НАУЧНИ ТРУДОВЕ НА
УНИВЕРСИТЕТ ПО ХРАНИТЕЛНИ
ТЕХНОЛОГИИ - ПЛОВДИВ
2016 г.
ТОМ 63, КНИЖКА 1

SCIENTIFIC WORKS OF
UNIVERSITY OF FOOD
TECHNOLOGIES
2016
VOLUME 63 ISSUE 1

Post-Fermentative Operations in the Technology of Non-Alcoholic Beer

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Running title: **Using of the Fructose Syrup and Polyclar®10 as a PVPP Preparation in the Technology of Non-alcoholic Beer**

Abstract
Non-alcoholic beer was the product of the scientific topic "Research on producing non-alcoholic beer" implemented by the University of Economics and Technical Industries, Hanoi, Vietnam. The beer has been produced at an industrial scale of 10.000 liters/batch. During the filtering process, Polyclar®10 as a PVPP preparation was used for separation of the polyphenols to prolong shelf-life of the finished beer. The appropriate proportion of the added Polyclar®10 was identified as 20 g/hl to guarantee the product's colloidal stability. Alcohol is an important flavor component and to improve the tasteless of non-alcoholic beer, fructose syrup was added in beer filtration process as a flavor enhancer. The suitable rate of the fructose syrup was determined to be 0.5% compared with filtered beer. The beer was bottled, pasteurized at same regime with bottles line in Ha Noi Brewery at 25 PU. Determination of the shelf-life of finished beer was based on the experiments of storing and analyzing the physico-chemical, microbiological indicators and sensory evaluation. The shelf-life of non-alcoholic beer was determined of 6 months.

Practical applications
Improving the tasteless and prolonging the shelf-life of non-alcoholic beer.

Key words: Non-alcoholic beer, Polyclar®10, flavor enhancer, shelf-life.

Introduction

After fermentation and maturation, the beer has to be improved by a number of processes like filtration, CO_2 saturation, bottling, pasteurization, labeling, packaging, warehousing. The stability of beer is the preservation of the organoleptic and physico-chemical properties for a specified period of time. During the storage of the beer different physico-chemical and biological processes run and cause visible quality modifications. The non-organic sediment (sludge) in beer is mainly due to the interaction between proteins, polyphenols, and trace amounts of carbohydrates and micronutrients (Bamforth, 1999). There are different models for describing the interaction between proteins and polyphenols and the generated complex systems. The distinctive polyphenols of the beer that are active in terms of the colloid destabilizing are proanthocyanidin representatives. These are the monomers, dimers, trimers and polymers of the catechin, the epicatechin and the gallocatechin, while the dimeric procyanidins which are dominant in relation to concentration are essential to the formation of turbidity. PVPP is a typical adsorbent for polyphenols and is a homopolymer of the venylpyrrolidone with high degree of polymerization and cross-linking. The adsorption of polyphenols by PVPP is irreversible during the beer processing (Kabzev & Ignatov, 2011).

To improve the beer organoleptic indicators the fructose syrup was added in beer filtration process. Within the usual filtration, the beer has not been released of microorganisms such as cultural and wild yeast and bacteria. The development of microorganisms in the bottled beer in all cases leads to biological clouding. The pasteurization is a heat treatment which is one of the safest ways for destruction of the microorganisms (Kabzev & Ignatov, 2011). Except the method 9.30 of EBC (Prediction of Shelf-Life of Beer) for determining the shelf-life of beer, the storage of samples under conditions similar to the real conditions for a period of 3-6 months and tracking the quality of beer have been practiced (Analitica - EBC, 2005).

Materials and methods

The non-alcoholic beer, produced on the principle of saccharification at high temperature and fermentation at low temperature for a long period of time in the Hanoi Brewery Plant after maturation has the following characteristics: 0.46% alcohol v/v; Initial extract 5.37%; Diacetyl 0.14 mg/dm^3; Polyphenol 76.2 mg/dm^3; Color 9.2 units EBC; Acidity of 1.2 ml NaOH 0.1N/10 ml beer; Bitter substances 23.6 BU; CO_2 4.5 g/dm^3; organoleptic assessment 17.0 (Ho, T. A., 2015).

Polyclar®10 is a PVPP (Polyvinyl polypyrrolidone) preparate with an American origin. Experiments for the separation of polyphenols during the beer filtration have been conducted in which besides the control sample (without Polyclar®10) the experimental variants have been formed with doses of 10 g/hl, 15 g/hl, 20 g/hl and 25 g/hl Polyclar®10 injected to a certain quantity of the filtered beer. The amount of polyphenols in the filtered beer has been determined under 9.11 EBC method as well as the sensitive nitrogen with the apparatus Analyser 2010 PPT (Analytica - EBC, 2005).

A Korean Fructose syrup is used for improving the beer flavor. The addition of the syrup in the beer is carried out in a stream by the dosing pump for kizelgurg (diatomit) during the filtration. The concentration of the syrup varies from 0% (control sample) to 0.3%; 0.5%; 0.7%; 1.0% relatively to the quantity of the filtered beer and the product has been assessed organoleptic.

After bottling, the beer goes through a pasteurizing apparatus with the following indicators: Initial inlet temperature: 5°C; Outlet/end temperature 34°C; The pasteurization temperature is 62°C for the time of 10 minutes; General pasteurizing units PU 25; Total time for the bottle passage is 34 minutes. For determining the sample shelf-life, they have been stored in a warehouse under conditions similar to the realization and the physico-chemical, microbiological indicators and the organoleptic assessment have been analyzed within a period of 3 – 6 months. The Analytical methods for the indicators follow the Vietnamese Standartization (TCVN) and the European Brewing Convention (EBC).

Results and discussion

Compared to the ordinary beers after fermentation and maturation non-alcoholic beer has a significantly lower alcohol concentration, higher content of the low molecular weight sugars absorbed by micro-organisms so it is necessary to improve the quality. Beer should be processed and pasteurized to extend the storage period.

1. The choice of an appropriate ratio of Polyclar®10 towards the filtered beer

Limpidity – one of the main organoleptic indicators of the beer that is being improved by filteration

with kyselgur. Under TCVN, beer must have the limpidity up to 0.5 EBC. In beer production Polyclar®10 is widely used during the filtration of beer for releasing the polyphenols against colloid clouding.

During the production of non-alcoholic beer a technology, significantly different to that used for ordinary beer has been applied and after maturation the beer has a chemical composition that strongly differs from that of the alcohol containing beers (Ho, T. A., 2015). The experiments have been conducted to five variants (from variant 1 to variant 5) with different amounts of Polyclar®10 added during filtration respectively of 0 g/hl (control sample) – 10 – 15 – 20 – 25 g/hl. The results from the dimension of polyphenols and the sensitive nitrogen for clouding in beer are presented in figure 1.

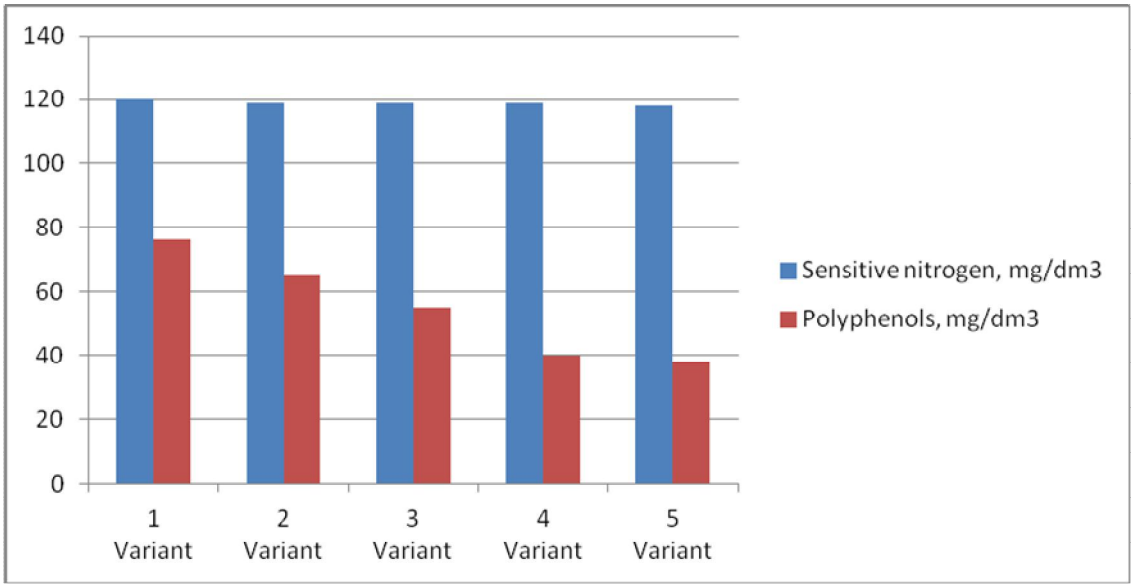

Fig. 1. Influence of the ratio of Polyclar®10 on the quality of beer

The results obtained on Fig. 1 show that the ratio of Polyclar®10 to the filtered beer has an insignificant effect on the quantity of sensitive proteins in beer, and the measured values of the sensitive nitrogen vary within a narrow interval between 118 – 120 mg/dm^3 within different doses of Polyclar®10. During filtration, however, a considerable amount of polyphenols has been absorbed on Polyclar®10, the concentration of polyphenols decreases from 76.2 mg/dm^3 in the control sample to 65 mg/dm^3 and 55 mg/dm^3 when the dose of Polyclar®10 has been increased up to 10-15 g/hl respectively. In the last two variants 4 and 5, where Polyclar®10 has been dosed respectively of 20 and 25 g/hl, the quantity of polyphenols reduced to 38 – 40 mg/dm^3, which is at about 50% of their initial concentration prior the treatment with Polyclar®10. There is a minor difference between the latter two variants which enables choosing the appropriate ratio of Polyclar®10 of 20 g/hl for the separation of polyphenols in the technology of non-alcoholic beer.

2. Selection of appropriate fructose syrup dose

The analysis of the non-alcoholic German OeTtinger beer shows that the alcohol content is up to 0.15% v/v, initial extract of 7.29%, the color intensity 7.8 EBC, bitterness 18.7 BU, acidity 1.5 ml NaOH 0.1N/10 ml beer. The analysts have not found out any significant distinctions in terms of flavor. The taste of beer, however, is sweet and gives a sense of a better residual taste that offsets the flat taste of the non-alcoholic beer. From the analysis of the sugar composition in HPLC, the following results have been established: Maltose: 33.91 g/dm^3; Glucose: 8.66 g/dm^3; Fructose: 2.71 g/dm^3. The sugar content of the beer is quite higher than the existing Japanese, Dutch and other non-alcoholic and regular beers (Ho, T. A., 2015). The sweet taste of the German OeTtinger beer has a positive influence and

gives basis for conditioning of the taste of the trial non-alcoholic beer.

Addition of Vietnamese maltose syrup and Korean fructose syrup during the filtration of beer has been experimented. The maltose syrup, however, has a low sweetness and very high viscosity due to the low degree of hydrolysis of the starch and it hampers her dissolution in the cold beer.

In cases when 0.3% fructose syrup has been added a sweeter taste than the control sample has established by the the tasting committee but the result was still not that impressive. When the concentration of the syrup was increased up to 0.5% the best organoleptic evaluations have been formed, the taste is harmonious and comes close to that of the German OeTtinger beer. A concentration of the syrup of 0.7% leads to an increased sweet aftertaste that affects negatively the remaining flavor and after a certain period of time a sour taste of the formed acids has been established. The use of 1.0% fructose syrup is the reason for the determined corn smell that comes from the raw materials of the syrup.

By the discussion of the obtained results a suitable concentration of the added fructose syrup of 0.5% to the amount of the filtered beer was chosen.

3. Determination of the shelf-life of the experimental non-alcoholic beer

Beer yeasts and some wild species as well as the lactic bacteria grow very easily in the bottled beer. The collective research work has found that the effectiveness of pasteurization regarding the destruction of the microorganisms reaches 99.35%. From a microbiological point of view, the experimental 25 PU are suitable. When the samples are treated in cold-warm-cold cycles and the measurement of the turbidity within the standard method 9.30 EBC, the experimental non-alcoholic beer was prognosticated to have a shelf-life of 6 months (Ho, T. A., 2015).

In practice, the samples of the pasteurized non-alcoholic beer have been stored and the physico-chemical and microbiological indicators have been monitored in six months. The results of the beer analysis before and after the storage are shown in table 1.

Table 1: Physico-chemical indicators of beer

№	Indicators	Unit	Analytical methods	Before storage	After storage
1	Alcohol	% v/v	Scaba	0.45	0.42
2	Apparent extract	%	Scaba	4.86	4.83
3	Real extract	%	Scaba	5.04	5.02
4	Initial extract	%	Scaba	5.75	5.71
5	Acidity (ml NaOH 0.1 N/10 ml beer)	ml	TCVN 5564:1991	1.20	1.20
6	Bitterness	BU	EBC 9.8	23.4	23.1
7	Polyphenol	mg/dm^3	EBC 9.11	76.1	76.0
8	Limpidity	EBC	Haffman	0.20	0.24
9	Diacetyl	mg/dm^3	9.24.1 EBC	0.14	0.14
10	Color	EBC	EBC 9.6	9.5	9.4
11	CO_2	g/dm^3	Haffman	4.5	4.2
12	pH	-	EBC 9.35	4.6	4.5
13	Foam stability (10-20-30)	s	Haffman	60-91-125	49-82-118

The obtained results from table 1 show that the pasteurization is the reason for the increasing of

the color intensity of 9.2 to 9.5 units EBC in beer, while the other indicators change insignificantly during the pasteurization.
From the data analysis of the beer before and after its 6 months storage it is determined that the physico-chemical indicators of beer remained with stable values. During the time of storage a slight reduction of the bitterness and the concentration of CO_2 has been observed, while the limpidity rises from 0.2 up to 0.24 EBC, and the reason probably is the inevitable coagulation of the protein-polyphenol complex. Minor differences in physico-chemical indicators result in the conjecture that the beer preserves its quality during the storage period.
The beer is being analyzed after the 6 month storage, in order to evaluate the beer stability in microbiological terms and the results are listed in table 2.

Table 2: Microbiological indicators of beer after its 6 month storage

№	Indicators	TCVN	Result
1	Total aerobic bacteria CFU/ml	TCVN 4884:2005 (ISO 4833:2003)	2
2	*E. coli*, CFU/ml	TCVN 6846:2007 (ISO 7251:2005)	0
3	*Staphylococcus aureus*, CFU/ml	TCVN 4830-3:2005 (ISO 6888-3:2003)	0
4	*Clostridium perfringens*, CFU/ml	TCVN 4991:2005 (ISO 7937:2004)	0

Data from table 2 show that the experimental non-alcoholic beer is stable from the microbiological point of view and the pasteurization has a sufficient impact. An organoleptic assessment of the stored beer is carried out in parallel after 3 and 6 months and the result is shown in table 3. The tasting committee was consisted from trained assessors from University of Economics and Technical Industries, Technical institute of brewing and Ha Noi brewery.

Table 3: Organoleptic indicators of beer

Test	Storage time	Taste	Fragrance	Foam	Limpidity, color	Note	Common assessments
Experimental beer	3 months	6.53	5.10	3.50	1.77	Harmonious flavour and fine foam	**16.90**
	6 months	6.43	5.00	3.45	1.68	Harmonious flavour and aroma, fine foam	**16.56**

Note: The assessments have been multiplied by the coefficient for importance of indicators.

Data from table 3 shows that the experimental beer after 6 months storage provides a good flavor and aroma, the organoleptic assessment after 3 and 6 months storage is equivalent to indicators of the control. The beer foam was stable and sensory indicators of the beer were benefited due to technology without intervention in beer after fermentation. In practice, the continuous storage up to 8 and 9 months have resulted in the expression of oxidative taste and aroma.
Compared to the ordinary alcoholic beers or the foreign non-alcoholic beer, the experimental beer have a shorter storage

period. The reason probably is due to the specific technology, which leads to the increased values of the higher molecular protein fractions that cause turbidity. (Ho, T. A., 2015, Plovdiv).

From the data analysis of the experiments, collective research work has established that the non-alcoholic beer preserves its physico-chemical, microbiological and organoleptic qualities within a period of six months.

Conclusion

The parameters of the post-fermentative operations in the technology of non-alcoholic beer are established as follows: The proper ratio of the added Polyclar®10 is 20 g/hl, the appropriate ratio of the fructose syrup is 0.5% compared to the amount of the filtered beer, the beer is pasteurized at mode with PU 25. The shelf-life of non-alcoholic beer has been determined to be six months.

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