Investigations of microbiological quality risks in non-alcoholic beer: growth potential of food-borne pathogens and alcohol formation by contaminant microbes

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Summary

In this study, the growth potential of food-borne pathogens and unintended alcohol formation by contaminant microbes were investigated. We focused on the NAB* production process by dealcoholization, as well as the post-packaging distribution and on-premise sales of NAB kegs*.
(*NAB sample: Alc.0.5 v/v%, pH4.6, 0.230 Mpa)

In NAB production by dealcoholization (Decarbonated NAB)
✓ The growth potential of food-borne pathogens was low in NAB sample at 6°C.
✓ Approximately 0.8% alcohol was formed by contaminant yeast during the period from the dealcoholization process to the completion of cooling. After cooling, the risk was controlled at 2°C in NAB sample.
→◆Process control and cold storage at around 2 °C is important after dealcoholization to prevent alcohol formation.

In the post-packaging distribution and on-premise sales of NAB kegs
✓ The growth potential of food-borne pathogens was low in NAB.
✓ The presence of preservatives tends to deactivate food-borne pathogens more rapidly.
✓ Alcohol formation was suppressed at low temperature for short period, although 0.5% or more alcohol was formed by contaminant yeast at room temperature for long period.
✓ The presence of sodium benzoate tends to suppress the alcohol formation.
→◆The management of the keg filling environment is important to suppress alcohol formation.
◆Adding sodium benzoate were also considered effective to control alcohol formation.
◆NAB keg should be stored at low temperature to prevent alcohol formation after keg opening.

Introduction

■Non-alcoholic beer (NAB)
- The definition of NAB differs from country to country. In many European countries, products are labeled as "non-alcoholic beer" when they contain less than Alc. 0.5 v/v%[*1].
- NAB market is expanding. Furthermore, NAB keg have begun to be sold in recent years.

■Microbial risks of NAB by dealcoholization
- Since NAB contains no or a very small amount of alcohol, it is considered that microorganisms grow more easily in NAB compared with traditional beer.
- The production process after dealcoholization is susceptible to microbial risks about the food-borne pathogens and alcohol formation, because of weaker gas pressure and alcohol.
- NAB keg is also more prone to microbial contamination after keg opening compared to cans and bottles. There are concerns about the potential growth of food-borne pathogens and unintended alcohol formation in on-premise consumption.

■In this study,
We investigated the growth potential of food-borne pathogens and alcohol formation by contaminant microbes in commercial NAB produced by dealcolization (Alc.0.5 v/v%, pH4.6, 0.230 MPa). Regarding the growth potential of food-borne pathogens during the cold storage, we focused on *E. coli* and *Salmonella sp.*, because they are considered to have stronger growth potential in NAB [*2].

Our goal is to find effective control measures against food-borne pathogens and alcohol-forming contaminants (below 1% ABV).

Results and Discussion

1. The microbial risks in NAB production by dealcoholization

1-1. Control measures of food-borne pathogens after dealcoholization process

Materials and Methods:

Strain	・*E. coli* NBRC3301 ・*Salmonella enterica sub sp. enterica* NBRC12529
Sample	Decarbonated NAB (pH4.6)
Condition*	・1000 cells/ml ・6°C, 100 days ・Aerobic incubation without agitation ・n=2

*** 6°C, 100 days**: After cooling process

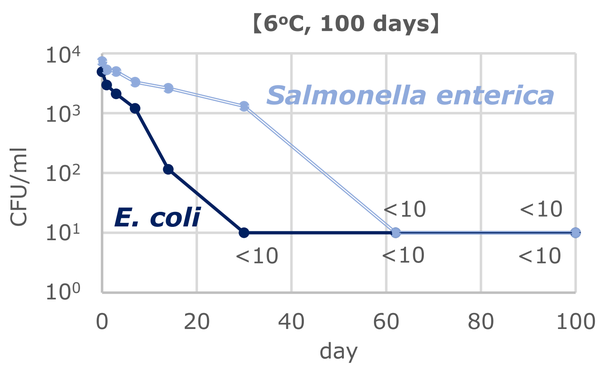

Fig. 1. The growth potential of *E. coli* and *Salmonella enterica* at 6 °C (n=2)

Results:
- No growth was observed with tested food-borne pathogens at 6°C for 100 days (Fig.1).
- *Salmonella enterica* decreased more slowly than *E. coli* in NAB (Fig.1).

1-2. Control of alcohol-forming contaminants

Materials and Methods:

Strain	Yeast and bacteria (Table 1)
Sample	Decarbonated NAB (pH4.6)
Condition*	・1000 cells/ml ・15°C, 5 days 2 or 4°C, 100 days ・Anaerobic incubation ・n=3

Table 1. Tested strains

Tested strains
(1) Brewing yeast (bottom)
(2) *Saccharomyces cerevisiae* (*S. cerevisiae*) NBRC565
(3) *S. cerevisiae* NCYC1236
(4) *S. cerevisiae var. diastaticus* IFO1440
(5) *S. cerevisiae var. diastaticus* ATCC13007^T
(6) *S. bayanus* NBRC11022
(7) *Brettanomyces naardenensis* (*B. naardenensis*) AGYC109
(8) *Candida parapsilosis* (*C. parapsilosis*) AGYC41
(9) *C. parapsilosis* AGYC176
(10) *Zygosaccharomyces rouxii* (*Z. rouxii*) NBRC1130^T
(11) *Z. rouxii* IFO0495
(12) *Z. fermentati* NBRC0479^T
(13) *Z. bailii* AGYC120
(14) *Levilactobacillus brevis* (*Lactobacillus brevis*) JCM1170
(15) *Levilactobacillus brevis* ABBC64
(16) *Secundilactobacillus paracollinoides* (*Lactobacillus paracollinoides*) JCM11969^T
(17) *Fructilactobacillus lindneri* (*Lactobacillus lindneri*) DSM20690^T
(18) *Pediococcus damnosus* ABBC478

***15°C, 5 days**
: During the period from the dealcoholization process to the completion of cooling

2 or 4°C, 100 days
: After cooling process

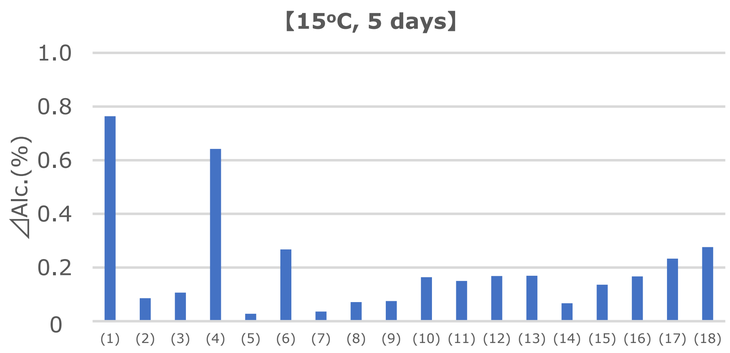

Fig. 2. Alcohol formation at 15°C after 5 days (n=3)

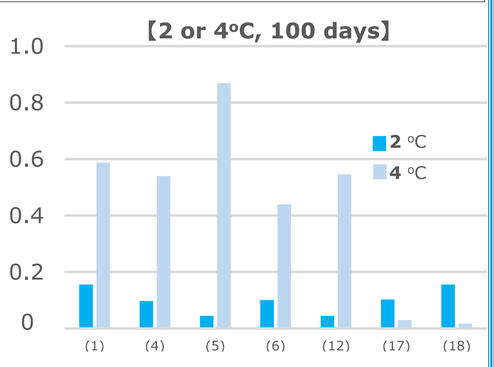

Fig. 3. Alcohol formation at 2 or 4°C after 100 days (n=3)

Results:
- After 5 days at 15°C, approximately 0.6 to 0.8% alcohol was formed by brewing yeast and *S. cerevisiae var. diastaticus* (Fig. 2).
- After 100 days at 2 °C, the alcohol formation was suppressed to 0.2% or less, although approximately 0.8% alcohol was formed by *S. cerevisiae var. diastaticus* at 4 °C (Fig. 3).

2. The microbial risks in the post-packaging distribution and on-premise sales of NAB kegs

2-1. The growth potential of food-borne pathogens

Materials and Methods:

Strain	・*E. coli* O157 CRA16244 CRA16039 CRA16040 ・*Salmonella enterica* CRA1868 CRA1947 CRA3736 ・*S. aureus* CRA2095 CRA1208 CRA11018 ・*Yersinia enterocolitica* CRA4103 CRA498 CRA499
Sample	Carbonated NAB (pH4.6) ・Control (without preservatives) ・Sodium benzoate (400 ppm) ・Potassium pyrosulfite (40 ppm)
Condition	・1000 ～10000 cells/ml ・25°C, 90 days ・n=2

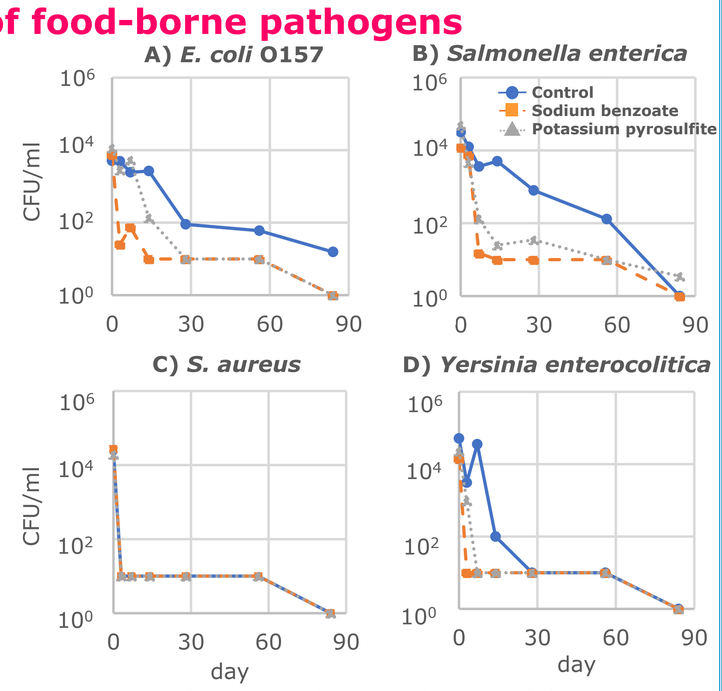

Fig. 4A-D. The growth potential of food-borne pathogens at 25 °C (n=2)

Results:
- No growth was observed with food-borne pathogens, independent of preservative addition (Fig.4).
- The presence of preservatives tends to deactivate *E. coli* O157, *Salmonella enterica,* and *Yersinia enterocolitica* more rapidly in NAB samples (Fig.4).

2-2. Alcohol formation and control of contaminant microbes

Materials and Methods:

Strain	Yeast and bacteria (Table 2)	
Sample	Carbonated NAB (pH4.6)	Decarbonated NAB (pH4.6)
	・Control (without preservatives) ・Sodium benzoate (400 ppm) ・Potassium pyrosulfite (40 ppm)	
Condition	・1000 cells/ml ・25°C, 3 months ・n=2	・1000 cells/ml ・4°C, 1 week 4°C, 2 weeks ・Anaerobic incubation ・n=2

Table 2. Tested strains

Tested strains
(1) Brewing yeast (bottom)
(2) *S. cerevisiae* NBRC565
(3) *S. cerevisiae var. diastaticus* IFO1440
(4) *S. cerevisiae var. diastaticus* ATCC13007^T
(5) *S. bayanus* NBRC11022
(6) *B. naardenensis* AGYC109
(7) *Dekkera anomala* (*D. anomala*) ATCC10562^T
(8) *D. anomala* NBRC0642
(9) *D. anomala* ATCC10559
(10) *Z. fermentati* NBRC0479^T
(11) *Z. bailii* AGYC120
(12) *Pediococcus damnosus* ABBC478

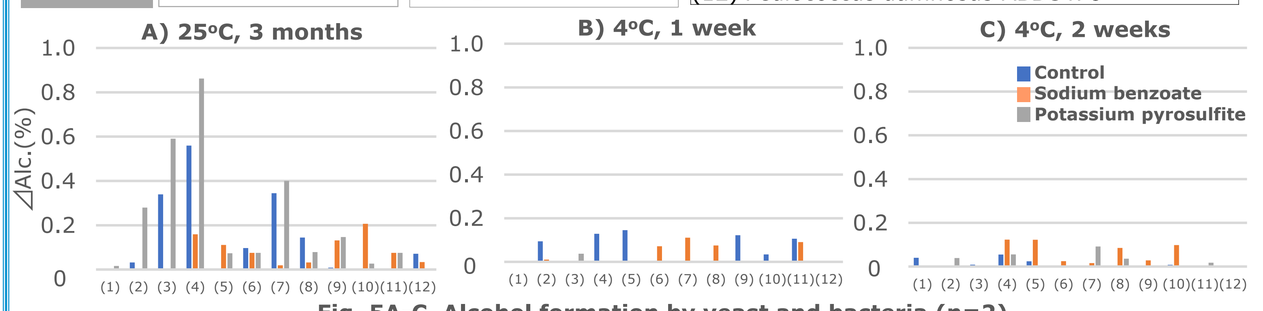

Fig. 5A-C. Alcohol formation by yeast and bacteria (n=2)

Results:
- After 3 months at 25 °C, 0.5% or more alcohol formation was observed in carbonated NAB samples. In contrast, with the addition of sodium benzoate, the maximum alcohol formation was 0.21% under otherwise identical storage conditions (Fig.5A).
- After 1 or 2 weeks at 4 °C, the alcohol formation was suppressed to 0.15% in decarbonate NAB samples (Fig.5B, 5C).

Conclusion

✓ **In NAB production**; The growth of *Salmonella enterica and E. coli* were controlled at 6°C or less after dealcoholization. Additionally, alcohol was formed by contaminant yeast during the period from the dealcoholization process to the completion of cooling. After cooling, alcohol formation was suppressed at 2 °C, although approximately 0.8% alcohol was formed at 4 °C.

✓ **In the NAB kegs**; The growth potential of food-borne pathogens was controlled. Alcohol formation by contaminant yeast was controlled at low temperature for short period. Although 0.5% or more alcohol was formed at room temperature for longer period, the alcohol formation was suppressed by adding sodium benzoate (400 ppm).

→ Controlling the production process with storage temperature at around 2°C after dealcoholization is important to suppress alcohol formation. Additionally, the microbiological management of the NAB keg filling environment is critical to suppress alcohol formation. If there are concerns about the filling environment, adding sodium benzoate is considered effective. Furthermore, NAB kegs should be stored at low temperature to prevent unintended alcohol formation after keg opening.

References

1. Nils Rettberg *et.al. Beverages*, ***8*** (1), 4 (2022). 2. Garry Menz *et.al. J. Food.Prot.*, ***74*** (10): 1670–1675 (2011) .

*Experiment 1-1. was conducted by Japan Food Research Laboratories (Japan).
Experiment 2-1. was conducted by Campden BRI (UK).