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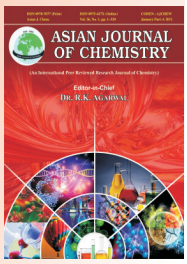

Preparation of L-Proline Chiral Bonded Silica Gels and Their Application in Beer†

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The chiral L-proline-silica gel was prepared by using silica gel with allyl glycidyl ether. When the coverage of L-proline surpassed 30 %, the adsorption rates of haze-active protein decreased. The benefits of L-proline-silica gel in beer stabilization were shown to specifically adsorb haze-active proteins and hardly affect beer foam stability.

Keywords: Silica gel, L-Proline, Chiral.

INTRODUCTION

Beers, fruit juices and wines all contain proteins and polyphenols that can combine to form colloidal suspensions that scatter light and make the product appear cloudy[1]. Clear beverages are generally intended to remain clear until they are purchased and consumed. Hazes or sediments may result from microbial growth, protein-polyphenol interaction. However, the most frequent cause of haze results from protein-polyphenol interaction in beer, wine and clear fruit juices. Whereas in beer, precipitate formation is mainly driven by the haze-active protein fraction which plays an important role[2].

The protein-polyphenol aggregate that can develop in bottled beer is referred to as chill-haze. Many studies have addressed chill-haze formation at molecular scale. It appears that a hydrophobic hordein fraction of malt combines with the polyphenols present to form a colloidal precipitate. Results from X-ray crystallography and NMR spectroscopy suggest that the protein-polyphenol interaction is driven initially by hydrophobic effects and is further stabilized by hydrogen bonding[3]. Other publications illustrate the involvement of proline residues on the protein-polyphenol interaction phenomenon. For example, the haze-forming potential of proteins has been reported to be directly related to their proline content.[4] During beer maturation, a major part of the protein-polyphenol complexes is precipitated out by cooling the liquid. In the subsequent clarification process, either the remaining polyphenols or the remaining proteins are removed from the beer[5]. Although PVPP is commonly used to remove remaining polyphenols, its use as a treatment has a number of disadvantages, including the high capital costs for PVPP regeneration and the inherent lowering of the natural antioxidant potential of the beer. Removal of the remaining haze active proteins is commonly achieved using silica gel. Although silica gel has been shown to specifically adsorb haze-active proteins, its selectivity of protein absorbing is undesired[6].

Herein, we describe L-proline chiral bonded silica gels selective adsorbent for the haze proteins. This new kind of absorbent by using silica gel as the carrier, L-proline as chiral ligands has a stable performance and high stereoselectivity for haze protein in beer. Furthermore, the new absorbent does not absorb other proteins and foam in beer and is a kind of effective beer stabilizer.

EXPERIMENTAL

All material from Aldrich and used as received except that silica gel was purchased from Anhui Liangchen Silicon Material Co., Ltd. IR (Perkin-Elmer, 2000 FTIR). The analysis of amino acids were performed with HPLC (Agilent HP1100), the proteins were purified from Mini-PROTEAN Tetra Cell (BIO-RAD). Elemental analysis was carried out with a Carlo Erba 1106 instrument.

General procedure (Proline-Silica Gel, PSG): In 30 mL of carbon tetrachloride was added, 3 g of silica gel, 4 mL of dimethylchlorosilane and 2 mL of pyridine, the mixture was refluxed for 5 h, filtrated and dried to give the silica hydride (SGH I). Then 4 mL of allyl glycidyl ether, 2 mg of hydrogen

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hexachloroplatinate and the silica hydride (SGH I) were added to 30 mL of isopropyl alcohol and the mixture obtained was stirred at 40 ºC for 24 h in a nitrogen atmosphere. After adding 2 mL of 1-octene to the resulting mixture, the mixture was stirred sequentially for 6 h under nitrogen atmosphere, filtrated and dried, giving the functionally modified silica gel (GSG II). The whole modified silica gel (GSG II) and 1 g of sodium L-proline were added to 30 mL of methanol, the mixture obtained was stirred at 60 ºC for 24 h, filtrated, washed with methanol and dried to give GSG III[7].

Silica hydride (SGH I): IR (ν_{max}, cm^{-1}): 2169 (Si-H), 1261 (Si-C), 1095 (Si-O), 1021 (Si-O), 883.5 (Si-H, variation of angles); elemental anal. (%): C 2.00, H 0.89.

γ-Glycidol-propyl-dimethylsilane-silica gel (GSG II): IR (ν_{max}, cm^{-1}): 2931 (C-H), 1263 (Si-C), 1178 (C-O-C), 1093 (Si-O), 1024 (Si-O); elemental anal. (%): C 5.36, H 1.23.

L-Proline-silica gel (PSG III): IR (ν_{max}, cm^{-1}): 3341 (OH), 2907 (C-H), 1742 (C=O), 1267 (Si-C), 1259 (C-O-C), 1124 (Si-O), 1033 (Si-O); elemental anal. (%): C 6.81, H 1.42, N 0.44.

RESULTS AND DISCUSSION

Herein, we wish to report our study on the synthesis and using of L-proline-silica gel. The approaches used to stabilize wines and fruit juices are similar to those employed in brewing (Table-1). Bentonite adsorption is more commonly applied for wine and juice making, while silica hydrogel use predominates for beer chill proofing, where removal of foam-active protein is undesirable. Polyphenol reduction can be accomplished in beer, fruit juices, or wine either by fining with a protein (most commonly gelatin) or by adsorption with polyvinylpolypyrrolidone (PVPP) (Fig. 1)[8].

TABLE-1
COMPARISON OF DIFFERENT STABILIZER IN BEER

Entry	Stabilizer	L-Proline of protein in beer liquid[a,b]	L-Proline of haze active protein (%)[a,b]
1	Gelatine, 400 mg	39.61	7.56
2	β-Glucanase, 400 mg	41.73	8.31
3	PVPP, 400 mg	37.45	10.43
4	Tannic acid, 400 mg	29.66	17.56
5	Bentonite, 400 mg	23.13	20.91
6	Silica gel, 400 mg	15.57	35.47
7[c]	PSG (1:29, w:w), 400 mg	17.42	48.11
8	PSG (1:19, w:w), 400 mg	11.35	39.63

(a) Yields of L-proline analysis operated on the beer haze separated from beer. (b) Samples were held at 80 ºC for 0.5 h. (c) PSG is absorbent of silica gel surface modified with L-proline.

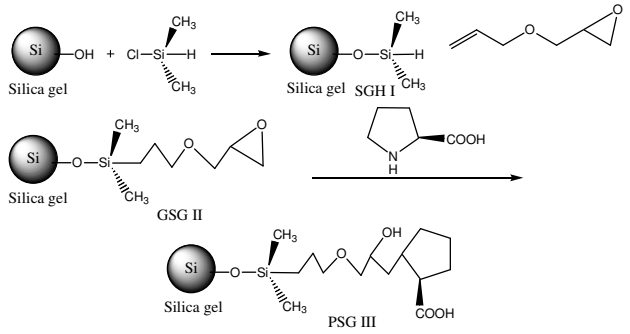

Fig. 1. Preparation routes of SGHI, GSGII and PSGIII. (a) Reaction temperature is 40-60 ºC, (b) reaction time is 24 h under nitrogen

Many commonly used methods for measuring protein or polyphenol do not give a good assessment of their haze-forming activity. The Bradford dye binding assay for protein, for example, hardly responds to beer haze-active protein, this is because Coomassie blue is highly biased toward basic and aromatic amino acids and these amino acids comprise only a small percentage of the haze-active protein[9].

Estimation of the relative amounts of "sensitive" or haze-active proteins in beer can be made by adding tannic acid (TA); this haze-active polyphenol combines with the haze-active protein in the sample to form haze that can be measured by light scattering. When this procedure was applied to both apple juice and beer the results are given in Tables 2 and 3. The data in Table-2 were produced by holding the sample-TA mixture at 25 ºC for 0.5 h before measurement with a ratio turbidimeter, while those in Table-3 were produced by holding at 80 ºC for 0.5 h. The two commercial apple juices showed a small (2-7 NTU) increase in haze as a result of the tannic acid addition at 25 ºC. The increase was larger for the mechanically clarified cider that had not been stabilized, which would have removed much of the haze-active protein or polyphenol. The beers, on the other hand, exhibited a very large increase in haze from the same tannic acid additions. The results were generally similar at 80 ºC, except for the cider. There are likely two temperature effects operating here[10].

TABLE-2
EFFECTS OF COVERAGE OF L-PROLINE ON ADSORPTION RATES TO PROTEIN IN BEER

Entry	Stabilizer	L-Proline of protein in beer liquid[a,b]	L-Proline of haze active protein (%)[a,b]
1	PSG (1:19, w:w), 400 mg	11.35	39.63
2	PSG (1:9, w:w), 400 mg	9.28	41.24
3	PSG (3:17, w:w), 400 mg	7.55	44.37
4	PSG (2:8, w:w), 400 mg	4.29	46.53
5	PSG (1:3, w:w), 400 mg	1.97	49.71
6	PSG (3:7, w:w), 400 mg	5.86	43.78
7	PSG (4:6, w:w), 400 mg	17.32	34.39
8	PSG (1:1, w:w), 400 mg	19.13	31.72

(a) Yields of L-proline analysis operated on the beer haze separated from beer. (b) Samples were held at 80 ºC for 0.5 h.

Higher temperature can dissolve loosely or freshly associated haze, called "chill haze" in brewing, leading to less light scattering. On the other hand, work with model systems showed that higher temperature incubation led to stronger protein polyphenol binding and more haze development, apparently because partial denaturation of the protein exposes additional polyphenol binding sites. In the case of beer, where the haze-active barley protein has typically been boiled for 90 min and is presumably thoroughly denatured, it is likely that heating at 80 ºC can contribute little additional access to polyphenol binding sites. Cider, on the other hand, has had little heat exposure (not even the pasteurization treatment commercial apple juices typically receive) and is more likely to show a temperature effect[11].

Amino acid analysis of haze-active protein (HAP) in beer: When the beer turbidity rises to about 1, we take 100 mL beer and filter it with 0.45 μm microporous membrane, then place turbid material and membrane into 20 mL of 2 % NaOH,

dissolve them for 10 min and detect the content of amino acids in the solution.

Analysis of amino acid of protein of additives adsorption in beer: Additives (400 mg) were added in the 2 L beer, constantly stirred solution for 1 h and stewed for 0.5 h. Filtering out additives with 10000 rpm (10 min, 3 ºC). Residue was eluted with ethanol-water (250 mL, 5%), centrifugated with 10000 rpm (10 min, 3 ºC), residue was again eluted with ethanol-water (200 mL, 5 %) and centrifugated with 10000 rpm (10 min, 3 ºC) and then residue was eluted with 2 % ammonia water. Collecting the upper clear liquid and it was neutralized to pH 7 with acetic acid. Preparing 50 mmol PBS the protein solution, the protein solution was dialyzed overnight, filtered it with membrane (0.20 μm) and concentrated to scruple with millipore 5 kDa and then detect the content of amino acids in the solution.

When these absorbents were applied to beer, the results were obtained in Table-1. It was found that gelatin, β-glucanase, PVPP, tannic acid and bentonite adsorption were obtained in low rates of adsorption (entries 1-5, Table-1). Although the yield of adsorption was high by using silica gel as absorbent for beer, removal of haze-active protein is undesirable (entry 6, Table-1). Inspiringly, PSG absorption rate was higher than the other absorbents for haze-active protein in beer (entries 7-8, Table-1).

After a comprehensive survey of the absorption conditions, PSG was considered as highly selective absorbent for removal of haze-active protein in beer. Subsequently, the ratios of L-proline and silica gel were examined using this method (**Scheme-I**). The results are listed in Table-2.

The PSG absorption has high selectivity for haze-active protein (HAP). This could be reason that chiral ligand exchange of L-proline helped to enhance selective absorption rate for haze-active protein. With amplifying the coverage of silica gel surfaces coated with L-proline, PSG absorption rates sharply increased for haze-active protein (entries 1-5, Table-2). However, the coverage of L-proline surpassed 30 %, the PSG adsorption rates decreased for haze-active protein (entry 6-8, Table-2). It was perhaps that hydrophobic group formed layer on the surface of silica gel and reduced PSG adsorption rates due to increasing bonding group.

Conclusion

We have demonstrated that L-proline-silica gel is safe and effective stabilizer in beer. The synthesis of L-proline-silica gel was carried out by employing silica gel, L-proline, allyl glycidyl ether and dimethylchlorosilane. When the coverage of L-proline surpassed 30 %, the adsorption rates of haze-active protein decreased. In this paper, the benefits of L-proline-silica gel in beer stabilization were shown to specifically adsorb haze-active proteins and hardly affect beer foam stability.

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