than that of maturation. Other trace components had no significant effect on ΔHw_{max}.

Fig. 3 shows the relationship between periods of maturation and maximum half width values attained by evaluating ΔHw_{max} in Fig. 2 on a basis of 43 vol% alcohol concentration. The evaluated maximum half widths, ΔHw_{max}, decrease as the period of maturation becomes longer.

Fig. 4 shows the relationship between periods of maturation and values of minimum proton exchange rate, R_{min}, estimated by Eq. (11) with the proton exchange rate constants k_a and k_b in Eqs. (13) and (14) usingΔHw_{max} in Fig. 3. As can be seen in Fig. 4, the proton exchange rate R_{min} increases as the period of maturation becomes longer, i.e. there is a correlation between R_{min} and maturation. The broken line in Fig. 4 indicates the minimum proton exchange rate of water. Some mature liquor samples had a value of minimum proton exchange rate faster than that of water. In contrast, the spin-lattice relaxation time T_1 has proved to be independent of maturation if the concentration of ethanol is constant (Okouchi et al., 1994b). It can therefore be conceived that maturation has no effect on molecular motion (rotation) in aqueous solution. Also, effect of the chemical shift by ^{1}H-NMR on maturation was not observed (Akaboshi & Ohkuma, 1984; Mizuno et al., 1995).

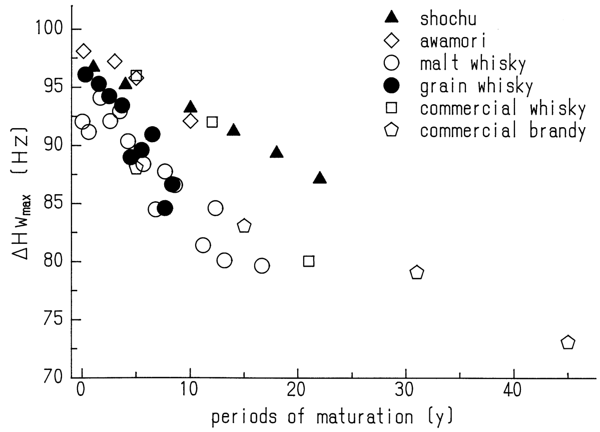

Fig. 3. Effects of maximum half widths, ΔHw_{max}, on the periods of maturation for maturation liquors based on 43% alcohol concentration.

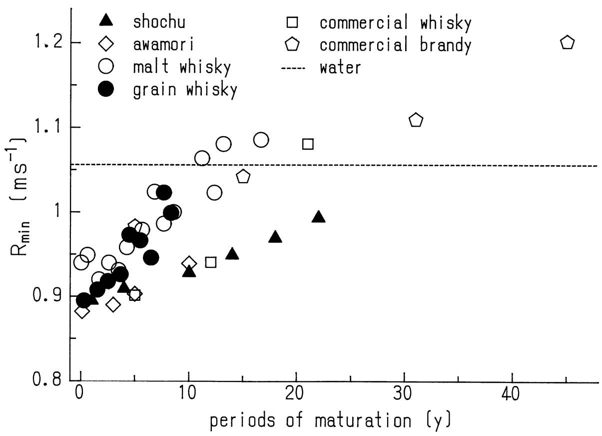

Fig. 4. Effects of minimum proton exchange rate, R_{min}, on the periods of maturation for maturation liquors based on 43% alcohol concentration.

4. Conclusion

From the above discussion, we think that the liquor maturation process modifies the solution structure so that the protons are exchanged/shifted readily, instead of affecting molecular rotational motions. Taking into consideration the work of Nishi and Saita (1994), suggesting that the degree of molecular clustering increases with maturation, it is presumed that alcohol and water molecules combinationally form a hydrogen-bonded network structure in which exchanges of protons are liable to occur, resulting in an increase in molecular cluster size. Although the details of molecular clustering by maturation are not yet apparent, a solution structure having a higher minimum proton exchange rate is considered to influence enzymic reactions and information transfer in an organism. We therefore conclued that favourable vital reactions of mice to mature liquor (Haseba et al., 1993) reflect solution structure modifications resulting from an increase in the minimum proton exchange rate.

References

Aishima, T., & Matsushita, K. (1988). Measurements of food aging by multinuclear NMR. In G. Charalambous (Ed.), *Frontiers of Flavor* (pp. 321–337). Amsterdam: Elsevier.

Aishima, T., Nishikawa, K., & Matsushita, K. (1991). Measurements of brandy aging using ^{17}O-NMR and small angle X-ray scattering. 25th Aji to Nioi no Sinpojiumu Ronbunsyu (pp. 61–64).

Akaboshi, R., & Ohkuma, K. (1984). NMR spectra of hydroxyl protons in aged spirits. *Nippon Nougeikagaku Kaishi, 58*, 357–365.

Baldwin, S., & Andreasen, A. A. (1974). Congener development in bourbon whisky matured at various proofs for twelve years. *J. AOAC., 57*, 940–950.

Caldin, E. F., & Long, G. (1954). The equilibrium between ethoxide and hydroxide ions in ethanol and ethanol–water mixtures. *J. Chem. Soc.*, 3737–3742.

Dratsaoglu, J., Hauber, S., Hertz, G., & Muller, J. (1990). Water proton exchange rates in solutions of strong electrolytes effected by H^+ and OH^- additions. *Z. Phys. Chem. Neue Folge., 168*, 12–42.

Guss, L. S., & Kolthoff, I. M. (1940). The distribution of protons between water and other solvents. *J. Am. Chem. Soc., 62*, 1494–1496.

Halle, B., & Karlstrom, G. (1983). Prototropic charge migration in water. Faraday Trans. 2. *J. Chem. Soc., 79*, 1031–1046.

Haseba, T., Matsushita, K., Asakura, T., Kameyama, K., Tamaki, T., Okouchi, S., Watanabe, T., & Uedaira, H. (1993). Diminution of biological reactivity of ethanol by changing the solution structure by weak ultrasonication. *Alcohol. Clin. Exp. Res., 17*, 963–967.

Luz, Z., Gill, D., & Meiboom, S. (1959). NMR study of protolysis kinetics in methanol and ethanol. *J. Chem. Phys., 30*, 1540–1545.

Matsushita, K., Nishina, M., & Hori, E. (1990). Metabolic process of the distilled spirit treated with ultrasonic wave-^{1}H-NMR spectroscopic study of rat serums. *Mag. Reson. Medicine, 1*, 56–59.

Meiboom, S. (1961). Nuclear magnetic resonance study of the proton transfer in water. *J. Chem. Phys., 34*, 375–388.

Mizuno, K., Miyashita, Y., Shinoda, Y., & Ogawa, H. (1995). NMR and FT-IR studies of hydrogen bonds in ethanol–water mixtures. *J. Phys. Chem., 99*, 3225–3228.

Nishi, N., & Saita, S. (1994). Clusters in whisky. *Kagaku to Kogyo, 47*, 168–171.

Nishi, N., Koga, K., Ohshima, Yamamoto, K., Nagashima, U., & Nagami, K. (1988). Molecular association in ethanol–water mixtures studied by mass spectrometric analysis of clusters generated through adiabatic expansion of liquid jets. *J. Am. Chem. Soc., 110*, 5246–5255.

Ohtsuka, K., Iki, I., & Totsuka, A. (1979). Types and components of alcoholic beverages—relation between aging grade and components of cognac brandy. *Jyouzou Kyokaishi, 74*, 549–554.

Okouchi, S., Ishihara, Y., & Uedaira, H. (1994a). ^{17}O-NMR of electrolyte aqueous solutions and tap water processed by commercial purifiers and reformers. *Japan Soc. Water Environ., 17*, 517–526.

Okouchi, S., Ishihara, Y., Inaba, M., & Uedaira, H. (1994b). ^{17}O-NMR line width of distilled spirits. *Nippon Nougeikagaku Kaishi, 68*, 1215–1218.

Okouchi, S., Yamanaka, K., Ishihara, Y., Yanaka, T., & Uedaira, H. (1994c). Relationships between water qualities and treatments in ultra pure water production system. *Water Sci. Tech., 30*, 237–241.

Okouchi, S., Ishihara, Y., Inaba, M., & Uedaira, H. (1995). Changes in proton exchange rate in commercial spririts with age. *Nippon Nougeikagaku Kaisi, 69*, 679–683.

Saita, S. (1993). Taste of whisky. *Hyoumenkagaku, 14*, 238–242.

Schoeneman, R. L., Dyer, R. H., & Earl, E. M. (1971). Analytical profile of straight bourbon whiskies. *J. AOAC., 54*, 1247–1261.

Tamaki, T., Matsushita, K., Hioka, K., & Takamiya, Y. (1986). ^{1}H and ^{17}O-NMR studies of distilled spirits: new methods for assessing the aging process of awamori. *Nippon Nougeikagaku Kaishi, 60*, 191–197.