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World Brewing Congress 2016

Investigating the effect of wort amino acids on lager yeast fermentation performance and beer flavour production through the application of Plackett-Burman Experimental Design

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World Brewing Congress
August 13-17, 2016
Sheraton Downtown Denver
Denver, CO 80202, U.S.A.

Introduction

Brewery wort is a complex yeast growth and fermentation medium, which contains an array of assimilable nitrogen. Amongst all the assimilable nitrogen, amino acids are the most abundant (1). Alterations related to nutritional parameters (e.g., malt varieties, novel adjuncts, adjunct composition, adjunct ratios or even mashing regimes, etc.) can lead to changes in the concentration of specific amino acid in wort. Amino acids composition and their utilization by yeast during wort fermentation influence both yeast fermentation performance and the flavour profile of the finished product (2, 3), which is therefore critical for maintaining product quality and consistency. This is particularly the case with multisite brewing. However, knowledge regarding the influences of wort amino acids on yeast performance and flavour profiles in beer fermentation was incomplete.

- Project Aims

This work was meant to investigate significant amino acids on yeast fermentation performance and major volatile production of an industrial lager yeast under conditions resembling beer fermentations.

Methods

- Static fermentation was carried out in glass tubes recommended by the European Brewery Convention. Experiments were performed using an industrial lager brewing strain, *S. pastorianus* TT-21, obtained from a Tsingtao brewery yeast storage vessel.
- Synthetic medium used for fermentation was composed with same amount of carbohydrate concentration, but different amino acids were added according to Design-Expert 8.0.6.1 (Stat-Ease, Minneapolis, MN, USA) by using Plackett-Burman design (PBD) (Table 1).

Table 1. Design of experiments of amino acids composition in synthetic medium. Concentration of amino acids in mg/L

Sample	Asp	Ser	Asn	Glu	Gly	His	Gln	Arg	Thr	Ala	Pro	Tyr	Val	Met	Lys	Ile	Leu	Phe	Trp	Dummy 1	Dummy 2	Dummy 3	Dummy 4
1	55	80	80	130	55	40	100	140	90	100	500	160	110	25	120	90	140	115	60	-1	1	-1	-1
2	55	120	80	65	30	40	100	140	60	140	350	160	110	25	75	60	200	170	60	1	1	-1	1
3	80	120	50	130	30	40	100	140	60	140	500	90	110	45	75	90	140	115	35	-1	1	1	1
4	80	120	80	65	55	20	100	200	60	100	500	160	110	25	120	60	200	115	35	-1	-1	1	1
5	55	120	80	65	30	40	50	200	60	100	350	90	150	45	120	90	200	115	60	-1	1	1	-1
6	55	80	50	65	55	40	100	200	90	100	500	90	150	45	75	60	200	170	35	-1	1	-1	1
7	55	120	50	130	30	20	50	140	90	140	500	160	150	25	120	60	200	170	35	-1	1	1	-1
8	55	120	50	130	55	20	50	200	90	100	350	160	110	45	75	60	140	115	60	1	1	1	1
9	80	80	80	65	55	40	50	140	90	140	350	90	150	25	120	60	140	115	35	1	1	1	1
10	55	80	50	65	30	20	50	140	60	100	350	90	110	25	75	60	140	115	35	-1	-1	-1	-1
11	80	80	80	130	30	20	100	200	60	100	500	90	150	25	75	60	140	170	60	1	1	1	-1
12	80	80	50	130	30	40	50	140	60	100	500	160	150	45	120	60	200	115	60	1	-1	-1	1
13	55	120	50	65	30	20	100	200	90	140	500	90	150	25	120	90	140	115	60	1	-1	-1	1
14	80	80	50	65	30	40	100	200	90	140	350	160	110	45	120	60	140	170	60	-1	-1	1	-1
15	55	120	80	130	55	40	50	200	60	140	500	90	110	45	120	60	140	170	35	1	-1	-1	-1
16	67.5	100	65	97.5	42.5	30	75	170	75	120	425	125	130	35	97.5	75	170	142.5	47.5	0	0	0	0
17	55	80	80	130	30	20	100	140	90	100	350	90	110	45	120	90	200	170	35	1	-1	1	1
18	55	80	80	65	55	20	50	140	60	140	500	160	150	45	75	90	140	170	60	-1	-1	1	1
19	80	120	80	130	55	20	100	140	90	140	350	90	150	45	75	60	200	115	60	-1	-1	-1	-1
20	80	120	50	65	55	40	50	140	90	100	500	90	110	25	75	90	200	170	60	1	-1	1	-1
21	80	80	50	130	55	20	50	200	60	140	350	90	110	25	120	90	200	170	60	-1	1	-1	1
22	80	120	80	130	30	40	50	200	90	100	350	160	150	25	75	90	140	170	35	-1	-1	-1	1
23	55	80	50	130	55	40	100	200	60	140	350	160	150	25	75	90	200	115	35	1	-1	1	-1
24	80	120	50	65	55	20	100	140	60	100	350	160	150	45	120	90	140	170	35	1	1	-1	-1
25	80	80	80	65	30	20	50	200	90	140	500	160	110	45	75	90	200	115	35	1	1	-1	-1
26	67.5	100	65	97.5	42.5	30	75	170	75	120	425	125	130	35	97.5	75	170	142.5	47.5	0	0	0	0

Results and Discussion

- **Fermentation characteristics**
  - The final RDF and alcohol concentration (% v/v) were similar for all fermentations, with RDF between 78.8~81.3% and alcohol concentration between 5.6~5.8% for all 26 tests. However, the maximum yeast cell counts in suspension were slightly different from each other dispersed from 3.0×10^7 to 4.0×10^7.
- **Flavour compounds production and yeast amino acids absorption**
  - The total higher alcohols and esters production were greatly affected by the amino acids composition of the synthetic medium, with total higher alcohols ranging from 124.08~196.07 mg/L and total esters 20.08~26.10 mg/L.
  - The total amino acids absorption of yeast were greatly affected by the amino acids composition of the synthetic medium varied from 58.4% to 77.2% .
  - The differences in flavour production and yeast amino acids absorption were not related to the total amount of amino acids in the synthetic medium.
- **Screening of important amino acids in flavour production by PBD**
  - The presence of glutamate acid and histidine would inhibit the synthesize of higher alcohols ($p < 0.5$).
  - The most significant effect on esters production was exercised by Leu, which could facilitate the formation of esters ($p < 0.5$).
  - The concentration of Glu, Pro and Ile presented a negative effect on the total absorption rate of amino acids. On the other hand, Lys had a positive effect on the use of amino acids by yeast ($p < 0.5$).

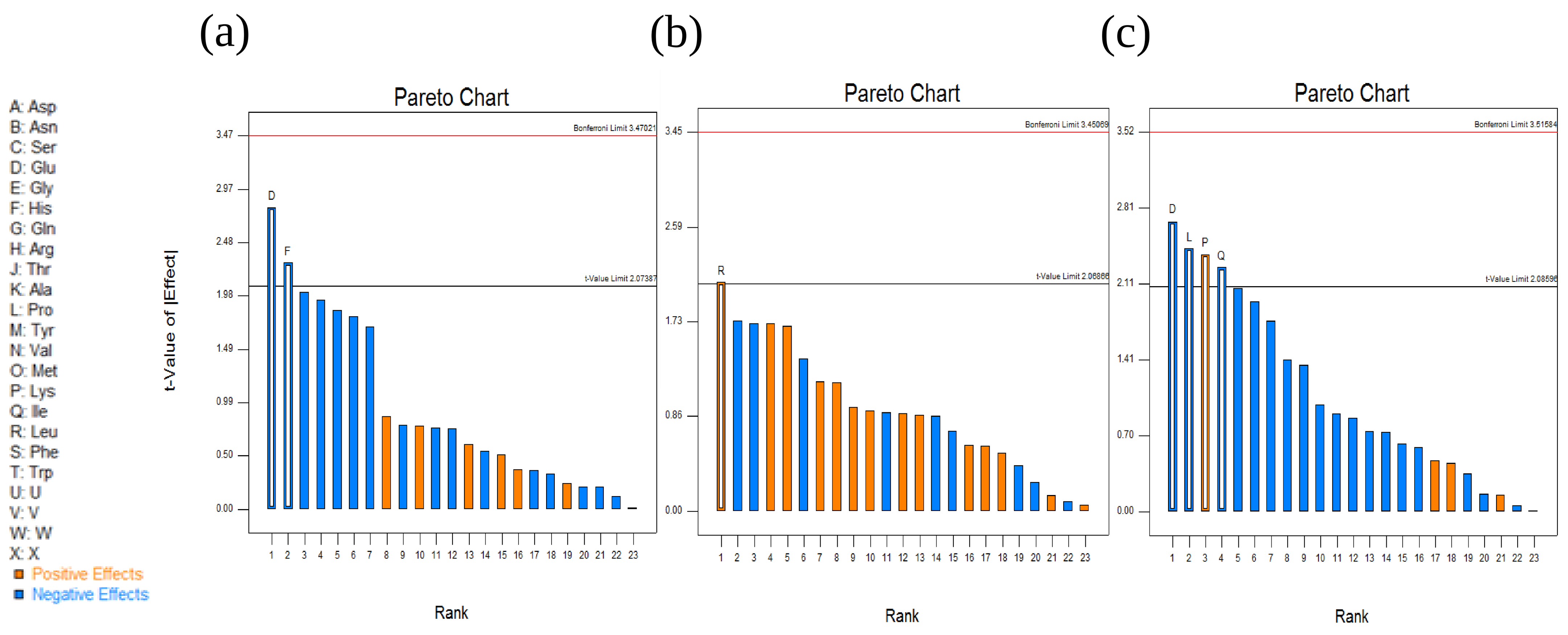

Fig. 1 Pareto Chart of final (a) Total higher alcohols (b) Total esters and (c) Total yeast amino acids absorption.

Conclusions

- The wort nitrogen composition and relative concentrations of each amino acids are important factors for the yeast fermentation performance and the production of flavour compounds.
- From a practical perspective, although the wort nitrogen is important to yeast growth and fermentation, not more is better. What is more critical is a reasonable match of different amino acids in wort. Understanding the relationships between the initial amino acids composition of wort and the evolution of aroma important compounds would be a powerful tool. The future applications of this information may drastically improve current regime of selecting malt and adjunct or their formula with desired amino acids in wort.

Ongoing……

- Researches on statistical analysis of the industrial scale data of batches of wort amino acids composition and the corresponding flavour of beer products.

Acknowledgements

- This work was supported by grants from the National High Technology Research and Development Program of China (863 Program, no. 2013AA102109), Science and technology planning project of People's Livelihood in Qingdao City (No. 14-2-3-49-NSH), the Program of Introducing Talents of Discipline to Universities (111 Project, no. 111-2-06), and a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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