Influence of Hop Resins on Freeze Injury to Escherichia coli

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The influence of hop resins on the freeze-thawing resistance of Escherichia coli K-12 IFO3301 was investigated. When the freeze-thawing treatment of the strain was carried out in nutrient broth containing 0.01% of hop resins, a great decrease in the viable cell count was observed. Especially, the decrease in the count on the desoxycholate agar plate was remarkable, and no viable cells were found after 24-h freezing. Further, an extension of the lag phase was observed when the strain after freeze-thawing treatment was incubated in the nutrient broth. Namely, the freeze-thawing injury to the strain was greatly enhanced by the addition of the hop resins. It was thought that the effective utilization of hop resins for hygienic control of frozen foods is feasible.

Keywords: hop resins, freeze injury, freeze-thawing treatment, Escherichia coli

Materials and Methods

Test materials and test strain Hop resins were purchased from the Cultor Food Science Co., Ltd., (Milwaukee, Wis.). This product was rich in the p-acid fraction, which was obtained by removing the y-acid (the main bitterness component) from a hop extract using a supercritical carbon dioxide extraction method. Food grade sodium hexametaphosphate (Taiheikagaku Co., Osaka) and glycerine (Wako Pure Chemical Co., Osaka) were also used as test materials. As a test strain, Escherichia coli K-12 IFO3301 (hereinafter referred to as E. coli) was used.

Measurement of viable cell count after freeze-thawing treatment The E. coli strain was incubated in nutrient broth at 30°C for 24 h and harvested by centrifugation (10,000×g, 15 min). The cells were washed twice with a sterile 40 mM phosphate buffer (pH 6.5) and suspended in nutrient broth containing a test material at 10^7 cfu/ml. This cell suspension (10 ml) in a test tube was frozen at -20°C. After one and 8 days, the frozen cell suspension was thawed at room temperature. The change in temperature is shown in Fig. 1 when the freeze-thawing treatment was carried out in nutrient broth. The surviving cells were enumerated by the following methods: 1. the standard plate count, 2. the desoxycholate agar count. The standard plate was incubated at 35°C for 48 h and the desoxycholate agar was incubated at 37°C for 24 h. These tests were performed by the pour plate method. The final concentrations of hop resins, sodium hexametaphosphate, and glycerine in the nutrient broth were 0.01, 1, and 10%, respectively. These concentrations of test materials have no effect on the growth of E. coli (Haas & Barsoumian, 1994; Mizobuchi & Sato, 1984; Schmalreck et al., 1975; Tutumi et al., 1976). Also, it was confirmed by organoleptic evaluation (flavor and taste) that these concentrations had no effect on foods (unpublished).

Measurement of lag time for growth The lag time for the growth of E. coli was examined in the nutrient broth medium after freeze-thawing treatment. The inoculum size in the nutrient broth was about 10^6 cfu/ml. The incubation was carried out with an automatic growth monitoring apparatus (Bioscanner; Ohtakeseisakusho Co., Tokyo), which continuously measured the absorbancy at 650 nm. The period from the start of incubation until the increase in the absorbency at 650 nm was regarded as the lag time for growth.

Results and Discussion

After the freeze-thawing treatment, the viable cell count of E. coli decreased as shown in Fig. 2. With the standard plate count method, the viable cells in the nutrient broth without test materials decreased from 10^7 cfu/ml to 10^6 cfu/ml after 8
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**Fig. 1.** Change in temperature during the freeze-thawing treatment (I: freezing treatment, II: thawing treatment) in the nutrient broth (——) and in the nutrient broth containing glycerin (—).

**Fig. 2.** Viable cell count of *E. coli* after the freeze-thawing treatment in the nutrient broth. The surviving cells were enumerated by the following methods: I. the standard plate count, II. the desoxycholate agar count. Test materials added were as follows: ×: No addition; •: Hop resins; ■: Sodium hexametaphosphate; ▲: Hop resins+Sodium hexametaphosphate.

days of freezing. With the addition of sodium hexametaphosphate, the cell count decreased from $10^7$ cfu/ml to $10^3$ cfu/ml; whereas, with hop resin, the count decreased to below 30 cfu/ml. It was not possible to form the colony when hop resins and sodium hexametaphosphate were used in combination. On the other hand, with the desoxycholate agar method, the viable cell count decreased from $10^7$ cfu/ml to $10^3$ cfu/ml after one day of freezing without test materials or with the addition of sodium hexametaphosphate. However, no viable cells were detected when hop resins were added.

Figure 3 shows the viable cell count of *E. coli* after treatment of the nutrient broth containing glycerine. With the standard agar method, the decrease in viable cell count was hardly observed after 8 days of freezing. With the desoxycholate agar method, the viable cell count did not change in the presence or absence of sodium hexametaphosphate,
whereas the viable cell count decreased to 10^9 cfu/ml with the addition of hop resins. Consequently, with hop resins (0.01%), the injury to E. coli by the freeze-thawing treatment was promoted, and the freezing-resistance was reduced even in the presence of glycerine, which is a cryoprotective agent against freezing degeneration.

Table 1 shows the lag time for the growth of freeze-treated E. coli, grown in nutrient broth. With the addition of hop resins, the lag phase of the E. coli was extended by about 4 h. This extension was about 1.2-fold compared to that without addition of a test material. A similar delay in growth was also observed in the nutrient broth containing glycerine (Table 2). That is, the lag phase time was extended about 1.4-fold by adding hop resins. On the other hand, no influence of sodium hexametaphosphate on the lag time was observed. However, the combined use of hop resins and sodium hexametaphosphate caused a synergistic injury action to E. coli, followed by extension of the lag phase time by about 1.6-fold.

Neither hop resins nor sodium hexametaphosphate has any antimicrobial activity against E. coli (Haas & Barsoumian, 1994; Tutumi et al., 1976). However, after the freeze-thawing treatment in the presence of these test materials, the viable cell count of E. coli was decreased. This effect was especially remarkable in the presence of hop resins. The viable cell count after the thawing was lower with the desoxycholate agar method than that with the standard agar method. This observation was similar to that reported by Speck (1970); Speck & Cowman (1971). They reported that E. coli which has suffered freeze injury lacked the ability to grow on a medium containing bile salts such as desoxycholate agar. It was considered that the injury was promoted in the presence of hop resins. Generally, most microorganisms undergo freeze injury during freeze-thawing treatments (Ostovar & Bremier, 1975; Schothorst, 1976; Speck & Ray, 1977; Warseck, 1973). Kaneda et al. (1985) reported that injury to the outer membrane of E. coli was promoted in the presence of sodium hexametaphosphate. The cell membrane became more porous in the presence of sodium hexametaphosphate, followed by the leakage of cell components. Presumably, hop resins may also affect the function of the cell membrane.

Recently, there has been a yearly increase in the consumption of frozen foods. Therefore, hygienic control becomes
more important. Microorganisms suffer from physiological or metabolic injury by freeze-thawing treatment, and the presence of the hop resins enhances this injury. The microbial viable cell count after thawing decreased greatly, and growth was remarkably retarded. From these findings, it is thought that application of this effect for hygienic control of frozen foods may be possible.

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