KVASNÝ PRŮMYSL

Kvasny prumysl (2025) 71: 1007–1014
https://doi.org/10.18832/kp2025.71.1007

Active Components in Kelp Beer and Its Anticoagulant Effect

Yunqian Cui [1*], Lei Zhu [1,2], Xiaocong Wu [1] and Xiangyu Xi [1]

[1] *School of Bioengineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, Shandong, China*

[2] *Tsingtao Brewery Co. Ltd., Qingdao 266071, Shandong, China*

**corresponding author: cuiyunqian@126.com*

Abstract

The brewing technology of kelp beer with kelp powder and its anticoagulant effect were explored in this study by pre-trials and laboratory-scale tests, the adding method and dosage of kelp powder were determined by analysis of anticoagulant effect and sensory evaluation of beers. Then, a 100 L pilot-scale brewing was successfully accomplished, and the physicochemical indexes and flavor components of final kelp beers were determined, the anticoagulant effect of kelp beer was assessed by measuring the values of activated partial thromboplatin time (APTT, 123.16±1.96 s), prothrombin time (PT, 32.80±0.26 s), and thrombin time (TT, 14.22± 0.51 s), which were higher than those of control beer. Finally, by the analysis of infrared spectroscopy, it was the polysaccharides and fucoidans originated from kelp powder in brewing that imparted the anticoagulant effect of kelp beer.

Keywords: kelp beer; polysaccharide; fucoidan; anticoagulant effect

1 Introduction

The kelp also called *Laminaria japonica* is a kind of brown seaweed cultured in China and many other countries and about 500,000 to 700,000 tons of dry kelp can be harvested in coastal areas of China every year (Zha et al., 2012). Kelp is rich in iodine, vitamins, minerals, proteins, fatty acids, mannitol, seaweed polyphenols and other nutrients and physiological active ingredients, in which there are also abundant *Laminaria* polysaccharides. So far, it is reported that there are four kinds of polysaccharide, i.e. alginate, cellulose, laminarin, and fucoidan (Ke et al., 2020; Abrahama et al., 2018), which have many biological functions, such as regulating immune systems, anti-tumor, decreasing hyperglycemia, anti-coagulant, regulating blood lipid, anti-radiation, anti-oxidation, anti-fatigue, anti-inflammatory and so on (Ke et al., 2020; Abirami et al., 2019; Abrahama et al., 2018; Islam et al., 2014; Wang et al., 2012; Zha et al., 2012; Zoysa et al., 2008). Consequently, kelp was applied to traditional food and complementary medicine in ten thousand years ago (Abirami et al., 2019; Bocanegra et al., 2009) and it was also documented as a drug in traditional Chinese medicine for over a thousand years (Wang et al., 2011). In recent decades, kelp has been attracting great interests of chemists and pharmacologists on account of the abundance of functional components and their specific health benefits (Wang et al., 2012), so more and more foods and beverages rich in kelp were researched and manufactured (Abirami et al., 2019).

Meantime, with the maturity of beer industry, the competition of the market and the demand of consumers for novelty, people began to tend to more varieties and more unique flavors of beer. Functional beer is being more and more favored by consumers because of its high nutrition and health benefits, thus the brewing technology and anticoagulant effect of kelp beer were investigated in this study. As far as we are concerned, there are fewer findings on the brewing of kelp beer and its anticoagulant effect.

Research Institute of Brewing and Malting
Published online: 15 April 2025

2 Materials and methods

2.1 Materials

The top-fermenting yeast DM303 was preserved at China-Germany Brewing Technical Center at Qilu University of Technology, Shandong, China. Pale ale barley malt (5.5–7.5 EBC, Harrington, cultivated in Canada) was purchased from China Yongshuntai Malt Co., Ltd. The bitter hop pellets (Qingdao Flower, α-acid 6%, harvested in 2021) were provided from Xinjiang Sapporo Agricultural Science & Development Co., Ltd (Xinjiang, China), and the aroma hop pellets (Saaz, α-acid 4.5%, harvested in 2021) were supplied from Yakima Chief (Washington, USA). Dried kelp (*Monostroma nitidum*) was purchased from Yantai Wanli Seaweed Food Co., Ltd (Shandong, China), which was dried and ground into powder.

2.2 Reagents

Kits for alcohol, bitterness, color, and residual sugars measurements were purchased from CDR s.r.l. (Florence, Italy). The kits for APTT, PT, and TT were provided by Shanghai Sun Biotechnology Co., Ltd (China). Other reagents were all of the analytical grades.

2.3 The brewing technology of kelp beers

A pre-trial was carried out to explore the potential anticoagulant effect of kelp beer, 0.05 g, 0.5 g, 1 g, and 1.5 g kelp powder were weighed and labeled as A, B, C and D respectively. Under the aseptic environment, they were added into 50 mL commercial Snow beer (8 °P, Snow beer group Binzhou brewery, Shandong, China) and refrigerated at 4 °C for 15 days.

In the laboratory-scale tests, the 14 °P brewer's wort was accomplished in 100 L pilot-scale mashing vessels as follows: the ratio of pale barley malt was 100%, and the infusion mashing was adopted: the mashing-in temperature was 45 °C (20 min), the proteolytic rest was 52 °C (40 min), the amylolytic rest was 65 °C (70 min), the lautering temperature was 78 °C; the boiling time was 70 min, and at 5 min before the end of boiling, the kelp powder was added to wort (Method 1). Yeast strain DM303 was pitched to the 14 °P cooled brewer's wort in a 2.5 L PET bottle, and the pitching rate was about 1×10^7 cells/mL. The primary fermentation was carried out at 20 °C. When the apparent extract was 4 °P or so, the kelp powder was added to fermenting broth (Method 2), and the open lid of PET bottle was bunged until the diacetyl content of the green beer was reduced below 0.1 mg/L. The temperature was then decreased to 0 °C in a refrigerator, and the secondary fermentation continued for more than five days.

In the pilot-scale experiments, kelp beers were brewed in 100 L fermenters according to the brewing technology optimized during laboratory-scale fermentation tests.

2.4 Analysis of the anticoagulant activity of beers

The anticoagulant activity was measured on a SL318 coagulation analyzer (Senlan Trade Co., Ltd, Jinan, Shandong, China) using the reagent kits of activated partial thromboplatin time (APTT), prothrombin time (PT), and thrombin time (TT); distilled water and heparin sodium were used as negative control and positive control, respectively (Liu et al., 2018).

2.5 Sensory evaluation of beers

The sensory evaluation of beers was assessed by a trained panel of 13 beer judges (9 males and 4 females) following the BJCP Beer Score sheet (https://legacy.bjcp.org/docs/SCP_Beer Score Sheet. pdf) from five aspects: appearance, aroma, flavor, mouthfeel, and overall impression. Beer judges were asked to score the samples of each session according to their preference (where the overall score is 50 points with appearance accounting for 3 points, aroma accounting for 12 points, flavor accounting for 20 points, mouthfeel accounting for 5 points, and overall impression accounting for 10 points).

2.6 Determination of physicochemical indexes in beer

The yeast concentration was detected every day using Countstar (Shanghai Ruiyu Biotech Co., Ltd, China) according to the manufacturer's protocol. The levels of diacetyl, alcohol, color, bitterness and residual sugars were detected using CDR BeerLab (CDR s.r.l, Florence, Italy), the pH was measured by a handheld pH meter, the foam stability and total acidity level were measured referring to the ASBC methods (ASBC, 2008; ASBC, 1942).

2.7 The detection of flavor compounds

The levels of flavor compounds (acetaldehyde, DMS, ethyl formate, ethyl acetate, isobutyl acetate, isoamyl acetate, ethyl caproate, ethyl caprylate, n-propanol, isobutanol, and isoamyl alcohol) were determined according to the method described by Wang et al. (2006).

2.8 Extraction of kelp polysaccharide

There are many ways to extract kelp polysaccharide. Because kelp polysaccharide is easy to dissolve in hot water, the water extraction method was adopted in this paper, and the residual polysaccharide could be still filtered once more. The obtained kelp polysaccharide was decolorized by ethanol and purified by fractional precipitation.

According to the method of Liu et al. (2018) and with minor revision, dried kelp was ground to power, 200 g kelp powder was added to 5 000 mL distilled water, the mixture was extracted in a 70 °C water bath for 5 h. The kelp extract was filtered using gauze. Then, the kelp extract was concentrated three times using rota-

ry evaporator. Next, 3.25 times volume of 95% ethanol was added to the concentrated kelp solution for 64 h, the supernatant was separated by centrifugation, and the obtained precipitate was dried in an oven to obtain the crude polysaccharide.

2.9 Extraction of kelp fucoidan

According to the method of Li et al. (2006) and with minor revision, the above crude polysaccharide was dissolved in water to prepare 2.5% polysaccharide solution, and to which 3 mol/L $CaCl_2$ was added to be stirred in water bath at 75 °C for 2 h, then the solution was filtered with gauze to obtain filtrate. 75% ethanol was added to the filtrate, which was then allowed to stand for 12 h and centrifuged at 5000 r for 15 min to obtain the precipitate. The precipitate was dried in an oven at 40 °C to gain fucoidan.

2.10 The analysis of polysaccharide structure in beer by Fourier Transform Infrared (FTIR)

The kelp beer and control beer were poured into the plate, frozen in a -20 °C refrigerator, and transferred into the freeze dryer for freeze-drying after freezing. FTIR spectroscopy of control beer, kelp beer, crude polysaccharide and fucoidan were recorded on a Nicolet Nexus 470 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The sample was mixed with KBr powder, ground, and pressed into 1-mm pellets for FTIR measurements in the frequency range of 500–4000 cm^{-1} (Liu et al., 2018).

2.11 Statistical analysis

The results presented in this work were the average of three independent experiments with the bars representing the standard deviation. An analysis of variance was performed using SPSS software (V16.0, SPSS) to test the significance of anti-coagulation activities of kelp beers and the concentrations of the flavor substances in final kelp beers at $p<0.05$. Once the analysis of variance determined that there was a significant difference among the means of each group, post hoc tests (Duncan's test) were used.

3 Results and discussion

3.1 Anticoagulant effect of pre-trials and laboratory-scale tests

For the pre-trials, the results in Table 1 indicated that the anticoagulant effect of beer with B and C was obviously increased compared with the control, while the enhancement effect of A and D was not significant, even D could not be completely dissolved in the beer, therefore the study on the anticoagulant effect of kelp beer was feasible and significant, and the dosage of kelp powder was set between B and C in the subsequent brewing experiments, which was 10 g/L, 15 mg/L, and 20 g/L, respectively. The specific adding method of kelp powder and its abbreviation were shown in Table 2.

For the laboratory-scale tests, the anticoagulant effects of water, control beer, and kelp beer were shown in Figure 1 (A, B, C).

Table 1 *Anticoagulant effect of pre-trials beer*

Control beer		Pre-trials beer			
		A	B	C	D
APTT(s)	50.20 ± 0.09	59.36 ± 1.30	61.57 ± 1.81	66.88 ± 0.56	52.77 ± 0.88
PT(s)	16.96 ± 0.32	21.37 ± 0.39	23.49± 1.23	26.61 ± 0.48	17.67 ± 0.21
TT(s)	8.35 ± 0.86	9.59 ± 0.24	9.97 ± 0.69	11.72 ± 1.67	8.92 ± 0.58

Table 2 *The abbreviation of corresponding sample*

Abbreviation	Adding method of kelp powder
W-10 (Method 1)	10 g/L kelp powder was added to wort 5 minutes before the end of boiling
W-15 (Method 1)	15 g/L kelp powder was added to wort 5 minutes before the end of boiling
W-20 (Method 1)	20 g/L kelp powder was added to wort 5 minutes before the end of boiling
B-10 (Method 2)	10 g/L kelp powder was added to the fermenting broth before lid was bunged
B-15 (Method 2)	15 g/L kelp powder was added to the fermenting broth before lid was bunged
B-20 (Method 2)	20 g/L kelp powder was added to the fermenting broth before lid was bunged
B-0	Beer sample without adding kelp powder (the control)

Figure 1A shows that, no matter in which stage the kelp powder was added, the APTT values of kelp beer were increased compared with water and control beer, and were positively correlated with the amount of kelp powder, in which the level of W-20 was the highest. For the beer that kelp powder was added 5 minutes before the end of boiling, the APTT values of W-20 were nearly one-third higher compared with W-15; for the beer that kelp powder was added to the fermenting broth before lid was bunged, there was no significant difference in APTT values between B-10 and B-15, but the APTT values of B-20 were about 2-fold than those of B-10 and B-15, and the enhancement effect of APTT was extremely significant. When the same dosage of kelp powder was 10 g/L, the APTT values of the kelp addition at fermenting stage were higher than those of at boiling stage.

Figure 1B shows that, no matter in which stage the kelp powder was added, the PT values of kelp beer were increased compared with water and control beer, and were positively correlated with the dosage of kelp powder, in which the level of W-20 was the highest. For the beer that kelp powder was added 5 minutes before the end of boiling, PT values were all increased in contrast to water and control beer; and for the beer that kelp powder was added to the fermenting broth before lid was bunged, the PT values of B-10 and B-15 were increased and have compatible levels compared with W-10, but the PT values of B-15 and B-20 were decreased compared with W-15 and W-20, respectively.

From Figure 1C, we can see that no matter in which stage the kelp powder was added, the TT values of kelp beer were reduced compared with water and control beer, and the level of W-10 was the lowest. For the beer that kelp powder was added 5 minutes before the end of boiling, TT values were gradually increased with the increase of kelp powder; however, for the beer that kelp powder was added to the fermenting broth before lid was bunged, the changes of TT value were not regular.

Together with the anticoagulant effects of the pre-trials and the laboratory-scale tests, the optimal way of adding kelp powder should be 5 minutes before the end of boiling instead of adding it to the fermenting broth before lid was bunged, and the dosage could be W-10, W-15, and W-20, i.e. 10 g/L, 15 g/L, and 20 g/L, respectively.

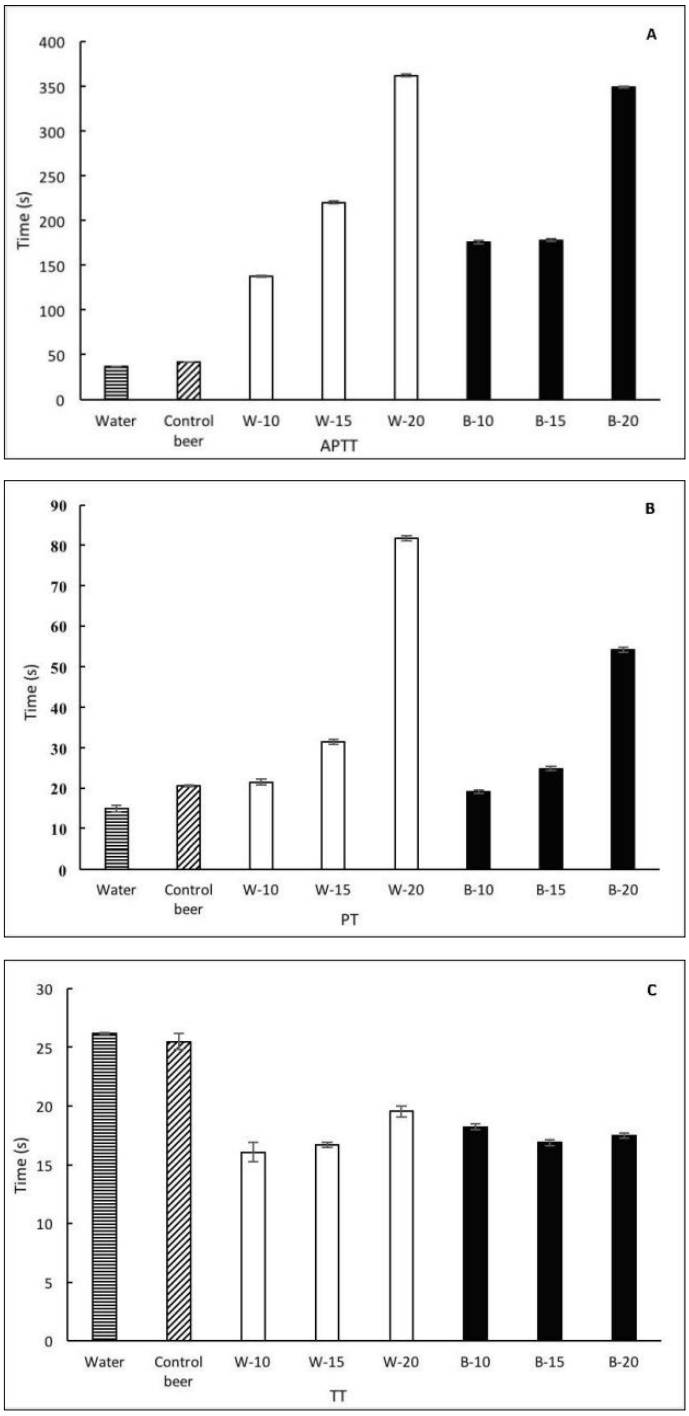

Figure 1 *Comparison of anticoagulant effect of APTT, PT and TT among water, control and kelp beer*

3.2 Sensory evaluation of laboratory- scale tests

Compared with the control beer, Figure 2 exhibited that the sensory score of W-10 was higher than that of the W-15 and W-20, which were 42.9, 35.8 and 27.7, respectively. With the increase of kelp powder, the seafood flavor rich in kelp beer led to its lower score and was not favored by most BJCP judges. It was due to higher sensory score and less kelp cost that the brewing technology of W-10 would be used in the subsequent pilot-scale experiments.

3.3 Sensory evaluation of laboratory-scale tests

It is a well-known fact that the diacetyl level is an important parameter in determining the maturation of beers. After it is reduced to below 0.1 mg/L (Pires et al., 2015), the temperature of beers decreasing till 0–2 °C. The variations of diacetyl in control beer and kelp beer were revealed in Figure 3, in which the reduction rate of diacetyl in kelp beer was faster than that in control beer because of more amino acids, glucoses, minerals and vitamins (Li et al., 2020; Saravana et al., 2018) in the wort brewed with kelp powder during production. The level of diacetyl in kelp beer was decreased rapidly to below 0.1 mg/L on the eighth day and began to cool down, while which was decreased to below 0.1 mg/L on the tenth day in control beer.

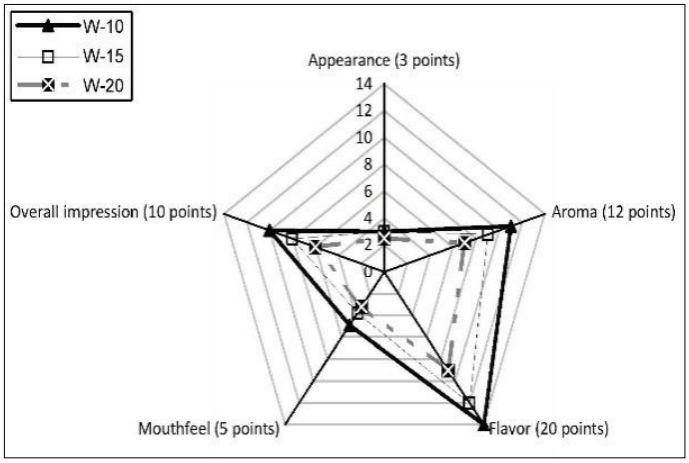

Figure 2 *Sensory evaluation of laboratory-scale tests*

3.4 Changes of yeast count in pilot-scale experiments

Figure 4 shows the changes of yeast count in the control beer and pilot-scale kelp beer which were similar in general.

For pilot-scale kelp beer, at the beginning of fermentation, the numbers of yeasts increased rapidly to reach the peak 1×10^8 cells/mL on the fifth day of fermentation, and then gradually decreased due to the bunging of fermentation tank and limited nutrients (Kucharczyk et al., 2018). On the tenth day of fermentation, the diacetyl level was reduced to within 0.1 mg/L (Figure 3), the temperature of young kelp beer was declined, then the yeast count would be dropped continuously to 2×10^6 cells/mL or so on the thirteenth day; but the control beer reached the maximum value 1.3×10^8 cells/mL on the sixth day of fermentation, on the eighth day of fermentation, the diacetyl level was reduced to within 0.1 mg/L (Figure 3), the temperature of young control beer was decreased, then the yeast count began to show a downward trend till 1.1×10^7 cells/mL on the fifteenth day due to similar reason with the kelp beer.

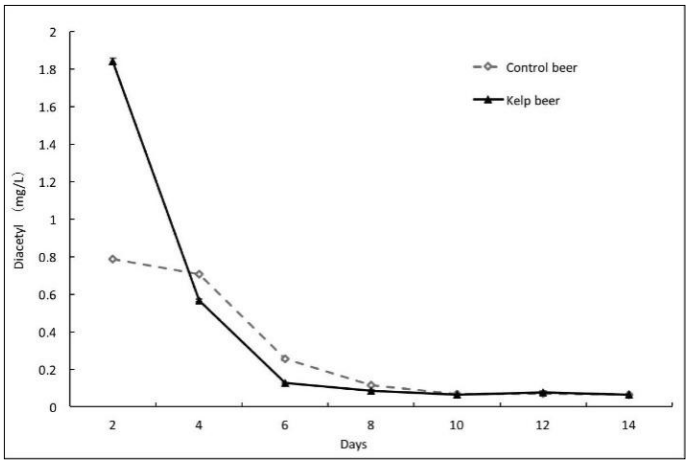

Figure 3 *Variations of diacetyl in pilot-scale experiments*

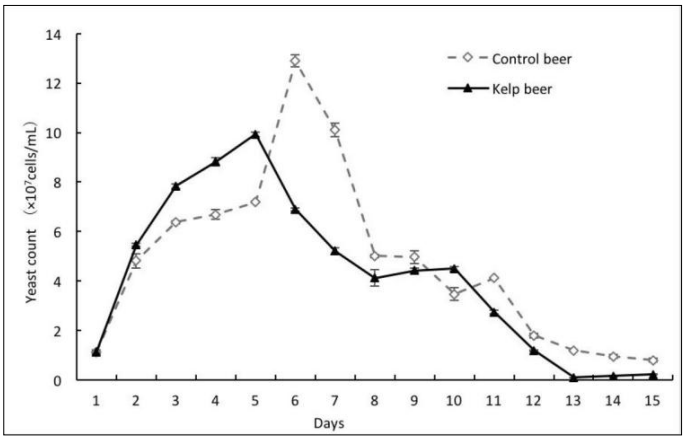

Figure 4 *Changes of yeast count in pilot-scale experiments*

3.5 Changes of alcohol content in pilot-scale experiments

The changes of alcohol content in the control beers and kelp beers were exhibited in Figure 5. When the original gravity of wort was reduced to 4.2 °P, the fermenter was bunged to make the yeast enter the anaerobic respiration stage and start the alcohol metabolism (Roos et al., 2019). During the first four days of fermentation, the alcohol content of the two experimental groups was increased rapidly, and then the rising trend tended to stabilize. After the young beer beg a decreasing temperature, the yeasts entered the decline period, and the rate of alcohol production was tended to slow down. Eventually, the alcohol content of control beer and kelp beer was 6.2 ± 0.02 mg/L and 6.1 ± 0.05 mg/L, respectively.

3.6 Determination of physicochemical indexes in pilot-scale experiments

The physicochemical indexes of pilot-scale experiments were reported in Table 3, the results of all physicochemical indexes in kelp beer were higher than those of in control beer, and both of them could also meet the current China's National Beer Standards GB 4927-2008 (GB4927-2008, 2008).

3.7 Detection of flavor components in pilot-scale experiments

The flavor components in pilot-scale experiments were shown in Table 4. The level of acetaldehyde and DMS in kelp beer was significantly lower than that of in control beer, which was beneficial to the overall flavor of both beers.

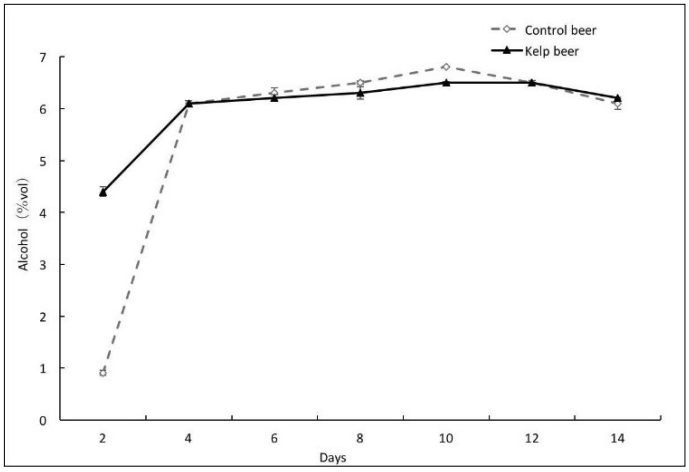

Figure 5 *Changes of alcohol content in pilot-scale experiments*

The total esters content of kelp beer and control beer was 47.83 mg/L and 44.89 mg/L, respectively, which was resulted in due to more amino acids, glucoses, minerals and vitamins (Li et al., 2020; Saravana et al., 2018) in wort when kelp powder was added at boiling stage, and thus the yeast vitality was enhanced to generate more esters. The higher alcohols concentration of kelp beer and control beer was similarly, which were 194.69 mg/L and 196.28 mg/L, respectively, though the higher alcohols concentration of kelp beer and control beer was both higher, yet the esters of them were also higher, the ratio of higher alcohols and esters was 4.07 and 4.37, respectively, which was within the optimum scope of (4.0–4.7):1 (Blanco et al., 2016; Procopio et al., 2011; Smogrovicova et al., 1999; Polednikova et al., 1993), and thus could still reduce the headache sense to people.

Table 3 *Physicochemical properties of pilot-scale experiments*

	Control beer	Kelp beer
Alcohol (%v/v)	6.10 ± 0.02	6.21 ± 0.05
Original gravity (˚P)	13.82 ± 0.07	14.10 ± 0.03
Color (EBC)	13.00 ± 0.03	17.00 ± 0.02
Bitterness (BU)	19.40 ± 0.05	22.90 ± 0.02
Turbidity (EBC)	115.00 ± 0.01	122.00 ± 0.10
pH	4.40 ± 0.01	4.50 ± 0.01
Total acidity (mL/100mL)	2.58 ± 0.30	2.59 ± 0.10
Residual sugar (g/L)	1.60 ± 0.11	2.10 ± 0.21

Table 4 *Flavor components in pilot-scale experiments*

Flavor compounds	Control beer (mg/L)	Kelp beer (mg/L)
Acetaldehyde	9.94 ± 0.02	3.04 ± 0.29
DMS	0.14 ± 0.13	0.12 ± 0.10
Ethyl formate	0.14 ± 0.09	0.17 ± 0.06
Ethyl acetate	39.66 ± 0.43	42.54 ± 0.64
Isoamyl acetate	4.61 ± 0.58	4.42 ± 0.28
Isobutyl acetate	0.38 ± 0.01	0.33 ± 0.0.11
Ethyl hexanoate	0.06 ± 0.01	0.12 ± 0.03
Ethyl octanoate	0.04 ± 0.21	0.25 ± 0.75
n-Propanol	32.98 ± 0.66	45.87 ± 0.88
Isobutanol	49.32 ± 0.89	47.16 ± 0.49
Isoamyl alcohol	113.98 ± 1.32	101.66 ± 0.08
Ratio of higher alcohols and esters	4.37	4.07

3.8 *Anticoagulant effect of pilot-scale experiments*

The anticoagulant effect of pilot-scale experiments was described in Table 5. Compared with water, the values of three anticoagulant effects in two beer groups were increased. Compared with control beer, the APTT values of kelp beer were nearly doubled and the increase effect was more significant ($p<0.05$), the PT and TT values of kelp beer were only increased slightly, but the increasing effect was not pronounced. In short, when kelp powder was added in beer brewing process, the anticoagulant effect of kelp beer was obviously improved in contrast with that of control beer.

In this study, the kelp beer was successfully brewed by adding 10 g/L kelp powder at 5 minutes before the end of boiling, then the anticoagulant effect of kelp beer was explored in detail by pre-trials, laboratory-scale tests and pilot-scale experiments, and finally anticoagulant effect of the kelp beer was proved to be attributed to the kelp polysaccharide and fucoidans, which were originated from the addition of kelp powder during brewing.

In addition, anticoagulant activities of kelp beer were also assessed by measuring the values of APTT (123.16 ± 1.96 s), PT (32.80 ± 0.26 s), and TT (14.22 ± 0.51 s), which were higher than those of the control beer. Gorinstein et al. (2007) found that people drinking moderate beer in the short term could indeed improve the anticoagulant effect their body. Consequently, the anticoagulant effect of kelp beer will be potentially beneficial to prophylaxis and treatment of thrombosis for people (Luo et al., 2013). Of course, after all the kelp beer is not a medicine and it can also not replace the role of medicine.

Table 5 *Anticoagulant index of beer in pilot-scale experiment*

	APTT(s)	PT(s)	TT(s)
Water	57.65 ± 1.07	22.96 ± 0.08	10.35 ± 0.39
Control beer	72.98 ± 1.52	31.68 ± 0.07	12.59 ± 0.24
Kelp beer	123.16 ± 1.96	32.80 ± 0.26	14.22 ± 0.51

The FTIR spectrum was exhibited in Figure 6, in which there were obvious and similar absorption peaks at 2930, 1607, 1300, 1257 and 889 cm^{-1} in kelp beer, kelp crude polysaccharide and fucoidans, but no corresponding absorption peaks in control beer, which indicated that polysaccharide and fucoidans were exist in kelp beer due to the addition of kelp powder in brewing, and imparted the potential anticoagulant effect of kelp beer, which is also accordance with Wang et al. (2012).

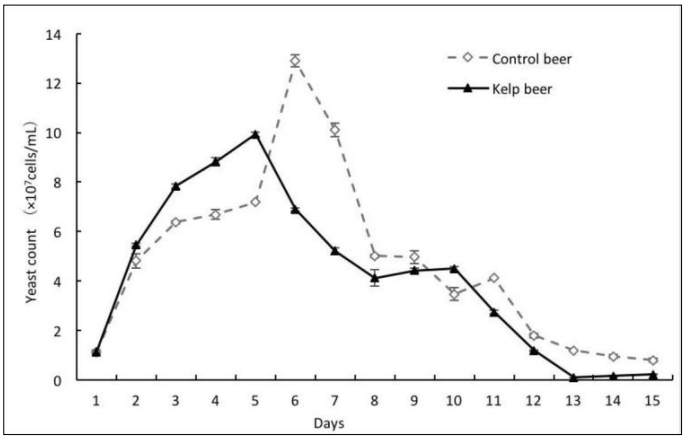

Figure 6 *The FTIR analysis of kelp beer and control beer*

In these 6 absorption peaks, the peak at 2930 cm^{-1} was from the characteristic C–H stretching vibrations of carbohydrates, the one at about 1607 cm^{-1} was the characteristic peak of carboxyl group caused by the asymmetric stretching vibration of C=O, the band at about 1300 cm^{-1} was attributed to the asymmetric and symmetric deformation vibrations of $-CH_3$ of fucose residue, the two signals appeared at 1257 cm^{-1} and 889 cm^{-1} could be assigned to the S=O asymmetric stretching vibration, symmetric C–O–S stretching vibration, indicating the presence of the sulfate groups from kelp (Li et al., 2022; Ning et al., 2022; Zha et al., 2012). With regards to the band at 2300 cm^{-1}, this signal of asymmetric stretching vibration should be arisen from the CO_2 in ambient air.

4 Conclusion

As far as we are concerned, there are few studies on the brewing technology kelp beer and its anticoagulant effect. In this study, the kelp beer was successfully brewed by adding 10 g/L kelp powder at 5 minutes before the end of boiling, then the anticoagulant effect of kelp beer was explored in detail by pre-trials, laboratory-scale tests and pilot-scale experiments, and finally anticoagulant effect of the kelp beer was proved to be attributed to the kelp polysaccharide and fucoidans, which were originated from the addition of kelp powder during brewing.

5 Acknowledgement

This study was funded by the General Program of Natural Science Foundation of Shandong Province (ZR2020MC205) and International Cooperation Program of Qilu University of Technology (Shandong Academy of Sciences) (QLUTGJHZ2018011).

The authors thank Professor Changheng Liu and Dr. Airong Jia from Shandong Academy of Sciences for constant support and guidance in kelp handling.

6 References

Abirami, R., Uma, T., Gaurav, R. (2019). Seaweed nutraceuticals and their therapeutic role in disease prevention. Food Science and Human Wellness, 8(3), 252–263. http://doi.org/10.1016/j.fshw.2019.08.001

Abrahama, R., Su, P., Puri. M., Raston, C., Zhang, W. (2018). Optimisation of biorefinery production of alginate, fucoidan and laminarin from brown seaweed *Durvillae apotatorum*. Algal Research, 38(2), 101389. http://doi.org/10.1016/j.algal. 2018.101389

ASBC (1942). Acidity and pH. Proceedings of the American Society of Brewing Chemists, 3, 103–104.

ASBC (2008). Foam stability of beer using the NIBEM-T meter. Journal of the American Society of Brewing Chemists,, 66(4), 271-272. http://doi.org/10.1094/ASBCJ-2008-1021-02

Beer Judge Certification Program (2015). https://legacy.bjcp.org/docs/SCP_Beer Score Sheet.pdf

Blanco, C., Iglesias, C., Montero O. (2016). Low-alcohol beers: flavor compounds, defects and improvement strategies. Critical Reviews in Food Science and Nutrition, 56(8), 1379-1388. http://doi.org/10.1080/10408398.2012.733979

Bocanegra, A., Bastida, S., Benedi, J., Ródenas, S. (2009). Characteristics and nutritional and cardiovascular-health properties of seaweeds. Journal of Medicinal Food, 12(2), 236–258. http://doi.org/10.1089/jmf.2008.0151

GB4927-2008 (2008). Chinese National Standard: Beer. Chinese Standard Press, Beijing.

Gorinstein, S., Caspi, A., Libman, I., Leontowicz, H., Leontowicz, M., Tashma, Z., Katrich, E., Jastrzebski, Z., Trakh-tenberg, S. (2007). Bioactivity of beer and its influence on human metabolism. International Journal of Food Sciences and Nutrition, 58(2), 94–107. http://doi.org/ 10.1080/09637480601108661

Islam, M., Ahmed,S., Kim, Y., Mun, H., Yang, C. (2014). Effect of sea tangle (*Laminaria japonica*) and charcoal supplementation as alternatives to antibiotics on growth performance and meat quality of ducks. Asian-Australasian Journal of Animal Sciences, 27(2), 217–224. http://doi.org/ 10.5713/ajas.2013.13314

Ke, S., Wei, B., Qiu, W., Zhou, T., Wang, S., Chen, J., Chen, J., Zhang, H., Jin, W., Wang, H. (2020). Structural characte-rization and α-glucosidase inhibitory and antioxidant activities of fucoidans extracted from *Saccharina japonica*. Chemistry & Biodiversity,17(7), e2000233. http://doi.org/10.1002/cbdv.202000233

Kucharczyk, K., Tuszyński, T. (2018). The effect of temperature on fermentation and beer volatiles at an industrial scale, Journal of the Institute of Brewing, 124(3), 230–235. http://doi.org/10.1002/jib.491

Li, B., Wei, X., Sun, J., Xu, S. (2006). Structural investigation of a fucoidan containing a fucose-free core from the brown seaweed, *Hizikia fusiforme*. Carbohydrate Research, 341(9), 1135–1146. http://doi.org/10.1016/j.carres.2006.03.035

Li, G., Row, K. (2020). Air assisted dispersive liquid-liquid microextraction (AA-DLLME) using hydrophilic-hydrophobic deep eutectic solvents for the isolation of monosaccharides and amino acids from kelp. Analytical Letters, 53(2), 188–202. http://doi.org/10.1080/00032719.2019.1643358

Li, P., Bai, J., Zhang, X., Yan, Z., He, P., Chen, Y. (2022). Structure and anticoagulant activity of a galactofuranose-containing sulfated polysaccharide from the green seaweed, codium isthmocladum. Molecules, 27(22), 8012. http://doi.org/10.3390/molecules27228012

Liu, X., Du, P., Liu, X., Cao, S., Qin, L., He, M., He, X., Mao, W. (2018). Anticoagulant properties of a green algal rhamnan-type sulfated polysaccharide and its low-molecular-weight fragments prepared by mild acid degradation. Marine Drugs, 16(11), 445. http://doi.org/10.3390/md16110445

Liu, X., Wang, S., Cao, S., He, X., Qin, L., He, M., Yang, Y., Hao, J., Mao, W. (2018). Structural characteristics and anticoagulant property in vitro and in vivo of a seaweed sulfated rhamnan. Marine Drugs, 16(7), 243. http://doi.org/10.3390/ md16070243

Luo, L., Wu, M., Xu, L., Lian, W., Xiang, J., Lu, F., Gao, N., Xiao, C., Wang, S., Zhao, J. (2013). Comparision of physicochemical characteristics and anticoagulant activities of polysaccharides from three sea cucumbers. Marine Drugs, 11(2), 399–417. http://doi.org/ 10.3390/md11020399

Ning, Z., Wang, P., Zuo, Z., Tao, X., Gao, L., Xu, C., Wang, Z., Wu, B., Gao, N., Zhao, J. (2022). A fucan sulfate with pentasaccharide repeating units from the sea cucumber holothuriafloridana and its anticoagulant activity. Marine Drugs, 20(6), 377. http://doi.org/10.3390/md20060377

Pires, E.J., Teixeira, J.A., Brányik, T., Brandão, T., Vicente, A.A. (2015). Continuous beer fermentation-diacetyl as a villain. Journal of The Institute of Brewing, 121(1), 55–61. http://doi.org/10.1002/jib.205

Poledníková, M., Voborský, J., Chládek, L., Sruma, T. (1993). Beer production using immobilized yeasts on pilot plant scale (in Czech). Kvasny prumysl, 39(1), 2–7. http://doi.org/10.18832/kp1993001

Procopio, S., Qian, F., Becker, T. (2011). Function and regulation of yeast genes involved in higher alcohol and ester metabolism during beverage fermentation. European Food Research and Technology, 233(5), 721–729. http://doi.org/10.1007/s 00217-011-1567-9

Roos, J., Vuyst, L. (2019). Microbial acidification, alcoholization, and aroma production during spontaneous lambic beer production. Journal of the Science of Food and Agriculture, 99(1), 25–38. http://doi.org/10.1002/jsfa.9291

Saravana, P., Cho, Y., Woo, H., Chun, B. (2008). Green and efficient extraction of polysaccharides from brown seaweed by adding deep eutectic solvent in subcritical water hydrolysis. Journal of Cleaner Production, 198(7), 1474–1484. http://doi.org/ 10.1016/j.jclepro.2018.07.151

Šmogrovičová, D., Dömény, Z. (1999). Beer volatile by-product formation at different fermentation temperature using immobilised yeasts. Process Biochemistry, 34(8), 785–794. http://doi.org/10.1016/S0032-9592(98)00154-X

Wang, D., Sun, J., Zhang, W., Jia, F., Yang, Y., Lin, Z., Feng, J., Pavlovic, M. (2006). Disruption of brewer's yeast alcohol dehydrogenase II gene and reduction of acetaldehyde content during brewery fermentation. Journal of the American Society of Brewing Chemists, 64(4), 195–201. http://doi.org/10.1094/ASBCJ-64-0195

Wang, J., Wang, F., Yun, H., Zhang, H., Zhang, Q. (2012). Effect and mechanism of fucoidan derivatives from *Laminaria japonica* in experimental adenine-induced chronic kidney disease. Journal of Ethnopharmacology, 139(3), 807–813. http://doi.org/10.1016/j.jep.2011.12.022

Wang, J., Zhang, Q., Zhang, Z., Hou, Y., Zhang, H. (2011). In-vitro anticoagulant activity of fucoidan derivatives from brown seaweed *Laminaria japonica*. Chinese Journal of Oceanology and Limnology, 29(3), 679–685. http://doi.org/10.1007/s00343-011-0181-9

Zha, X., Xiao, J., Zhang, H., Wang, J., Pan, L., Yang, X., Luo, J. (2012). Polysaccharides in *Laminaria japonica* (LP): extraction, physicochemical properties and their hypolipidemic activities in diet-induced mouse model of athero-sclerosis. Food Chemistry, 134(1), 244–252. http://doi.org/10.1016/j.foodchem.2012.02.129

Zoysa, M., Nikapitiya, C., Jeon, Y., Lee, Y., Lee, J. (2008). Anticoagulant activity of sulfated polysaccharide isolated from fermented brown seaweed *Sargassum fulvellum*. Journal of Applied Phycology, 20(1), 67–74. http://doi.org/10.1007/s10811-007-9182-7