

Enrichment of Vanillin in Barley Malt by Hydrolysis in High Temperature and High Pressure Steam

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ABSTRACT: Malted barley was treated with saturated steam at temperatures ranging from 180 to 220 °C, that is, high temperature and high pressure steam (HHS), and a control sample exposed to saturated liquid water in the same temperature range. The malt treated in steam was extracted with water (70 °C) for 15 min after drying. Vanillin which gave beer preferable flavor was detected in all malt extracts, both after treatment with steam and in effluent from malt exposed to liquid water. Hydrolysis of ingredients in the malt occurred even in steam. The treatment of natural products in HHS could be a new thermal technique to provide a special ingredient for food industry, for example, malt for beer.

KEYWORDS: High temperature and high pressure steam, HHS, Hydrolysis, Malt, Phenolic compounds, Subcritical water, Vanillin

INTRODUCTION

The type of beer depends chiefly on the brewing method and the raw materials used. Barley malt is the most important ingredient for brewing beer, and there are many special kinds of malt, for example, caramel malt, Munich malt, and amber malt to modify the beer's flavor, plus several variations in brewing conditions.

The conventional special malts listed above are produced by heating in air: so-called kilning and/or roasting. Generally, the temperature of the kilning and roasting ranges from 80 to 200 °C at ambient pressure in air. In these heating processes, some burnt and sweet flavors are produced by thermal decomposition, oxidation, and the Maillard reaction, each with its effect on beer flavor. To generate a new and pleasant beer flavor, a different kind of special malt must be prepared, with a different set of components. To convert the ingredients' natural polymers such as polysaccharides and proteins into small fragments, hydrolysis is quite useful because the natural polymers (polysaccharides, proteins, and lignin) are poly condensation compounds.

It is well-known that natural polymers can be hydrolyzed by liquid water in subcritical conditions (Figure 1, liquid phase) without an acid catalyst.^{1–18} This high reactivity of liquid water at high temperature (subcritical water) is the result of the water's higher dissociation constant compared with ambient-conditions water. The higher concentration of both hydronium and hydroxide ions leads to a significant increase in nucleophilicity, accelerating hydrolysis.^{1,2} This special quality of subcritical water has inspired many studies of the hydrolysis of monosaccharides,^{3,4} cellulose,^{5–8} malto-oligosaccharides,^{9,10} amino acids,¹¹ and lignin^{12–15} in subcritical water. Also, it is well-known that some phenolic compounds such as vanillin are generated by the hydrolysis of lignin in subcritical water.^{16–18}

This hydrolysis in subcritical water brings fragment compounds such as vanillin into the water phase and could be viewed as reactive extraction. For example, the lignin in malt can be hydrolyzed into small fragments and the fragments

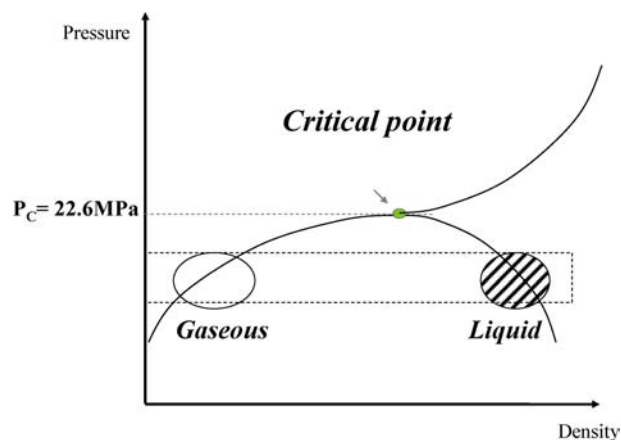


Figure 1. Phase diagram of water including sub- and supercritical regions. Subcritical region is wrapped by dashed line. The regions in this study are shown by circles and the saturated steam is shown by a white out and liquid water is shown by a diagonal.

simultaneously extracted into water during the treatment. To produce a special malt that includes small molecular fragments via hydrolysis rather than by thermal decomposition in air, subcritical liquid water treatment is not appropriate because the special malt must retain fragments such as vanillin.

It would be considered that hydrolysis in the gaseous phase is quite slow because of the low water density, reduced dielectric constant, and ionic products, and only thermal decomposition proceeds at a meaningful rate. However, it was recently reported that high temperature (~ 350 °C) and high pressure steam is a good reactant for the hydrolysis of artificial polymers.¹⁹ Because of a poor solubility of steam for

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Table 1. Physical Properties of Water at High Temperature and High Pressure

	T, °C	P, MPa	density, kg/m ³	viscosity, mPa·s	dielectric constant	ion product
saturated	180	1.00	5.160	0.015	1.04	2.04×10^{-46}
steam	200	1.55	7.860	0.016	1.06	7.18×10^{-43}
	220	2.32	11.62	0.017	1.09	1.11×10^{-39}
liquid water	180	1.00	887.0	0.149	38.2	4.00×10^{-12}
	200	1.55	864.7	0.134	34.7	5.15×10^{-12}
	220	2.32	840.3	0.121	31.5	6.05×10^{-12}

nonvolatile organic compounds (such as saccharides, amino acids, and phenolic compounds), natural products in a solid matrix would only be fragmented via hydrolysis in steam (if it would occur with a meaningful rate) rather than reactive-extraction like liquid water treatment.^{12,13} Thus, the treatment in steam can be considered a new technique for special malt production if the treated malt has unique properties. Some phenolic compounds have desirable aromatic flavors. For example, the sweet vanilla flavor of vanillin might be a pleasant flavor addition for beer. There are no reports of natural compounds being treated in steam at 180 °C~200 °C.

In the present study, barley malt was treated in steam at 180 to 220 °C, that is, high temperature and high pressure steam (HHS), and barley malt was also exposed to liquid water in the same temperature range for comparison. The malt treated in steam was extracted with hot water (70 °C) for 15 min (after drying) and the extracts were analyzed for vanillin. After the liquid water treatment, we also analyzed vanillin in the water to determine the hydrolysis products of malt.

MATERIALS AND METHODS

Materials. Barley malt (*Hordeum vulgare* (L.) "Scarlet") was used in the present study. It is a quite usual ingredient for brewing beer. Milli-Q water (Millipore, MA) was well substituted by N₂ gas, and used as solvent for the reaction.

Procedures of High Temperature and High Pressure Treatment. In the present study, we investigated the ability of saturated steam to hydrolyze components of the malt. To compare the reaction in saturated steam, we also performed malt treatment in liquid water. Pressure–density diagram and the physical properties of pure water are shown in Figure 1 and Table 1, respectively.

A 400 mL batch type reactor (AKICO, Japan) was used for the treatment. Figure 2 shows a schematic diagram how to perform experiments in saturated steam and liquid water. When

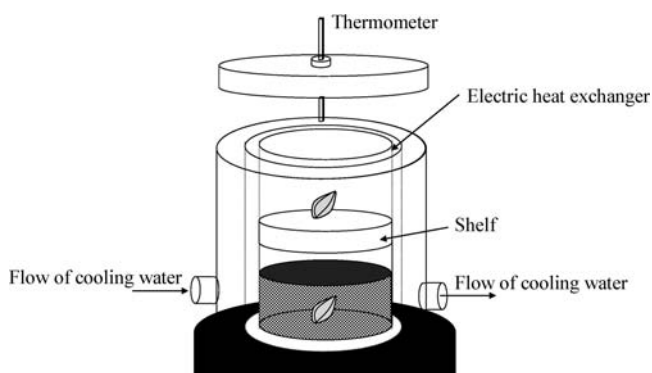


Figure 2. Schematic diagram of apparatus. The sample was putted on the shelf in the treatment in saturated steam. For the treatment in liquid water, the sample was loaded in the apparatus without the shelf.

the sample was treated in saturated steam, 30 g of malt, and 300 mL of water were loaded into the reactor and placed on the shelf to avoid contact with liquid water, as shown in Figure 2. For treatment in liquid water, the same amount of malt was placed in 300 mL of water, also as shown in Figure 2. In both case, air in the container was well substituted by N₂ gas before the reaction. The temperature inside the reactor was controlled in the range from 180 and 220 °C and the pressure was at the corresponding vapor pressure of saturated steam (see in Figure 1 and Table 1). The temperature-rising rate was set to the maximum capacity of the device, about 40–50 °C/min. The samples were treated for 1 min both in saturated steam and liquid water.

Analyses. Figure 3 shows the preparation procedures for analytical samples for both steam- and water-treated cases. The

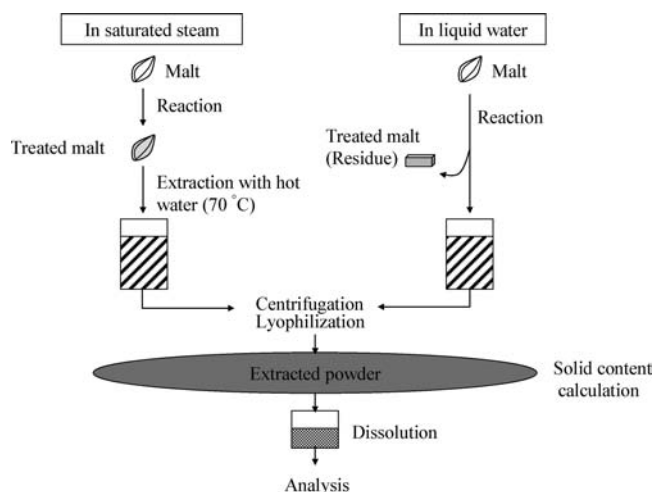


Figure 3. Flowchart of experiments. Barley malt was treated in the saturated steam or in the liquid water and processed to the extracted powder for the analysis of vanillin.

malt treated in saturated steam was recovered from the reactor and dried in ambient atmosphere for a day. The dried malt was extracted with 300 mL of hot water (70 °C) for 15 min. The obtained aqueous solution was centrifuged at room temperature for 10 min and the supernatant was filtered and lyophilized. The weight of dried sample was measured for calculation of the solid content, and the sample was redissolved with 300 mL of water. The solution was subjected to analysis of vanillin. The solubilized compounds were obtained from the original malt in the same manner as described above. When the malt was treated in liquid water, the solid residue (the treated malt) and the compounds solubilized in water were recovered after the reaction. After removing the residue, the recovered aqueous solution was centrifuged and supernatant was lyophilized. The dried powder was redissolved with 300 mL of water and the solution was analyzed.

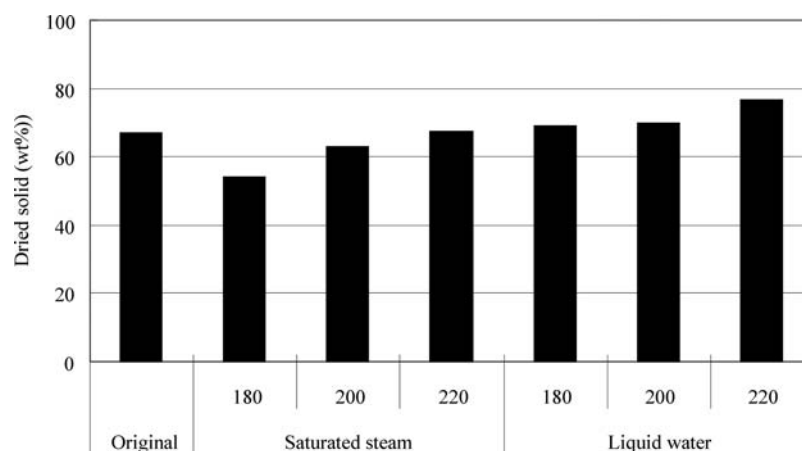


Figure 4. Yield of dried solid from barley malt of the treatment in saturated steam and liquid water.

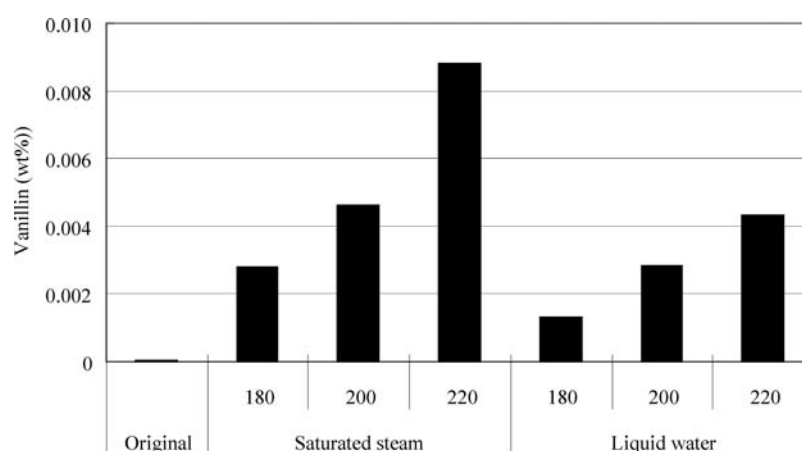


Figure 5. Yield of vanillin from barley malt of the treatment in saturated steam and liquid water.

In the present study, we focused on vanillin, hydrolysate of lignin. Vanillin was separated by HPLC (LC-10AD, Shimadzu, Japan) on a column of Deverosil-C30-UG5 4.6 × 150 mm (Nomura Chemical, Japan). Solvent A was 0.05% trifluoroacetic acid aqueous solution, solvent B was 90% acetonitrile containing 0.05% trifluoroacetic acid, and the gradient of solvents was 0% solvent B to 90% solvent B over 0 to 20 min at a flow rate of 1 mL/min. Vanillin was detected by a UV-vis detector at 280 nm. Authentic vanillin (Nacalai Tesque, Japan) was used as the reference standards.

Definitions. We evaluated the amount of dried solid after lyophilization based on the weight of the original barley malt. The yield of vanillin was also calculated based on the weight of the original malt (dry basis).

$$\begin{aligned} \text{yield of dried solid (wt\%)} \\ &= \frac{\text{weight of the extracted powder (g)}}{\text{weight of the original malt (g)}} \times 100 \end{aligned}$$

$$\begin{aligned} \text{yield of vanillin (wt\%)} \\ &= \frac{\text{weight of vanillin (g)}}{\text{weight of the original malt (g)}} \times 100 \end{aligned}$$

RESULTS AND DISCUSSION

The Yields of Products. Figure 4 shows the yield of dried solid obtained from the sample treated with saturated steam or liquid water. The yield of dried solid obtained from the sample treated with saturated steam slightly decreased compared with that of original malt, while the yield from liquid water at each temperature was always higher than that of original malt. The yield of dried solid increased with increasing temperature regardless of the reaction atmosphere.

The yields of vanillin, which is a representative phenolic compound derived mainly from lignin, are shown in Figure 5. From the original malt, no vanillin was extracted after 15 min in hot water. Treatment with saturated steam yielded vanillin even at 180 °C, and the yield increased significantly with increasing temperature. Compared with treatment by liquid water, the yield of vanillin from saturated steam was always higher. This is the first report described the formation of vanillin by saturated steam processing.

Special Features of Reaction in Saturated Steam. The total yield of water-soluble components (namely the dried solid shown in Figure 4) from original (untreated) malt was 66 wt %. The yield of dried solid increased by the treatment of malt in liquid water (Figure 4, 68 wt % to 76 wt % at 180 to 220 °C) and the yield of vanillin (Figure 5) increased with increasing temperature.

The reaction in saturated steam displays some differences from that in liquid water described above. The yield of the total

solubilized components (i.e., the dried solid) was a little lower (Figure 4, 54 wt % to 66 wt % at 180–220 °C) compared with liquid water treatment, while the yield of vanillin was remarkably higher (Figure 5).

It has been reported that some phenolic compounds including vanillin could be derived via hydrolysis of lignin without catalyst in subcritical (liquid water) and supercritical water.^{16–18} Similarly, vanillin was obtained from the treatment of malt in liquid water, and vanillin presumably formed via hydrolysis of lignin. The yield of dried solid increased at higher temperature both in saturated steam and liquid water. It was shown that the natural polymers such as polysaccharides and proteins in malt were also hydrolyzed both in liquid water, as reported previously,^{3–15} and saturated steam, and converted to water-soluble components.

It is noteworthy that the yield of vanillin was much higher, particularly at higher temperature, which is clear evidence that saturated steam is more suitable to hydrolyze lignin to vanillin even though its density is quite low. The dried solids consist of several water-soluble components, and they are more hydrophilic than vanillin. It was suggested that the hydrophilic components were relatively easier to decompose in liquid water than in saturated steam. In this case, the differences between saturated steam and liquid water were not simply due to their different properties such as density, dielectric constant, diffusivity, and so on.

Finally, we illustrate the reaction pathway in saturated steam and liquid water at high temperature and high pressure in Figure 6. In this figure, the lignin, polysaccharide (such as

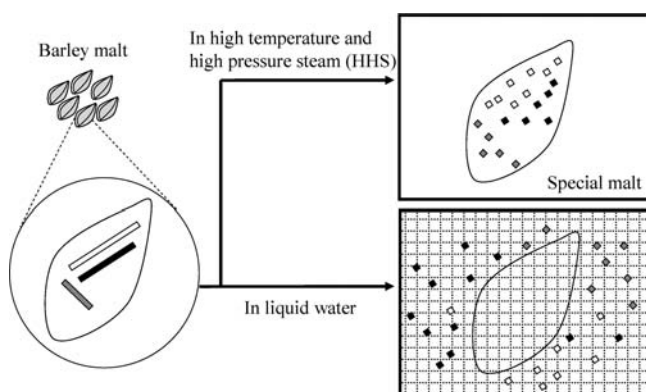


Figure 6. Image of differences between the reaction of barley malt in liquid and gaseous phase. Lignin, polysaccharide and protein in the malt are shown with the black, gray, and white bar. Hydrolyzed or decomposed products are shown as the small squares (the color of the squares are the same as its polymer).

dietary fiber) and protein in the malt are indicated with black, gray, and white bars. These natural polymers are hydrolyzed into vanillin (also other phenolic compounds), mono- and oligo-saccharides, and amino acids. These small compounds are shown as small squares (colors to match the polymer). In the present study, we first showed that saturated steam can hydrolyze natural polymers, with the hydrolyzed small compounds remaining in the malt. Thus our study shows that hydrolysis inside the malt, driven by HHS, would be a new way to make a different special malt as a new raw material to brew a new beer. Furthermore, treated ingredients can be used for food production in general, not only for beer but also tea, coffee, or any other food or beverage.

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Notes

The authors declare no competing financial interest.

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