Food Sci. Biotechnol. Vol. 14, No. 3, pp. 358 ~ 362 (2005)

Food Science and Biotechnology

Evaluation of pH-sensitive Eudragit E100 Microcapsules Containing Nisin for Controlling the Ripening of *Kimchi*

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Abstract Eudragit E100 microcapsules containing nisin were prepared and employed to control the ripening of *kimchi*. The recovery yields of microcapsules without/with nisin ranged from 93.53 to 94.61 % and 92.85 to 94.09 %, respectively. The particle size of microcapsules decreased (>200 to 100 μm) as the amount of aluminium tristearate increased from 6.0 to 15 %. The microcapsules were morphologically spherical and possessed rough surface. Nisin was completely released from the microcapsules within a day at pH 3.0 and within two days at pH 4.0, 5.0, and 6.0, respectively, whereas half the amount of nisin was released at pH 7.0 within two days. During fermentation of *kimchi* with microcapsules containing nisin, the pH decrease was retarded which resulted in a constant pH of approximately 4.2. The pH of 4.2 was optimal for ripening of *kimchi* for a longer period of time when compared with samples without nisin.

Keywords: Eudragit E100, microcapsules, nisin, *kimchi*

Introduction

Kimchi is a traditional Korean vegetable food fermented by various microorganisms present in the raw materials and ingredients of *kimchi*, such as cabbages, radishes, red hot pepper, garlic, fish sauce, and ginger. Lactic acid bacteria (LAB) play the most important role in the fermentation process. The quality of *kimchi* can be effectively controlled by changing fermentation conditions such as temperature, concentration of salt and sugar, time duration for fermentation process, and number of microflora. Overripening is the most serious problem during the storage of *kimchi*. Proliferation of LAB during fermentation of *kimchi* results in the overripening of *kimchi*, hence control of the bacterial proliferation is needed without reducing the quality of *kimchi*.

Nisin is a bacteriocin and is a 3,500-Da peptide produced by *Lactococcus lactis* subsp. *Lactis*. Nisin generally inhibits gram-positive organisms, such as *Lactobacillus plantarum* that plays an important role in the overripening of *kimchi*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium botulinum* (1-3). It is a hydrophobic protein (4), and has been termed as Generally Recognized As Safe (GRAS) status by the US FDA.

Microencapsulation is one of the means to achieve controlled release of the core materials. Eventhough the inhibition of LAB in food products such as wine and beer has been shown in recent studies (5, 6) and controlled-release technology, particularly microencapsulation has been used in food industry to improve the food qualities (7-11), none of these studies have reported on the effects of microencapsulation of nisin on the storage and qualities of *kimchi*. Microencapsulation of nisin may cause a slow, time-release of nisin into the environment, so it may remain at high concentrations for an extended period of time. Hence, microencapsulation also results in extending the activity of nisin, a minimal use of nisin necessary to retard overripening of *kimchi*, and lowered cost of operation process.

Eudragit E, a copolymer of dimethylaminoethyl methacrylate and methacrylic ester, which is insoluble above pH 5.0 has been widely used for drug delivery system to the stomach (12-15). Hence, Eudragit E must be capable of releasing nisin after an optimal ripening condition (about pH 4.2) of *kimchi* to retard the overripening of *kimchi*.

The objective of the present study was to evaluate the feasibility of microencapsulation of nisin with pH sensitive-Eudragit E100 to extend the shelf life of *kimchi*.

Materials and Methods

Materials Eudragit E100 was obtained from Röhm Pharma GmbH (Darmstadt, Germany). Nisin was purchased from Sigma (St. Louis, MO, USA). *Kimchi* was a product of Hankuk Nongsusan Co. (Haman, Korea). Aluminium tristearate and agar were purchased from Junsei Chemical Co. (Tokyo, Japan). MRS (DeMan, Rogosa and Sharpe) broth for the growth of selective lactic acid bacteria and plate count agar (PCA) for the growth of total aerobic bacteria were purchased from DIFCO (MA, USA). Other reagents were all of analytical grade and were used as received.

Preparation of microcapsules Microcapsules containing

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Received April 6, 2005; accepted May 9, 2005

nisin were prepared by the evaporation process in an oil phase to solve the problems of swelling and fragility, as reported earlier. The process is advantageous in terms of being simplice and with the possibilities of giving mass-production (16). In previous studies, Eudragit E showed good reproducibility for the preparation of microcapsules, similar to Eudragit RS and RL (12, 13, 16). Therefore, Eudragit E100 (1.0 g) was chosen for the present study. It was dissolved in acetone (10 mL), and aluminium tristearate as a dispersion agent was added in various amounts (6, 9, 12, and 15 %, w/w). The mixture was agitated in a stirrer (Lab Stirrer, MS-2800, TOPS Co., Seoul, Korea) with three propellers for about 20 min, until it was completely dissolved and then nisin (4 mg) was added and stirred for 1 min. The mixture was then poured into liquid paraffin (200 mL), which was previously cooled to 10°C and stirred at 300 rpm. During this period, the temperature was gradually increased to 35°C and acetone was completely removed by evaporation. The micro-capsules were collected by filtration with a filter paper (Whatman No. 3), washed with 50 mL of *n*-hexane five times, and dried in a desiccator. The dried microcapsules were passed through a standard sieve (100 μm-500 μm) and stored in a glass vial at room temperature until they were used.

Scanning electron microscopy The shape and size of microcapsules were examined by scanning electron microscopy (SEM). The dried microcapsules were coated with pure gold under vaccum (0.1 Torr) at high voltage (800-1500 V and 8 mA) by using an ion coater (Eiko Engineering IB-3, Japan) for 5 min and then observed under scanning electron microscope (ABT-32, Topcon Co., Japan) at 15 Kv.

Effect of pH values on the release of nisin from the microcapsules In order to evaluate controlled release of nisin from the microcapsules under various pH conditions, 10 mg of microcapsules containing nisin was added to 3 mLof 100 mM sodium phosphate-citrate buffer solutions adjusted to pH 3, 4, 5, 6 and 7, and the mixture was stored at 8°C. The buffer solutions containing the microcapsules with nisin were filtered with a filter paper (Whatman No. 3), and then the antimicrobial activities of nisin released from the microcapsules were assayed by spot-on lawn method by observing the size of clear inhibitory zones. Fifty μl of indicator strain, *Micrococcus flavus* ATCC 10240, grown on nutrient agar at 30°C for 6 hrs was inoculated into the soft agar (7 mL) was poured over agar plates, allowed to cool and dried for 4 hrs (indicator lawn). Five μL of nisin solution and PBS (Phosphate buffered solution) (5 μL) as a control were spotted on seeded dried agar surfaces (indicator lawn) and the plates were incubated at 37°C for 24 hr. Plates were observed for clear zones around spotted places every 24 hr until 9 days. The antimicrobial activities of nisin were determined by the percentage of the size (mm in diameter) of the inhibition halo and were compared with that of the control (10, 17).

Effects of microcapsules containing nisin on *kimchi* fermentation Effects of Eudragit E microcapsules containing nisin on the fermentation of *kimchi* were studied by observing changes in pH and populations of total microorganisms and *Lactobacillus* species. Fresh *kimchi* was homogenized in a blender, sterilized with alcohol and the homogenate was squeezed with a cloth to obtain *kimchi* juice (solution). Each of microcapsules containing nisin (0, 50, 100, and 300 ppm), prepared with 12% aluminium tristearate were added to the *kimchi* solution and stored at 20°C for 8 days. Changes in pH values were observed with a pH meter, and populations (CFU/mL) of total microorganisms and *Lactobacillus* species were determined by plating duplicate decimal dilutions of samples on total plate count agar and MRS plates, respectively.

Statistical analysis All measurements were done in triplicate, and Student's t-test was used to determine the difference between mean values ($p<0.05$) (16).

Results and Discussion

Preparation of microcapsules The evaporation process in an oil phase by using an acetone/liquid paraffin system was used in this study to prepare Eudragit E100 micro-capsules containing nisin. Characteristics of the micro-capsules obtained by the evaporation process in an oil phase, such as amount, shape and size were found to be affected by the concentration of polymer, amount of the dispersing agent, shape of propeller and rotation speed of the stirrer.

In this study, microcapsules were produced with a polymer concentration of 10% (w/v), 300 rpm of rotation speed, three propellers, by solubilization of the polymer in acetone and in the presence of various amounts of aluminium tristearate (6, 9, 12, and 15%). Acetone was necessary to solubilize the polymer, as it may not affect nisin activity in causing inhibition of microbial proliferation. Squillante *et al.* also reported that the addition of acetone had no effect on enzyme activity of β-galctosidase in the same preparation process (18). It is critically important to add aluminium tristearate for the formation of microcapsules, as it is a dispersing agent and may reduce the interfacial tension between Eudragit E particles and liquid paraffin (dispersion medium) during the preparation of a suspension, which will promote the formation of microcapsules by enhancing the dispersion and preventing the electrification and flocculation of the Eudragit E particles (12, 13, 19). Moreover, it has been reported that microcapsules can be formed by using less amount of aluminium tristearate with Eudragit E than Eudragit L and S (12).

The recovery yield was calculated as the ratio of the final weight of microcapsules produced to the total weight of all the materials used to prepare microcapsules. The recovery yields of microcapsules without and with nisin ranged from 93.53 to 94.61% and from 92.85 to 94.09 %, respectively, depending on the amounts of aluminium tristearate (Table 1). These results demonstrated that there were no distinct differences in the recovery yields, not only between the microcapsules without and with nisin, but also with various amounts of aluminium tristearate in the formulations.

Microcapsules size and morphology Various amounts

Table 1. Recovery yield of Eudragit E microcapsules

Eudragit E (mg)	Nisin (mg)	Recovery yield (%) Amount of aluminium tristearate (mg) 60	90	120	150
1000	0	93.53	94.61	93.57	93.95
	4	94.09	92.85	93.72	93.95

(w/w) of aluminium tristearate, 6.0, 9.0, 12, and 15% were used to investigate the effect of aluminium tristearate on microencapsulation. Table 2 shows that the size of microcapsules was affected by the incorporation of aluminium tristearate. When nisin was introduced into the microencapsulation system, the sizes of the particles were mostly beyond 200 μm at aluminium tristearate concentration of 6.0%. However, at 15% of aluminium tristearate, 55.27, 30.78, 11.93 and 0.75% the spherical microcapsules were distributed in size of above 200 μm, 150-200 μm, 100-150 μm and below 100 μm, respectively.

These findings show that the particle size of spherical microcapsules became smaller as the amount of aluminium tristearate increased from 6 to 15%. In addition, the similar trends were also observed in case where nisin was not added, indicating that the addition of nisin had no effect on the particle sizes of microcapsules. In a similar study, Han *et al.* (16) reported that the sizes of most of the Eudragit microcapsules were 337-637 μm and became smaller with increasing amount of aluminium tristearate employed for the preparation of Eudragit microcapsules containing β-lactam antibiotics. The effect of a dispersion agent on the size of microcapsules has also been demonstrated in a previous study (19).

The SEM of Eudragit E100 microcapsules containing nisin are shown in Fig. 1. The microcapsules, regardless of the amount of aluminium tristearate, were spherical and showed rough surfaces. The SEM results also revealed that the size of microcapsules decreased as the amount of aluminium tristearate increased.

pH dependent release of nisin Fig. 2 demonstrates the dissolution pattern of Eudragit E microcapsules containing nisin by using 100 mM sodium phosphate-citrate buffer as the dissolution medium at various pH values ranging from 3.0 to 7.0. Eudragit E 100 is soluble below pH 5.0, which

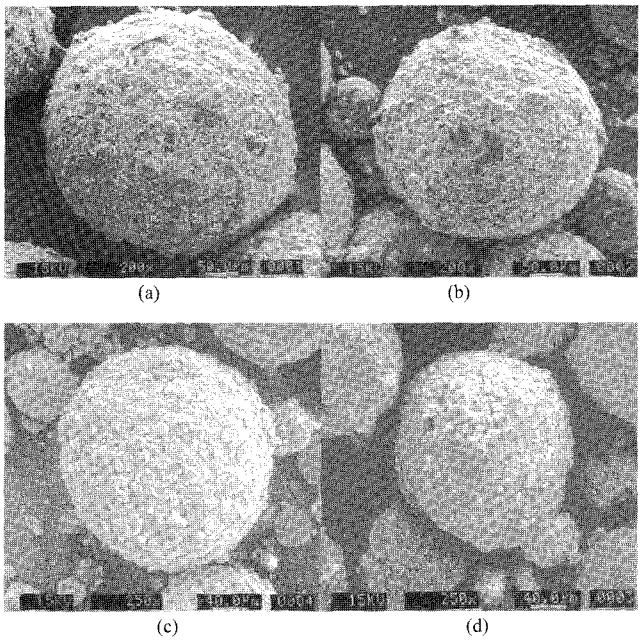

Fig. 1. Scanning electron micrographs of Eudragit E100 microcapsules with (a) 6, (b) 9, (c) 12 and (d) 15% of aluminium tristearate.

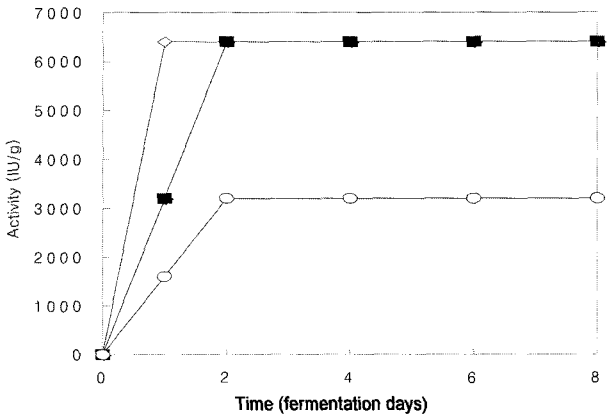

Fig. 2. Dissolution of Eudragit E microcapsules containing nisin with 12% aluminium tristearate at various pH values (from 3.0 to 7.0) of the dissolution medium. Nisin containing Eudragit microcapsules were incubated at 8°C in 100 mM sodium phosphate-citrate buffer of pH: ◇, 3 ; ◆, 4; □, 5; ■, 6; and ○, 7.

Table 2. Effects of aluminium tristearate on the particle size distribution of Eudragit E 100 microcapsules

Aluminium tristearate content (%)	Size (μm) >200	150-200	100-150	<100
	- Nisin			
6	98.26	0.35	0.46	0
9	86.16	9.05	2.24	0.43
12	65.99	21.63	13.49	1.02
15	55.27	30.78	11.93	0.75
	+ Nisin			
6	98.96	0.10	0	0
9	86.12	8.98	2.30	0.31
12	77.83	19.41	0.61	0
15	59.36	32.55	7.12	1.07

results in theoretical release of nisin in the environment where pH is less than 5.0. It was reported that Eudragit E dissolves below pH 5.0 and releases the encapsulated drug (14). In this study, nisin was completely released from the microcapsules within a day at pH 3.0 and within two days at pH 4.0, 5.0, and 6.0, respectively, followed by the dissolution of Eudragit E microcapsules; whereas half of the nisin was released at pH 7.0 within two days when compared with pH 4.0, 5.0, and 6.0, and kept for 8 days. These results were not exactly consistent with the fact that Eudragit E can be dissolved below critical pH of 5.0. The nisin release from the Eudragit E microcapsules at pH 6.0 and 7.0 above the critical pH might be caused by the swelling of Eudragit E (accelerated by Eudragit E ionization), since the swelling could cause dissociation of Eudragit E (20). However, the amount of nisin released at

pH 7.0 was much less than that at pH 3.0, 4.0, 5.0 and 6.0. These results demonstrate the dissolution pattern of Eudragit E microcapsules containing nisin was dependent on the pH values of the dissolution medium and ensure nisin release in an acidic environment. This indicated the feasibility of nisin microencapsulation with Eudragit E to control the nisin release in the environment related to the ripening of *kimchi*.

Effects of microcapsules containing nisin on the quality of *kimchi* during fermentation *Kimchi* quality can be effectively controlled by desirable microorganisms and various fermentation conditions. The effects of Eudragit E microcapsules containing nisin on the *kimchi* quality during fermentation were investigated by observing changes in pH (Fig. 3) and populations of total microorganisms and *Lactobacillus* species (Fig. 4). The *kimchi* solution with microcapsules containing nisin was stored at 20°C for up to 8 days, and then changes in pH and populations of total microorganisms and *Lactobacillus* species were monitored. In Fig. 3, the greater amounts of Eudragit E microcapsules at the starting day of experiment implied the higher pH values, which may be due to a basic property of Eudragit E (14). The pH values decreased rapidly within 3 days due to the production of organic acids through the growth of microorganisms during *kimchi* fermentation, regardless of the amount of microcapsules in the range of 0 to 300 ppm of nisin. However, after 3 days, the pH values did not decrease apparently irrespective of the amount of microcapsules, thereby maintaining the optimal pH condition (about 4.2) for the ripening of *kimchi*. As a result, the overripening of *kimchi* was retarded. The retardation of over ripening of kimchi could be due to release of nisin from the microcapsules in *kimchi* solution. Thus microbial growth was inhibited, resulting in a reduction in organic acid producing microorganisms during fermentation.

Moreover, this fact is could also be explained from Fig. 4. Fig. 4 (a) shows the effect of microcapsules containing nisin on total number of microorganisms in *kimchi* during fermentation. The number of total microorganisms mostly increased upto 3 days of storage, while the increase in total microorganisms was delayed, and then there was rather little reduction in bacterial counts from 3 to 8 days of storage. Furthermore, the growth pattern of *Lactobacillus* species in *kimchi* into which nisin-containing microcapsules were added was similar to that of total microorganisms (Fig. 4 (b)). As mentioned above, these results were due to the controlled release of nisin from the microcapsules as the pH values decreased during *kimchi* fermentation. Delaying the growth of *Lactobacillus* species during *kimchi* fermentation is essential to preserve the optimal quality of *kimchi* for a longer period of time. Deterioration of *Kimchi* takes place, since homofermentavtive *Lactobacillus plantarum* strains produce excessive organic acids and soften the texture of *kimchi* after the optimum fermentation period, which is referred to as overfermentation (2). Therefore, these strains need to be controlled for the good quality of *kimchi*. Nisin is considered to be better than organic acids for controlling the growth of *Lactobacillus* species, which could tolerate in an acidic pH condition by maintaining a pH difference between the microbe's

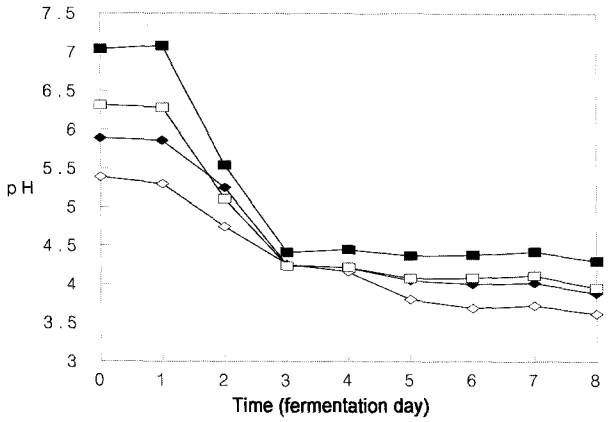

Fig. 3. Effects of Eudragit E microcapsules containing nisin on changes in pH values of *kimchi* during fermentation at 20°C for upto 8 days. Nisin contents of Eudragit E are as follows: ◇, 0 ppm; ◆, 50 ppm; □, 100 ppm; and ■, 300 ppm. *Kimchi* was fermented at 20°C, and values are the average of triplicate.

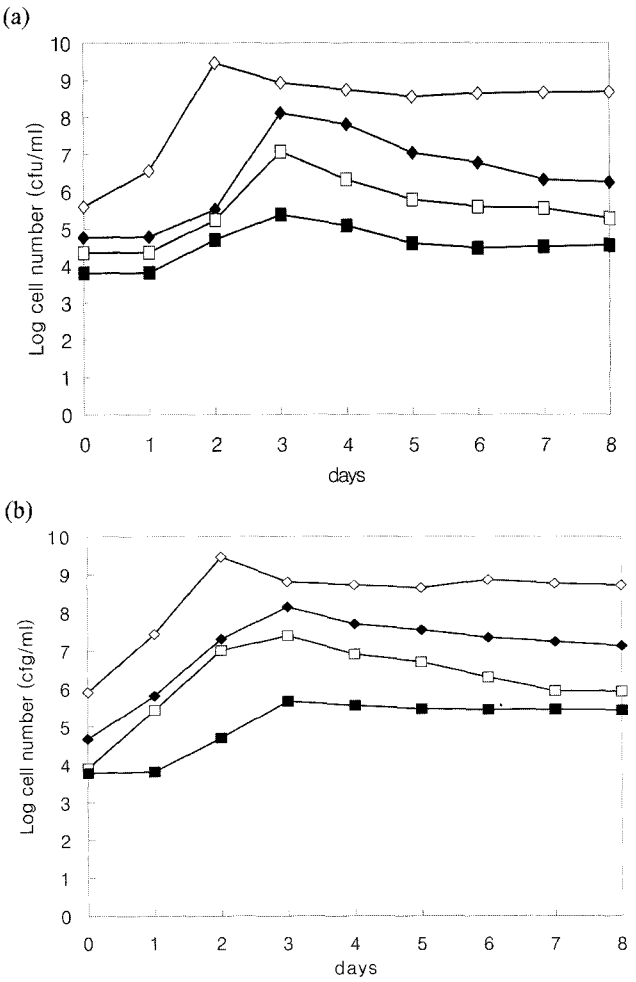

Fig. 4. Effects of Eudragit E microcapsules containing nisin on changes in populations of total microorganisms (a) and *Lactobacillus* species (b) in *kimchi* during fermentation at 20 °C for upto 8 days. Nisin contents of Eudragit E are as follows: ◇, 0 ppm; ◆, 50 ppm; □, 100 ppm; and ■, 300 ppm. *Kimchi* was fermented at 20°C, and values are the average of triplicate.

cytoplasm and the environment via a unique pH homeostasis (21, 22).

This study shows that Eudragit E100 microcapsules containing nisin could maintain high quality of *kimchi* for a longer period of time by controlling the growth of total microorganisms and *Lactobacillus* species, which play significant roles in *kimchi* fermentation. To improve the feasibility of Eudragit E100 for the sustained-release of nisin, further researches may need to be studied on combining various kinds of Eudragit such as E, L, S, RS and RL at suitable ratios or on novel pH sensitive polymer.

Acknowledgments

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea. Han-Soo Kim and Seong-Chun Jo received scholarship from the Brain Korea 21 Program of the Korean Ministry of Education.

References

1. Ryu HJ, Chung CH, Kyung KH. Evaluation of nisin as a preservative to prevent over-acidification of *kimchi*. Food Sci. Biotechnol. 7: 205-208 (1998)
2. Mheen TI, Kwon TW. Effect of temperature and salt concentration on *kimchi* fermentation. Korean J. Food Sci. Technol. 16: 443-450 (1984)
3. Nettles CG, Barefoot SF. Biochemical and genetic characteristics of bacteriocins of food-associated lactic acid bacteria. J. Food Prot. 56: 338-356 (1993)
4. Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. 12: 39-86 (1993)
5. Daeschel MA, Jung DS, Watson BT. Controlling wine malolactic fermentation with nisin and nisin-resistant strains of *Leuconostoc oenos*. Appl. Environ. Microbiol. 57: 601-603 (1991)
6. Ogden K, Tubb RS. Inhibition of beer-spoilage lactic acid bacteria by nisin. J. I. Brewing. 91: 390-392 (1985)
7. Bakan JB. Microencapsulation of food and related products. Food Technol. 27: 34 (1973)
8. Brazel CS. Microencapsulation: offering solutions for the food industry. Cereal Foods World. 44: 388-390 (1999)
9. Dziezark JD. Microencapsulation and encapsulated ingredients. Food Technol. 42: 135 (1988)
10. Hoover HG, Steenson LR. Screening methods for detection bacteriocin activity. pp. 23-27. In: Bacteriocins of lactic acid bacteria. Academic Press, New York, USA (1993)
11. Kim SJ, Park GB, Kang CB, Park SD, Jung MY, Kim JO, Ha YL. Improvement of oxidative stability of conjugated linoleic acid (CLA) by microencapsulation in cyclodextrins. J. Agric. Food Chem. 48: 3922-3929 (2000)
12. Goto S, Kawata M, Nakamura M, Aoyama T. Eudragit E, L and S (acrylic resins) microcapsules as pH sensitive release preparation of ketoprofen. J. Microencapsul. 3: 305-316 (1986a)
13. Goto S, Kawata M, Nakamura M, Aoyama T. Eudragit RS and RL (acrylic resins) microcapsules as pH insensitive release and sustained release preparations of ketoprofen. J. Microencapsul. 3: 293-304 (1986b)
14. Leopold CS, Eikeler D. Eudragit E as coating material for the pH-controlled drug release in the topical treatment of inflammatory bowel disease (IBD). J. Drug Target. 6: 85-9 (1998)
15. Ramadan EM, El-Helw A, El-Said Y. Effects of encapsulation of mefenamic and with cationic Eudragit E on its bioavailability and gastric ulcerogenic activity in rabbits. J. Microencapsul. 4: 125-132 (1987)
16. Han K, Shin DS, Jee UK, Chung YB. Preparation and evaluation of sustained-release Eudragit microcapsules containing β-lactam antibiotics. J. Kor. Pharm. Sci. 22: 267-279 (1992)
17. Cha DS, Chinnan MS. Emerging role of nisin in food and packaging systems. Food Sci. Biotechnol. 12: 206-212 (2003)
18. Squillante E, Morshed GS, Bagchi, Mehta KA. Microencapsulation of β-galctosidase with Eudragit L-100. J. Microencapsul. 20: 153-167 (2003)
19. Kawata M, Nakamura M, Goto S, Aoyama T. Preparation and dissolution pattern of Eudragit RS microcapsules containing ketoprofen. Chem. Pharm. Bull. 34: 2618-2623 (1986)
20. Buonaguidi M, Carelli V, Colo GD, Nannipieri E, Serafini MF. Evaluation of a pH-sensitive semi-interpenetrating polymer network for control of GI drug delivery. Int. J. Pharm. 147: 1-10 (1997)
21. McDonald LC, Fleming HP, Hassan H.M. Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. Appl. Environ. Microbiol. 56: 2120-212 (1990)
22. Nannen NL, Hutkins RW. Intracellular pH effects in lactic acid bacteria. J. Dairy Sci. 74: 741-746 (1991)