Fermentation of *Monascus purpureus* on Bacterial Cellulose-nata and the Color Stability of *Monascus*-nata Complex

F. SHEU, C.L. WANG, AND Y.T. SHYU

ABSTRACT: Nata, a bacterial cellulose produced by *Acetobacter aceti ssp. xylinum*, was colored by means of fermentation with *Monascus purpureus*. Scanning electron microscopy (SEM) observations showed that the *Monascus* mycelium could grow through the cellulose network of nata. Rice powder as a major carbon source and monosodium glutamate (MSG) as a nitrogen source gave an appealing coloration after 12 d of fermentation at 30 °C. Compared to dyed nata, the color of the *Monascus*-nata complex had better resistance to washing, heat, freezing, acidification, and alkalization. A 66.1% decolorization was found under irradiation with 366 nm ultraviolet light after 36 h. The *Monascus*-nata complex has the potential to be a new vegetarian foodstuff.

Key words: *Monascus purpureus*, bacterial cellulose, nata

Introduction

NATA, A WHITE GELATINOUS BACTERIAL CELLULOSE, IS PRODUCED by *Acetobacter aceti ssp. xylinum* through fermentation of fruit juices. Nata is considered one of the traditional holiday foods among Philippine people and is also popular in other Asian countries, including Indonesia, Japan and Taiwan, due to its distinctly soft texture and high fiber content (Okiyama and others 1992a; Roberfroid 1993). The biogenesis and fermentation for nata production have been well studied (Cannon and others 1991; Banzon and others 1990; Okiyama and others 1992a; Embuscado and others 1994). However, few studies have reported on the coloring of nata. The coloring of nata can improve its appearance and provide more variety of applications as a foodstuff. Due to its uniform 1↦4β-glucan structure, nata is difficult to dye with only hydroxyl groups available.

Traditionally grown on steamed rice, bread, brans, and cereal meats, *Monascus purpureus* produces true red mycelium and pigments through solid culture and has been used in the Orient for many centuries to color and flavor food and beverages (Johns and Stuart 1991; Chen and Johns 1993). The fermentative conditions influencing the pigment production have been extensively studied (Su 1978; Wong and others 1981; Lin and Demain 1991; Chen and Johns 1993). Pigments from *M. purpureus* are very stable and suitable for the use as a food additive (Fink-Gremmels and others 1991; Fabre and others 1993; Juzlova and others 1996). Moreover, the *Monascus* fungi contains therapeutic metabolites such as monakolin K (mevinolin) (Juzlova and others 1996), which is regarded as a healthful nutritious food in Asia.

The objective of this study was to develop a new foodstuff *Monascus*-nata complex which was prepared by fermenting with bacterial cellulose (nata) with *M. purpureus*. The structure of *Monascus*-nata complex was observed using scanning electron microscopy (SEM). The parameters affecting the fermentation and the coloration of *M. purpureus* were studied. The color stability of this complex was also examined and compared with pigment-dyed nata.

Results and Discussion

Structure of *Monascus*-nata complex

For structural observation, *M. purpureus* was fermented on pieces of nata for 12 d at 30 °C until the red color appeared. Fig. 1 shows the SEM micrograph of nata, *Monascus* mycelium and *Monascus*-nata complex. The capillaries of the nata network (about 0.5 to 1 μm, Fig. 1C) (Okiyama and others 1992b) were wider than the diameter of *Monascus* mycelium (about 0.1 to 0.5 μm, Fig. 1B). Therefore, the *Monascus* fungi could extend its mycelium through the capillaries of nata to be held in the network structure for further growth. After fermentation, a new structure composed mainly of mycelium network was formed. The original cellulose network of nata still filled the empty space between the mycelium (Fig. 1A). However, the mycelium of *M. purpureus* could not grow well outside the piece of nata without the structural support of nata in a shaking environment. The porous network structure of nata is probably helpful for the initial growth of *M. purpureus*, and the original shape of the nata was observed to limit the final shape of *Monascus*-nata complex.

According to the SEM micrography (Fig. 1), the permanent red of *Monascus*-nata complex was probably due to the red mycelium of *M. purpureus*. Water-soluble pigments produced extracellularly by *M. purpureus* (Lin and others 1992) were not believed to be a major contributor to coloration of the complex. This was due to the much lighter color of the fermentation broth than the complex. A lack of bonding between the water-soluble pigment and free hydroxyl groups of cellulose was a possible reason for this.

Fermentation conditions for coloration

Carbon source, nitrogen source, and pH have been shown to influence pigment production by *Monascus purpureus* (Su 1978; Wong and others 1981; Lin and Demain 1991, Chen and Johns 1993). Therefore, the effect of these compounds on coloration of *Monascus*-nata complex was studied. When glucose, rice powder, maltose, or sucrose was used as a sole carbon source (5%), the

© 2000 Institute of Food Technologists

most appealing and bright red color was observed with the rice powder medium after 12 d (Fig. 2A). Fermentation using maltose and glucose as carbon sources yielded very dark liver color while sucrose medium produced a light and uneven red, especially in a crosscut observation. For nitrogen source experiment, 1.5 % MSG medium produced an appealing red appearance while other nitrogen sources produced faint or foggy reds (Fig. 2B). Additionally, initial pH value (3.5 to 10.5) and temperature (15 to 35 °C) had no significant effect ($p > 0.05$) on coloration (data not shown). Minor inorganic elements (calcium, potassium, zinc and manganese) did not show any obvious effect on coloration (data not shown). This result might be due to a crude carbon source, rice powder, which, generally composed of 82% carbon, 17% water, 0.4% nitrogen, 0.3% phosphoric, and 0.3% potassium (Juliano 1986) was used as a major carbon source during fermentation.

Fig. 3 shows the progress of coloration on surface and center of the *Monascus*-nata complex. The red coloration in the center proceeded slower than on the surface. After 12 d of fermentation, red *Monascus*-nata pieces could be obtained with a fully red surface, and the central point appeared a ruby color. The results generally followed previous research (Wong and others 1982; Lin and Demain 1991, Chen and Johns 1993). However, it must be noted that growth and pigment production are strain-specific among *Monascus purpureus* (Lin and Demain 1991). According to the results above, further experiments of color stability were carried out using a medium composed of 5% rice powder and 1.5% MSG for fermentation at 30 °C for 12 d.

Color stability

The color stability of *Monascus*-nata complex was examined and compared with *Monascus* pigment and artificial pigment (Cochineal Red A) dyed nata pieces. The complex showed good resistance to washing decoloration (Fig. 4), 93.3% and 96.8% coloration were retained after 5 d of washing on the surface and the central point, respectively. In contrast, only 61.6 % of coloration on *Monascus* pigment-dyed nata and 27.8 % of coloration of artificial pigment-dyed nata were retained. These results also showed that the complex was only partially dyed by the extracellular pigments secreted by *M. purpureus* during fermentation. There was no appreciable color change of any sample after autoclaving, −20 °C freezing or pH 2.5 acidification (data not shown). However, at pH 12.5, the complex and *Monascus* pigment-dyed nata were significantly ($p < 0.05$) decolorized by 9.6 % and 7.2 %, respectively. This result was in contrast to a study by Lin and others (1992). In their report, pigments produced by *M. purpureus* were stable to pH 12.

Fading of *Monascus*-nata complex was observed under irradiation with 366 nm ultraviolet light for 36 h (Fig. 5). A 66.1 % decoloration of the complex was greater than the nata dyed by water-soluble *Monascus* pigment. The UV decolorization of cell-bound *Monascus* pigment and the inferior UV resistance to the water-soluble *Monascus* pigment had been reported previously (Sweeny and others 1981; Lin and others 1992). Therefore, packaging of the complex is important to avoid the UV decoloration by sunlight.

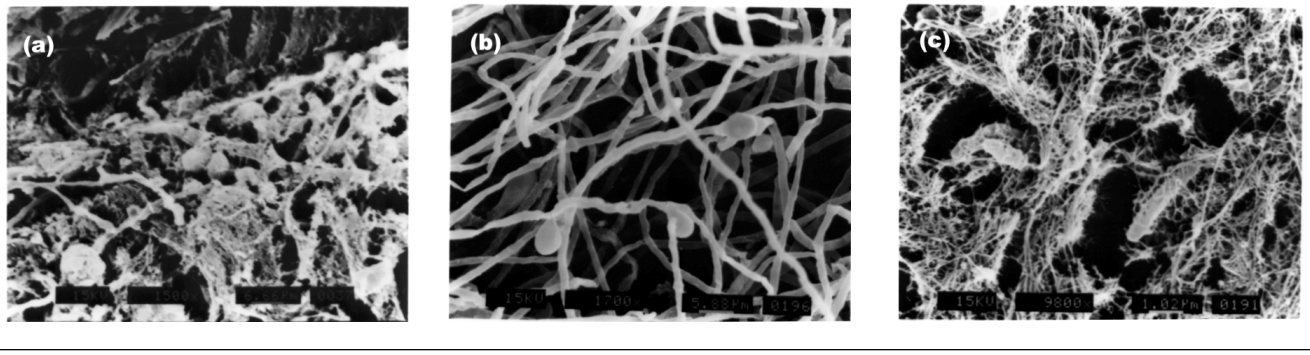

Fig. 1—SEM micrography of (A) *Monascus*-nata complex (1500×); (B) mycelium of *Monascus purpureus* (1700×); (C) the cellulose network of nata (9800 ×).

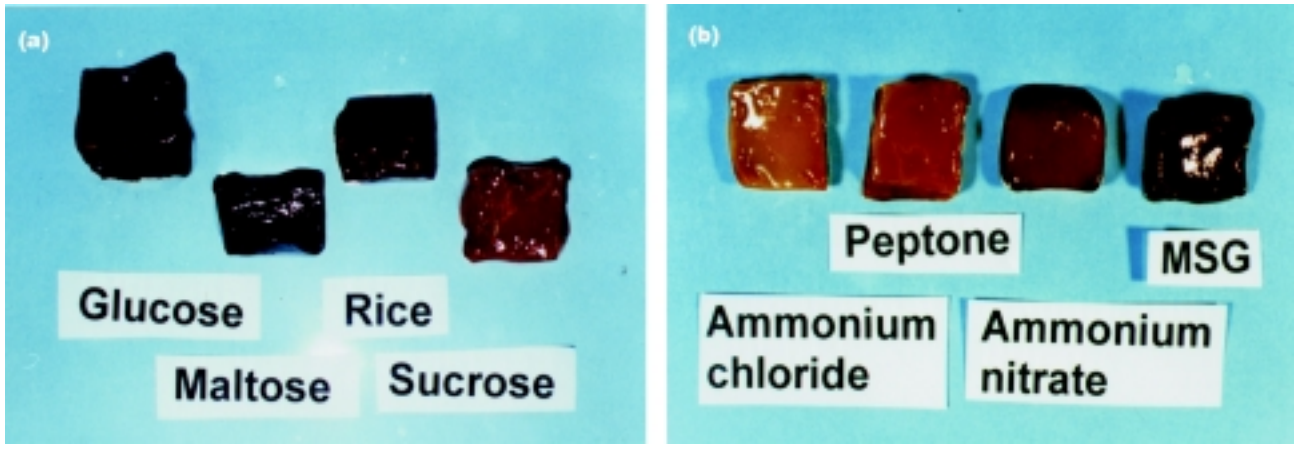

Fig. 2—Influences of (A) different carbon sources and (B) different nitrogen sources on the coloration of *Monascus*-nata complex. For carbon source experiments, MSG (15 g/L) was used as a nitrogen source; for nitrogen source experiments, rice powder was used as a carbon source.

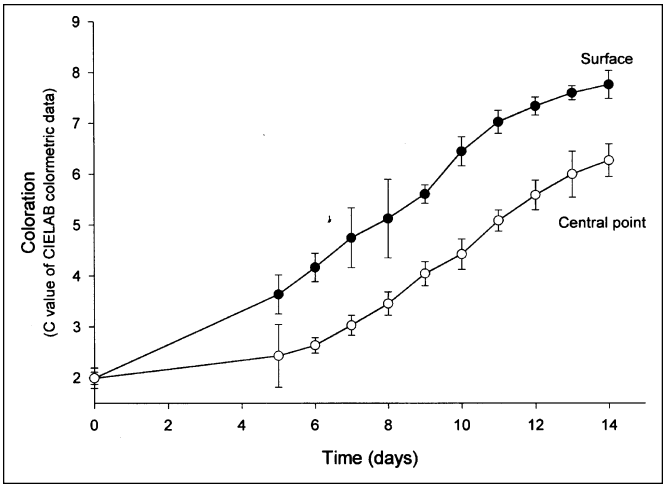

Fig. 3—The coloration on the surface (●) and central point (○) of *Monascus*-nata pieces during fermentation, with the C value of CIELAB colormetric data. *Monascus purpureus* was fermented on 2 × 2 × 1.5 cm³ nata pieces in a medium consisting of 5% rice power and 1.5% MSG at 30 °C. Vertical bar represents S. D. (n = 3).

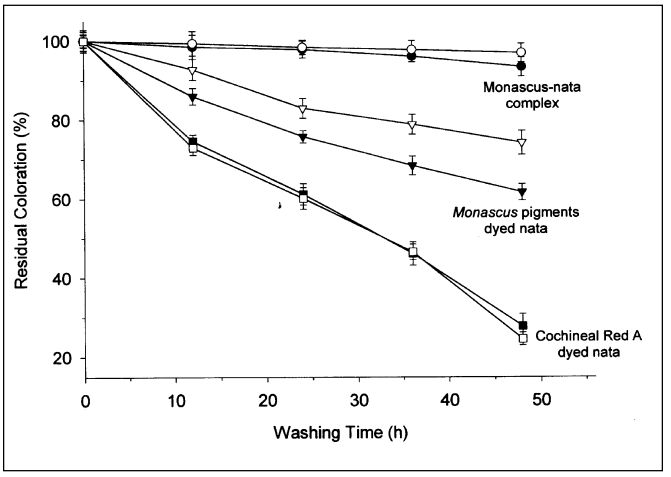

Fig. 4—Decoloration by washing on the surface (●) or central point (○) of *Monascus*-nata complex, the surface (▼) or central point (▽) of *Monascus* pigment dyed nata and the surface (■) or central point (□) of Cochineal Red A dyed nata. *Monascus*-nata complex was produced by fermenting *M. purpureus* on 2 × 2 × 1.5 cm nata pieces using a medium composing of 5% rice powder and 1.5% MSG at 30 °C for 12 d. The residual coloration was calculated based on the C value of CIELAB colormetric data. Vertical bar represent S. D. (n = 3).

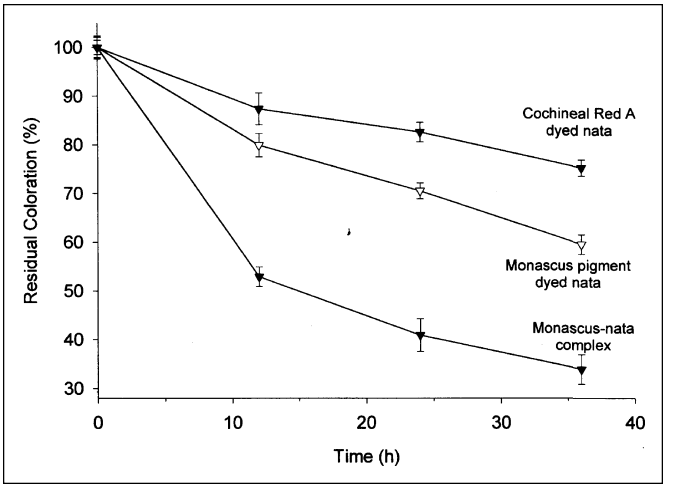

Fig. 5—Decoloration by UV irradiation on *Monascus*-nata complex (●), *Monascus* pigment dyed nata (▼) and Cochineal Red A dyed nata (■). *Monascus*-nata complex was produced by fermenting *M. purpureus* on 2 × 2 × 1.5 cm nata pieces using a medium composing of 5% rice powder and 1.5% MSG at 30 °C for 12 d. The residual coloration was calculated based on the C value of CIELAB colormetric data. Vertical bar represents S. D. (n = 3).

Conclusions

THE PRODUCTION OF A NEW *MONASCUS*-NATA COMPLEX achieved the indelible coloration of nata. The coloration of this complex was influenced by the carbon and nitrogen sources during fermentation, but other parameters were not found to affect it significantly. In addition, the complex showed better color stability than Cochineal Red A or *Monascus* pigments dyed nata in the resistance to washing, autoclaving, acidification, and alkalization, but inferior in UV resistance. The mycelium is regarded as the major contributor for color.

Monascus-nata complex, which combined the properties of nata and *Monascus* fungi, showed the potential to be a new foodstuff as vegetarian meat or seafood replacement. Growth of *Monascus* mycelium did not impart a flavor on this new product and made it to be a good base as a flavor-added food. The color and texture of the complex were like liver, lean meat, or tuna meat (sashimi). It also provides high fiber content, limited calories, and healthful nutrients. Moreover, the waste broth of fermentation could be further used for a source of water-soluble pigments.

Materials and Methods

Materials and chemicals

Bacterial cellulose (nata) in acetic acid was a gift from the Cana Food Company (Tainan, Taiwan). The nata was cut into 2 cm × 2 cm × 1.5 cm pieces and then washed with running water for 4 h at room temperature to remove the acetic acid residue before fermentation.

Water-soluble *Monascus* pigment powder was obtained from Zhejiang Chemicals Corp. (Hangzhou, China). Artificial red pigment Cochineal Red A (PONCEAU 4R) was obtained from E. Merck (Schuchardt, Germany).

Nata pieces were soaked in a solution containing 1 % Cochineal Red A pigment or 1 % *Monascus* pigment for 5 d. The pigment-dyed nata was used for the experiments of color stability.

Organism and cultivation

Monascus purpureus Went CCRC3150 was obtained from CCRC (Culture Collection and Research Center, Ching-Chu, Taiwan) and maintained on potato dextrose agar (PDA) slopes (Difco Lab., Detroit, Mich., U.S.A.) at 4 °C. A distilled water suspension from 6-d old PDA slope of *M. purpureus* grown at 30 °C was used for inoculation.

Fermentation

Glucose-monosodium glutamate (MSG) medium described by Su (1978) was used with a glucose concentration of 50 g/L. When required, maltose, rice power, or sucrose (50 g/L) was substituted for glucose, whereas peptone, ammonium nitrate, or ammonium chloride (15 g/L) was substituted for MSG. The pH of the medium was adjusted using 1 M HCl or 1 M NaOH. Ten nata pieces were put into the medium (150 mL) in a 500

mL flask. After sterilization at 121 °C for 20 min, each flask was inoculated with 2.0 mL of *M. purpureus* in distilled water. Fermentation was carried out on a rotary shaker at 150 rpm at 30 °C.

Structure observation

The method of Okiyama and others (1992b) was modified and used for the sample preparation. After 5 d of fermentation, the *Monascus*-nata complex was immersed in 5% glutaraldehyde overnight. The sample was dried using serially diluted ethanol (50 % to 100%) and anhydrous acetone, and then lyophilized using a SPI-DRY™ critical point dryer (Structure Probe Inc., West Chester, Pa., U.S.A.). The dried sample was removed from the specimen stubs and was coated using a SC502 SEM coating system (Bio-Rad Laboratories Inc., Chicago, Ill., U.S.A.). A SEM-ABT-60 (Topcon Co., Tokyo, Japan) scanning electron microscope was used for observation at 15 kV.

Color determination

A CIELAB colorimetry system, which was described in detail by Fabre and others (1993), was used for color determination. Coloration was determined with a Color-Pen™ handy color difference photometer (Dr. Bruno Lange GmbH, Berlin, Germany), which measured the spectrum of reflected light and converted it to a set of color coordinates (L, a and b values). The C value, calculated from the Eq 1, is a measure of the saturation or purity of the color.

$$C = (a^2 + b^2)^{1/2} \quad (1)$$

The percentage (%) of decoloration was calculated from the relationship:

$$\text{Percent (\%)} = (C_{\text{after treatment}} / C_{\text{before treatment}}) \times 100 \quad (2)$$

The measurement of each *Monascus*-nata complex sample was repeated 10 times.

Examination of the color stability

The color resistance of *Monascus*-nata complex and dyed nata to washing, heat, freezing, acidification, alkalization, and UV treatments were compared. For washing resistance evaluation, samples were washed under running tap water without any detergent at a constant flow rate of 10 L/h for 48 h. To examine the thermal stability of the color, the samples were autoclaved at 121 °C for 1 h or frozen at −20 °C for 5 d before analysis. For acidification and alkalization experiments, the samples were soaked in solutions at pH 2.5 or 12.5 for 5 d. To study the UV resistance, samples were exposed to ultraviolet light (366 nm) with a U60-110 Ultra-Violet lamp (Jepson Bolton & Co Ltd, Watford Herts, U.K.) for 36 h. The residual coloration on the surface of each sample was determined.

Statistical analysis

Each treatment was carried out in triplicate (n = 3), and all the experiments were repeated at least twice. Since the results of these experiments are similar, the result of only one experiment is shown in this manuscript. Significance of data was analyzed by one dimensional analysis of variance. Difference among the mean values was tested using the Least Significant Difference (LSD) multiple range test (Steel and Torrie 1980). Values were considered significant when $p < 0.05$.

References

Banzon JA, Gonzalez ON, Dedeon SY, Sanchez OC. 1990. Nata de coco. In: Banzon JA, editor. Coconut as food. Diliman, Quezon City, Philippines: Philippine Coconut Research and Development Foundation. p 185-190.

Cannon RE, Anderson SM. 1991. Biogenesis of bacterial cellulose. Crit. Rev. Microbiol. 17(6):435-447.

Chen M, Johns MR. 1993. Effect of pH and nitrogen source on pigment production by *Monascus purpureus*. Appl. Microbiol. Biotechnol. 40(1):132-138.

Embuscado ME, Marks JS, BeMiller JN. 1994. Bacterial cellulose. I. Factors affecting the production of cellulose by *Acetobacter xylinum*. Food Hydrocolloid. 8(5):407-418.

Fabre CE, Santreer AL, Loret MO, Baberian R, Pareilleux A, Goma G, Blanc PJ. 1993. Production and food applications of the red pigments of *Monascus* ruber. J. Food Sci. 58(5):1099-1102

Fink-Gremmels J, Dresel J, LeistnerL. 1991. Use of *Monascus* extracts as an alternative to nitrite in meat products. Fleischwirtsch. 71:1184-1186.

Johns MR, Stuart D.M. 1991. Production of pigments by *Monascus purpureus* in solid culture. J. Ind. Microbiol. 8(1):23-28.

Juliano BO. 1986. Rice properties and processing. Food Rev. Int. 1(3):423-445.

Juzlova P, Martinkova L, KrenV. 1996. Secondary metabolites of the fungus *Monascus*: a review. J. Ind. Microbiol. 16(3):163-170.

Lin TF, Yakushijin K, Buchi GH, Demain AL. 1992. Formation of water-soluble *Monascus* red pigments by biological and semi-synthetic process. J. Ind. Microbiol. 9(3):173-179

Lin TF, Demain AL. 1991. Effect of nutrition of *Monascus sp.* on formation of red pigments. Appl. Microbiol. Biotech. 36(1):70-75

Okiyama A, Motoki M, Yamanaka S. 1992a. Bacterial cellulose I. Two-stage fermentation process for cellulose production by *Acetobacter aceti*. Food Hydrocolloid 6(5):471-477.

Okiyama A, Motoki M, Yamanaka S. 1992b. Bacterial cellulose II. Processing of the gelatinous cellulose for food mterials. Food Hydrocolloid 6(5):479-487.

Roberfroid M. 1993. Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. Crit. Rev. Food Sci. Nutr. 33:103-148.

Steel RG, Torrie JH. 1980. Principles and Procedures of Statistics. 2nd ed. New York: McGraw-Hill Book Co. 633 p.

Su YC. 1978. The production of *Monascus* pigments (in Chinese). Food Sci. 5: 4A-17A.

Sweeny JG, Estrada-Valdes MC, Iacobucci GA, Sato H, Sakamura S. 1981. Photoprotection of the red pigments of *Monascus anka* in aqueous media by 1,4,6-trihydroxynaphalene. J. Agric. Food Chem. 29(6):1189-1193.

Wong HC, Lin YC, Koehler PE. 1981. Regulation of growth and pigmentation of *Monascus purpurus* by carbon and nitrogen concentration. Mycologia 73(4):649-654.

JFS 1999-0416 accepted 4/5/99; revised 8/30/99; accepted 12/2/99.

Author Sheu is affiliated with the Department of Pharmacology/Toxicology, University of California, Davis CA 95616. Authors Wang and Shyu are affiliated with the Department of Horticulture, National Taiwan University, No. 140, Section 4, Keelung Road, Taipei, Taiwan 10660, Republic of China. Direct inquiries to author Shyu (E-mail : tedshyu@ccms.ntu.edu.tw).