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# Modulation of Bacteria with a Combination of Natural Products in KIMCHI Fermentation

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Three kinds of bacteria that influence Kimchi fermentation, *Lactobacillus plantarium* for acidity, *Leuconostoc mesenteroides* for ripening Kimchi, and *Pichia membranifaciens* for decreasing Kimchi quality, were regulated by natural products including *Theae folium, Taraxacum coreanum, Brassica juncea, Astragali radix, Gynostemma pentaphyllum, Camellia japonica, Agaricus blazei,* and *Cordyceps militaris*. The common prescription combined *T. folium, T. coreanum* and *C. militaris* and simultaneously regulated these 3 bacteria as follows: the growth of *L. plantarium* and *P. membranifaciens* were inhibited and *L. mesenteroides* was promoted. The most effective mixing ratio was *T. folium: T. coreanum: C. militaris* = 3:2:1. With this new prescription, deep flavor, extended preservation, and a special taste are expected in the Kimchi due to these natural products.

**Key Words** : *Kimchi fermentative bacteria, natural products combination, common prescription, T. folium, T. coreanum, C. militaris*

## INTRODUCTION

The taste of Kimchi is developed by fermentation of saccharides to lactic acid and other organic acids by lactic acid bacteria, which results in the special flavor and taste. Kimchi requires a maturation temperature and preservation period at 2-7℃ for 2-3 weeksto reach pH 4.3 for more delicious flavor and for vitamin production [1].

But as time passes, the taste can worsen due to acidification, softness of texture, and bad odors. To preserve the freshness of the Kimchi, additives [2-4], preservatives [5], pH buffering agents [6, 7], bacteriocin [8] and chitosan [9, 10], which is not safe for consumption, canbe used. Currently, the cold chain method is applied to Kimchi preservation [11]. Thirty bacteria and yeast have been applied to Kimchi fermentation, such as the G(+) bacteria [8], *L. brevis, L. plantarium, L. latis, L. mesenteroides, P. cerevisiae* [12], *S. faeculis*, and *E. faecalis*, the G(-) bacteria, *P. nigrifaciens* and *B. macerans*, and the yeast *Zygosaccharomyces*, which produces organic acids [13]. The time course of G(+) and G(-) behavior is an important index of Kimchi fermentation. Our purpose is to preserve the Kimchi by controlling these bacteria with natural products as sources of antibacterial, antifungal, antioxidative [14] and anti-angiogenesis effects [15, 16] such as *T. folium, T. coreanum, G. pentaphyllum, B. juncea, A. radix, C. japonica flower, C. japonica leaf, C. japonica* pericarp, *C. militaris*, and *A. blazei*. To this end, *Lactobacillus* sp. [13] should be controlled to protect against an acidic taste, *Leuconostoc* sp. [7] should be promoted for delicious and abundant taste, and *Pichia* sp. [7] should be controlled to decrease the septic white membrane.

Some common prescriptions were constructed to simultaneously control these 3 kinds of Kimchi fermentative bacteria using natural products to preserve the delicious, ripening taste of Kimchi.

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## MATERIALS AND METHODS

### Natural Products

Green tea was supplied by Okrojeda company. *A. radix* was purchased from an herbal market. *G. pentaphyllum, T. coreanum, C. japonica* flower, *C. japonica* leaf, *C. japonica* *pericarp*, and *B. juncea* were obtained from Dolsan, and *C. militaris* and *A. blazei* were supplied by a mushroom farm at Dolsan, Yosu-city.

### Extraction of Natural Products

Natural products (300 g) were extracted with 1500 mL of D.W. in a reflux flask for 1hr, and concentrated to 500 mL. Each natural product was used alone, orin combination at a suitable mixing ratio, for experimental purposes.

### Strains and Culture

*L. plantarium* KCTC 3108, *L. mesenteroides* KCTC 3722, and *P. membranifaciens* KCTC7628 were purchased from Gene Bank (Seoul, Korea), and cultured with MRS + 0.5% Lactose (pH 7.6), Tomato juice media, and Malt extract [17] at 25℃ and 150 rpm.

### Modulation of Kimchi Fermentative Strains

Regulation of Kimchi fermentative strains was investigated by the difference in O.D. at 660 nm using a spectrophotometer (UV-2101PC, Shimadzu, Japan) every 6 hr. Strains were cultured in 250-mL flasks that contained sample (5 mL) and medium (95 mL) at 25℃.

### Preparation of Kimchi Juice

Kimchi is made from Chinese cabbage and radish mixed with various resources such as hot pepper, garlic, ginger, jotgal, onions and salt as a control kimchi, and a common prescribed kimchi added in control kimchi with natural productscombination, fermented at different temperature, 4°C, 10°C and 20°C. Every 7days, 300g of each kimchi is crushed with mixer and filtered through a Whatman No. 2 paper, and centrifuged at 5,000 rpm for 10 min, the supernatant was used as a kimchi juice.

### Measurement of pH, Salinity and Acidity

pH and salinity were measured with Consort C803 (Turnhout, Belgium), and acidity was followed Vanderzant's methods[18] using Kimchi juice.

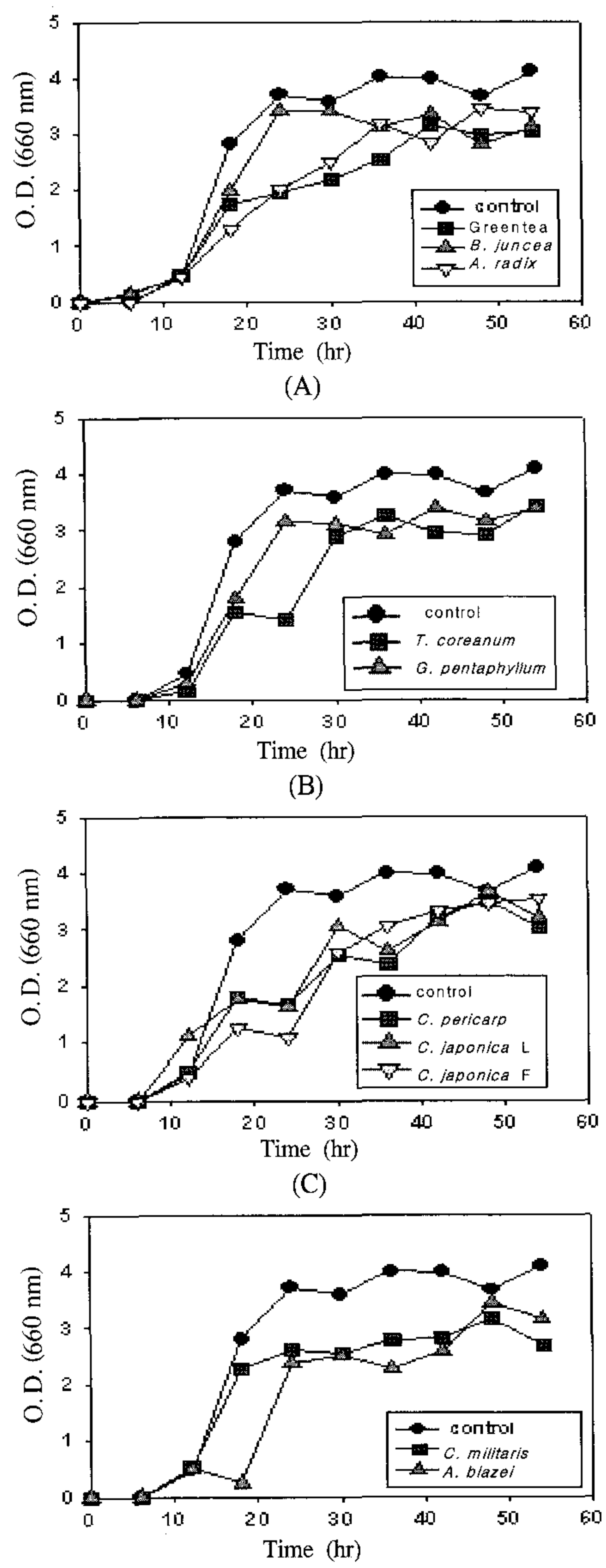
### Counting strains

For selecting *Leuconostoc* and *Lactobacillus* strains from fermented vegetables, *Lactobacilli*MRS (Difco Laboratories; Detroit, MI, USA) agar medium containing 0.1% bromphenol bilu(BPB)[19], for selection *Entrococcus* and *Pediococcus*, MRS agar containing 0.5% bile salt, azide dextrose agar (Difco)[20], for *Lactococcus*, KF *Streptococcus* agar(Difco) and M17 containing 0.002% bromophenol purple(BCP)[21], for yeast, potato dextrose agar(Difco) controlled as a pH 3.5 with 10% tartaric acid[21], and total number of bacteria was followed by Jang's method[21]using Gram staining.

## RESULTS AND DISCUSSION

### Selection of Natural Products for Growth Inhibitory Effect of *L. plantarium*

Fig. 1 shows the growth inhibitory effects of natural products on *L. plantarium* [7, 22-25] including *T. folium, B. juncea, A. radix, T. coreanum, G. pentaphyllum, C. japonica* flower, *C. japonica* leaf, *C. japonica* pericarp, *C. militaris*, and *A. blazei*. Of these natural products, *T. folium, T. coreanum, C. japonica* pericarp and *C. militaris* exhibited higher controlling effects on *L. plantarium*, which would decrease the sour taste of Kimchi. Another paper suggested that *G. ularensis, S. chinensis*, and *A. sessliflorum* would serve the same purpose [24], but these natural products are not suitable for food, except *G. ularensis*.




(A) (B) (C) (D)

**Figure 1.** The growth inhibitory effects of natural products against *L. plantarium*. *T. folium, T. coreanum, C. japonica* pericarp and *C. militaris* exhibited higher controlling effects on *L. plantarium.*

### Selection of Natural Products for Growth-Promoting Effects on *L. mesenteroides*

The growth of *L. mesenteroides,* which give flavor to Kimchi, was promoted by natural products such as *T. folium, T. coreanum,* and *C. militaris*(Fig. 2). Lee [24] showed a controlling effect for *L. mesenteroides* and not a promoting effect with *A. tuberosum* extract this bacterium provides a deep flavor by producing mannitol [26-27]. Park [17] also indicated that *S. baicalensis* and *P. amurense* extracts could control *L. mesenteroides.*

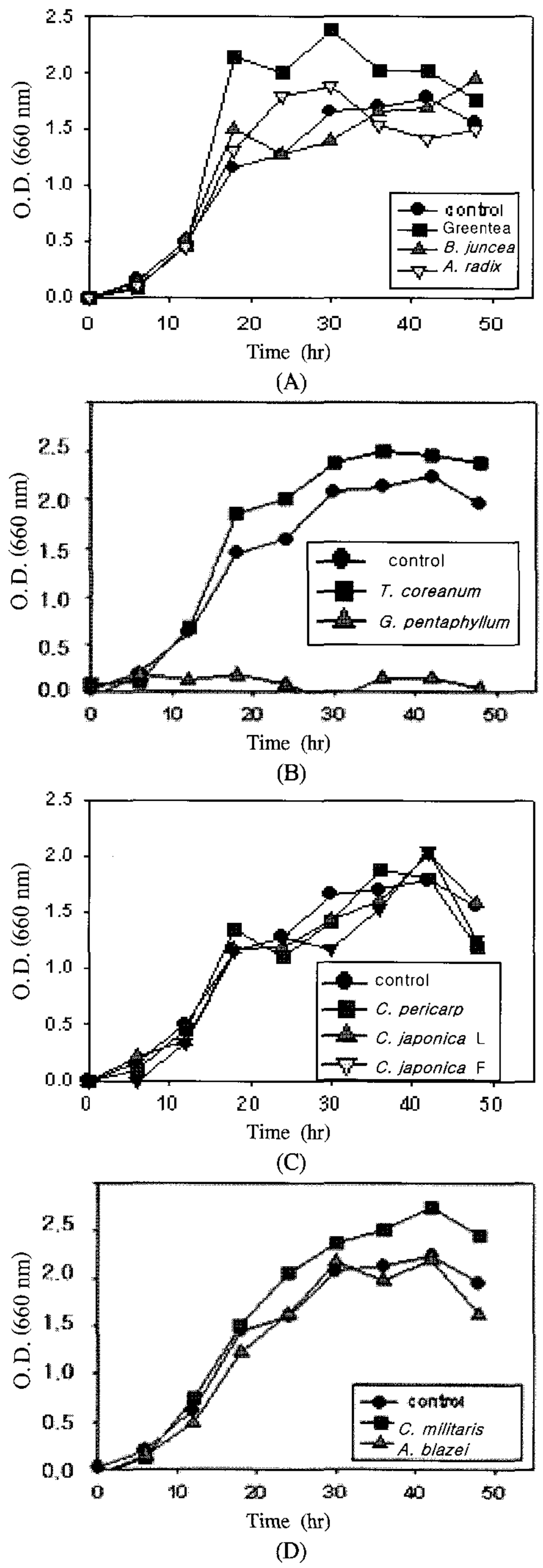



**Figure 2.** The growth-promoting effects of natural products against *L. mesenteroides.* The growth of *L. mesenteroides* was promoted by *T. folium, T. coreanum,* and *C. militaris.*

### Selection of Natural Products for Growth Inhibitory Effect of P. membranifaciens

Fig. 3 exhibites the growth of *P. membranifaciens* was inhibited by natural products such as *T. folium, T. coreanum, G. pentaphyllum, C. japonica* flower, *C. japonica* leaf, *C. japonica* pericarp, and *C. militaris,* which would decrease the septic white membrane during Kimchi fermentation. The septic effect of this white membrane is not good for appearance, produces a bad odor and decreases Kimchi flavor and preservation duration.

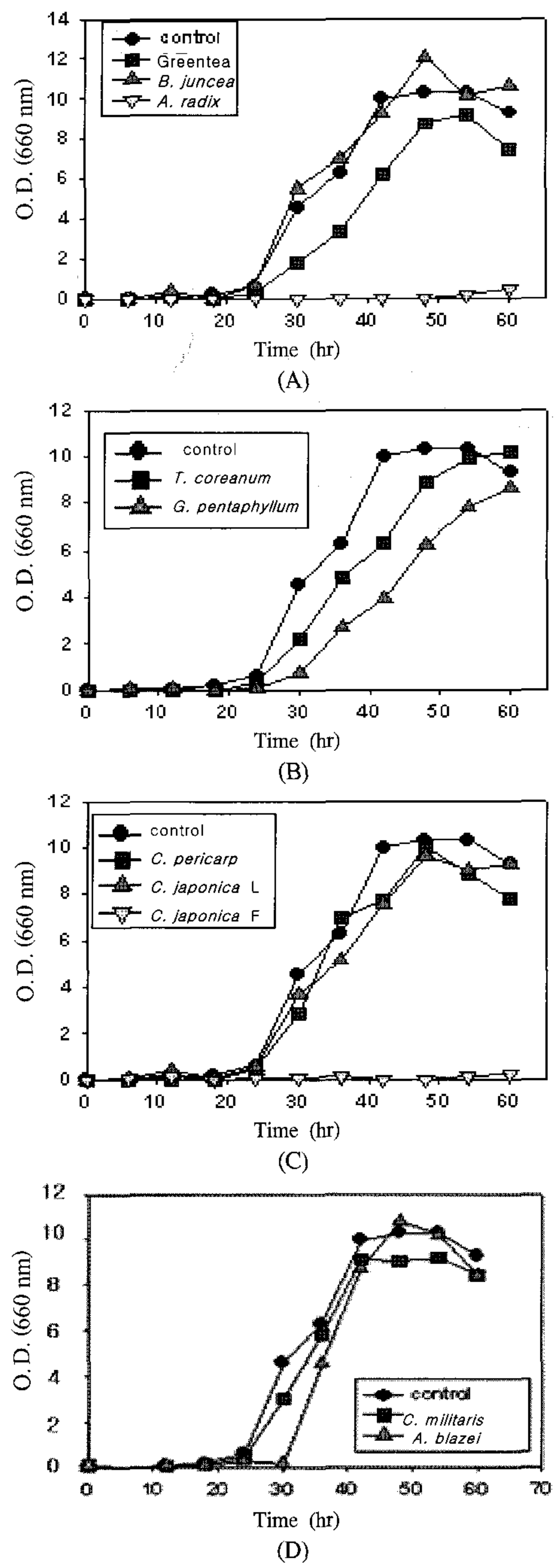



**Figure 3.** The growth-inhibitory effects of natural products against *P. membranifaciens.* The growth of *P. membranifaciens* was inhibited by *T. folium, T. coreanum, G. pentaphyllum, C. japonica* flower, *C. japonica* leaf, *C. japonica* pericarp, and *C. militaris.*

### Construction of A Common Prescription to Modulate Kimchi Fermentative Bacteria.

The following combinations of natural products were prepared: a.) control; b.) *T. folium, A. radix* and *C. militaris* c.) *T. Folium, T. coreanum* and *C. militaris* d.) *T. folium, T. coreanum* and *A. radix* e.) *T. folium, T. coreanum, A. radix* and *C. militaris* f.) *T. folium* and *C. militaris* g.) *T. folium* and *T. coreanum*. Combination C is the best prescription for the combined inhibitory/promoting effects on the Kimchi fermentative bacteria, *L. plantarium, L. mesenteroides,* and *P. membranifaciens*(Fig. 4).

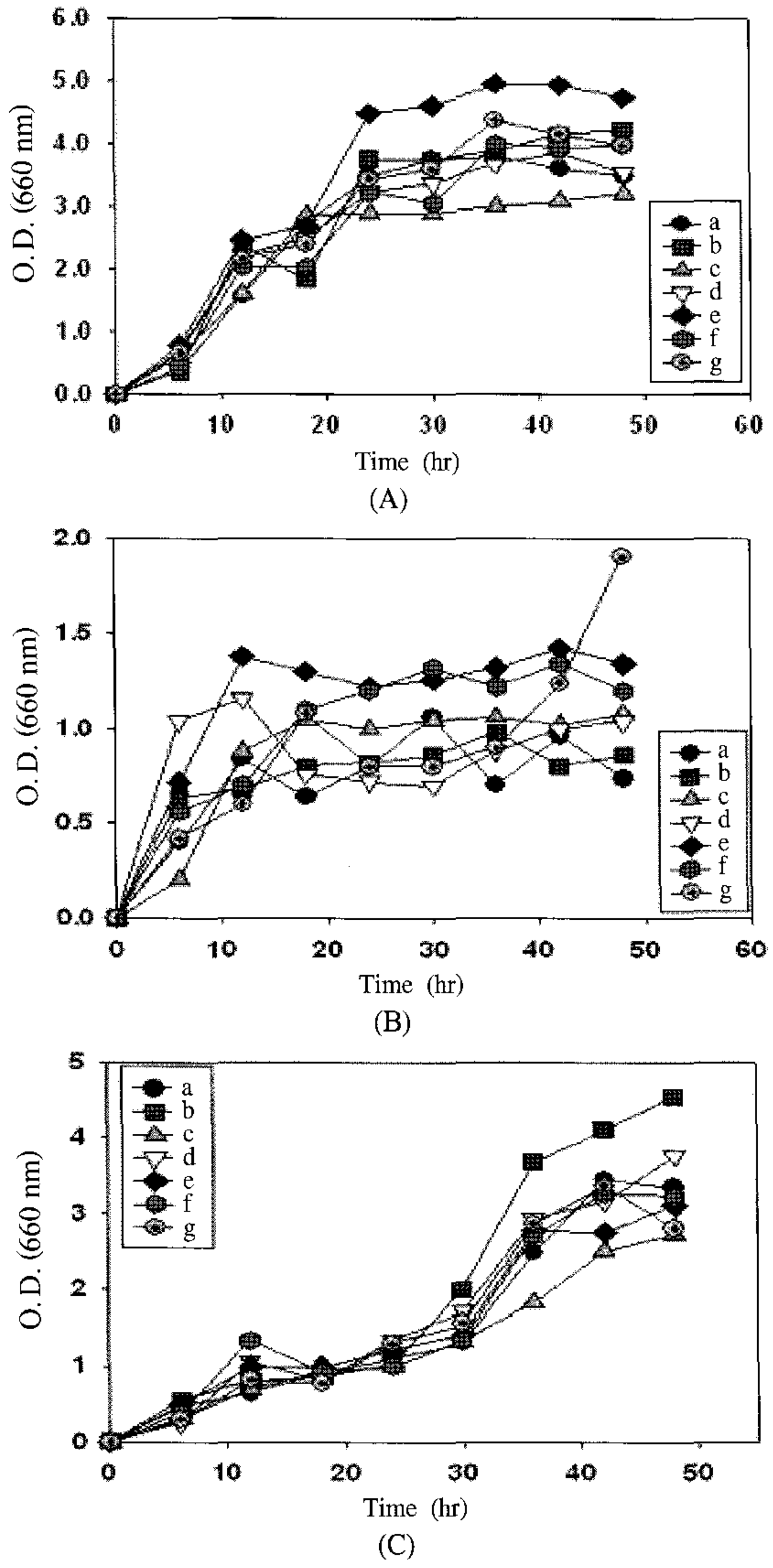



**Figure 4**. Common prescription to control 3 kinds of bacteria that influence Kimchi fermentation: (a) growth inhibition of *L. plantarium* (b) growth promotion of *L. mesenteroides* (c) growth inhibition of *P. membranifaciens.*

### Optimum Combination Ratio for The Common Prescription

When the growth rates ofthe three bacteria were compared under conditions with natural products mixed of *T. folium, T. coreanum,* and *C. militaris* with mixing ratio as 1:1:1, 2:1:1, 1:2:1, 1:1:2, 3:2:1, 2:3:1, 1:3:2, and 1:1:2. The ratios of 1:1:1 and 3:2:1 exhibited higher growth promoting effects for *L. mesenteroides* (Fig. 5). For growth inhibition of *L. plantarium*, the ratios 1:2:1 and 3:2:1 showed growth inhibitory effects (Fig. 6), and for *P. membranifaciens*, the ratio of 3:2:1 showed growth inhibitory effect (Fig. 7). From these results, the best ratio is 3:2:1, which simultaneously modulated all 3 strains.

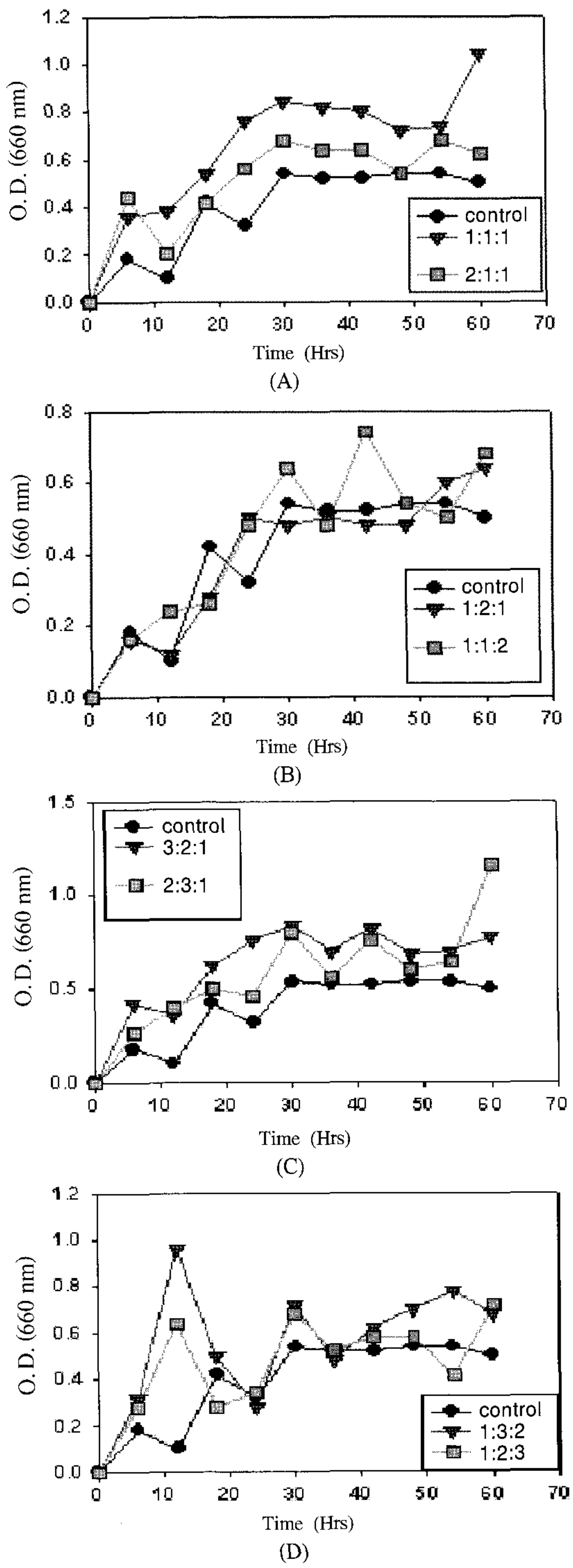



**Figure 5**. The most effective mixing ratio of natural products for promoting *L. mesenteroides* growth. *T. folium: T. coreanum: C. militaris*, the ratios of 1:1:1 and 3:2:1 exhibited higher growth promoting effects for *L. mesenteroides.*

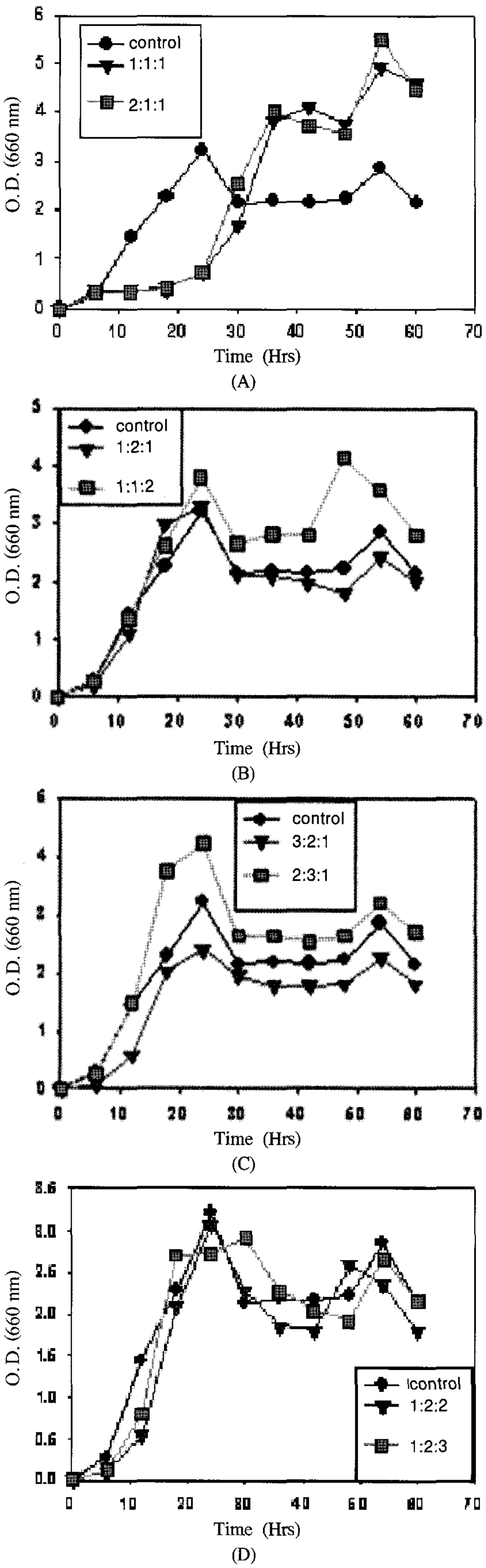



**Figure 6**. The most effective ratio of natural products to control *L. plantarium*. For growth inhibition of *L. plantarium*, the ratios 1:2:1 and 3:2:1 showed growth inhibitory effects.

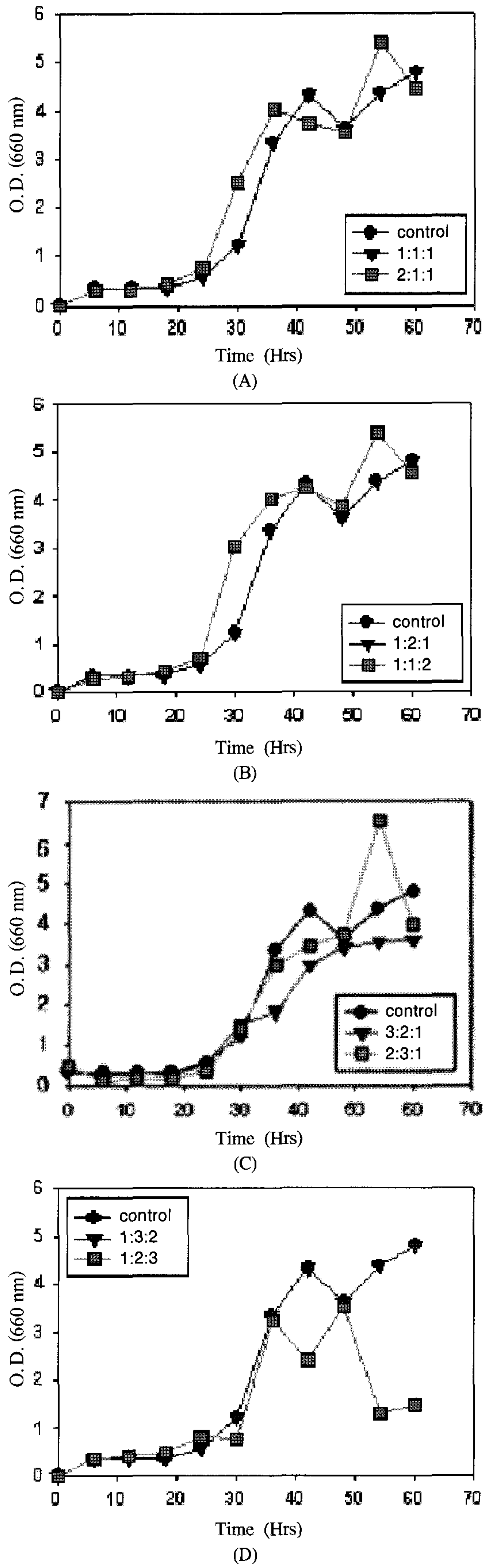



**Figure 7**. The most effective ratio of natural products to control *Pichia* sp. For *P. membranifaciens*, the ratio of 3:2:1 showed growth inhibitory effect.

## Characteristics of Kimchi Prepared with Common Prescription

### Variation of pH, Acidity and Salinity

Kimchi fermentation factors[28-29] prepared with common prescription. were changed a various direction. For that, pH [30-31], acidity and salinity were investigated at various preserving temperatures, 4℃, 10℃ and 20℃. For pH, at each temperature, Fig. 8(a) shows pH of common prescribed kimchi is higher compared with each control, which was expected to prevent souring of the Kimchi taste [32].

As with pH, for acidity, Fig. 8(b) expresses acidity of common prescribed kimchi is lower than that of controls, indicating that acids were not fully produced with the common prescription and that it can extend the duration of preservation.

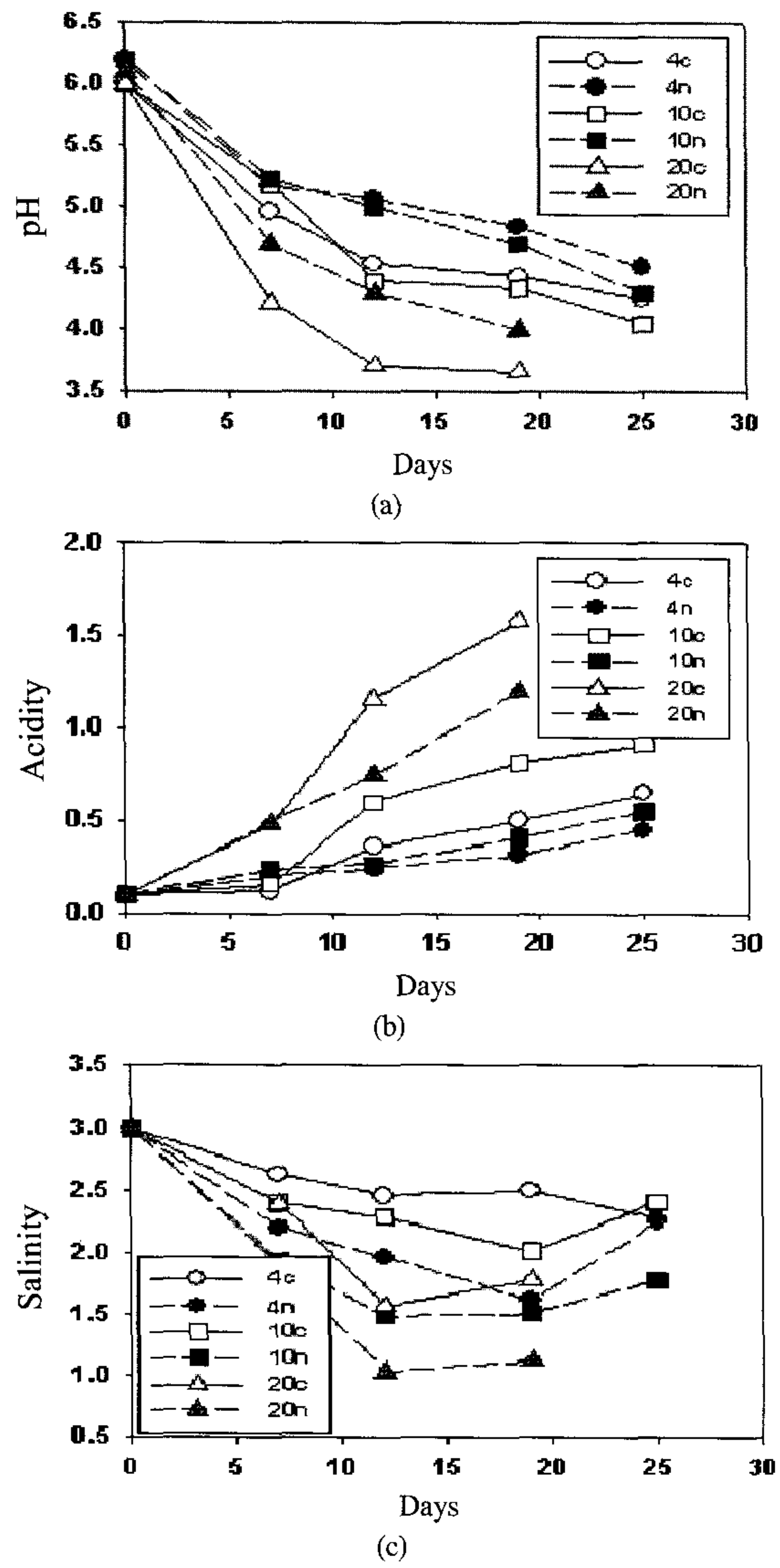



**Figure 8**. pH (a), acidity (b), and salinity (c) changes in Kimchi prepared with natural products according to the preservation temperatures and the number of days. 4c, 10c, and 20c : control, 4n, 10n, and 20n : natural product of each preserving temperature. pH, acidity and salinity were modulated excellently resulted in preventing sour taste, extending the duration of preservation, and preparing lower salt Kimchi.

Fig. 8(c) shows that salinity of this new type of Kimchi is tended to be lower with the natural products than that of control according to preserving temperature therefore, this common prescription would be helpful in preparing lower salt Kimchi[33].

### Changes of Kimchi Fermentative Bacteria.

*L. plantarium* did not appear in Kimchi preserved at 4℃ [Fig. 9(a)], and these bacteria were found in lower numbers ($3.8 \times 10^5$ and $4.8 \times 10^5$) than in control ($7 \times 10^5$ and $10 \times 10^5$) after 7 days in Kimchi preserved at 10℃, and 20℃, respectively. Therefore, this common prescription successfully controlled *L. plantarium* compared to control, which is important for reducing the sour taste and increasing long-term preservation.

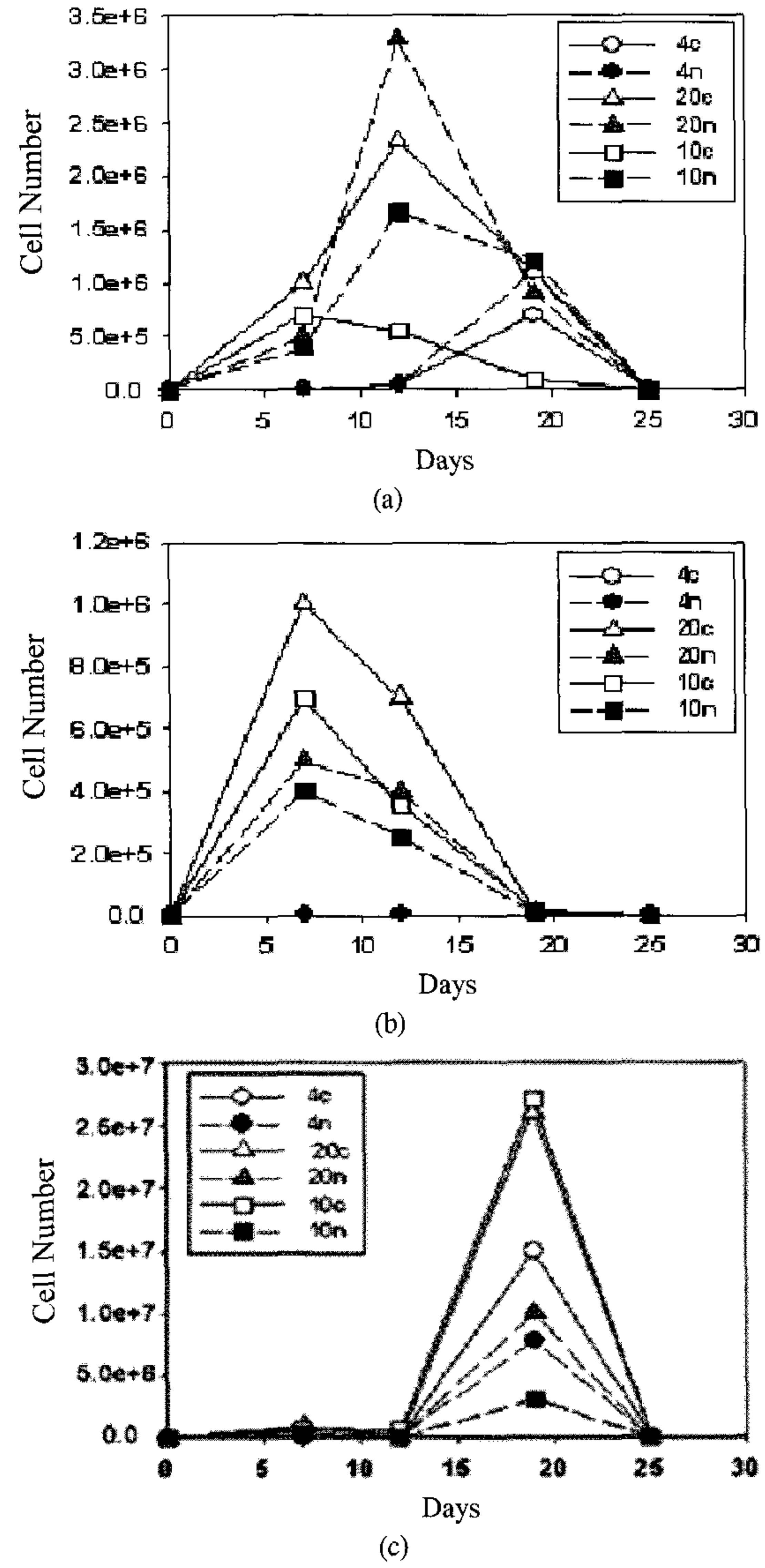



**Figure 9**. Controlling the number of *Lactobacillus* sp. (a), *Leuconostoc* sp. (b), *Pichia* sp. (c) in Kimchi according to preservation temperature and days. 4c, 10c, and 20c : control, 4n, 10n, and 20n : natural productof each preserving temperature. Controlled *L. plantarium* and *P. membranifaciens* reducd the sour taste and increased long-term preservation, and promoted *L. mesenteroides* improved the flavor of the new type of Kimchi.

*L. mesenteroides* was found in higher numbers ($1.5 \times 10^6$ and $3.3 \times 10^6$) than in control ($0.5 \times 10^6$ and $2.3 \times 10^6$) in Kimchi preserved at 10℃ and 20°C after 12 days [Fig. 9(b)], respectively. Also, in Kimchi preserved at 20℃, *L. mesenteroides* was 6.6 times ($3.3 \times 10^6$) higher than control at 4℃ ($5 \times 10^5$) and 10℃ ($5 \times 10^5$).

Therefore, this common prescription promoted growth of *L. mesenteroides*, which should improve the flavor of the new type of Kimchi.

In the last part of the fermentation process, *P. membranifaciens* was higher, with more than $15 \times 10^6$ in control at all 3 preserving temperatures [Fig. 9(c)]. These bacteria produce a white membrane over the Kimchi, caused by its decomposition. But in Kimchi prepared with the common prescription, the number of *P. membranifaciens* decreased remarkably [Fig. 9(c)] at each preserving temperature. This is expected to increase the duration of preservation and keep the flavor of the Kimchi.

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