CHARACTERIZATION AND EVALUATION OF CAROTENOID CLEAVAGE ENZYMES IN DIFFERENT PROCESSING STAGES OF JAPANESE GREEN TEA

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Summary

Several carotenoid degradation products such as β-ionone are important in development of high quality green tea flavor. The effects of tea manufacturing on the formation of carotenoid derived aroma compounds in green tea are still not sufficiently understood. Carotenoid cleavage enzymes in green tea are characterized by a high temperature optimum.

Up to now, it was not clear, if these specialized enzymes in green tea influence carotenoid degradation during manufacturing. We could prove existence, activity, and importance of carotenoid cleavage enzymes during Japanese green tea manufacturing and putatively even in readily processed green tea.

Keywords

carotenoid degradation, flavor, enzyme, thermo stability

Introduction

Several carotenoid cleavage products are important in development of high quality green tea flavor. Flavoursome teas are known to be produced from tea leaves with high carotenoid contents. However, apart from chemical formation of aroma active norisoprenoids, oxidative enzymatic cleavage in general is an important flavor formation pathway. Only a limited number of carotenoid cleavage enzymes from plants are isolated and/or characterized. Carotenoid cleavage enzymes responsible for the degradation of neoxanthin to abscisic acid, a plant hormone, have been isolated from maize and Arabidopsis thaliana⁵. In addition, carotenoid cleavage enzymes were cloned from red crocus (Crocus sativus), and isolated from the cytosol of quince (Cydonia oblonga) and star fruit (Averrhoa carambola)². Finally, we could already show for the first time that carotenoid cleavage enzymes are present in fresh tea leaves (Camellia sinensis). Additionally the enzyme could be isolated and partially characterized².

Limited data about the temperature dependence of carotenoid cleavage enzyme activity are available. The temperature optimum in star fruit is 45°C and in quince it is 50°C or even higher⁵. Furthermore we have the evidence that the temperature optimum of carotenoid cleavage enzymes in Japanese green tea is even higher. Therefore we assume that these enzymes are highly active during high temperature treatment of tea leaves during manufacturing.

Materials and Methods

Plant material

Green tea leaves (Camellia sinensis) were provided from the National Institute of Vegetable and Tea Science, Kanaya, Shizuoka prefecture, Japan.
Isolation of the enzyme

Temperature has a strong influence on enzymatic activity. In order to assess temperature effects on carotenoid cleavage enzymes during manufacturing we prepared samples using various steaming durations and different processing steps. Each time the tea leaves were transferred into potassium rich Tris-buffer, homogenized, and centrifuged. The proteins were first precipitated with 70 % and subsequently treated with 95 % chilled acetone. The resulting precipitates were filtered (0.2 μm), rediluted in water, frozen and lyophilized to obtain a stable dry protein powder. A first purification step could be achieved by adding 70 % of acetone because two thirds of the total protein content could already be separated from the desired enzymes.

Further clean up was done by FPLC, using a gel filtration column (HiPrep Sephacryl S-200 26/60, Amersham Bioscience). The protein concentration was monitored at 280 nm and the enzyme activity was measured as described by Fleischmann et al. (3,4). Running conditions were: half concentrated extraction buffer with addition of 100 mM sodium chloride, flow rate 0.8 mL, and fraction size 10 mL. Figure 1 shows the UV-chromatogram (line) of the FPLC-separation. The second line (dots + line) shows the relative carotenoid cleavage activity of each FPLC fraction.

Results and Discussion

Exemplarily we will discuss the enzymatic cleavage activity in commercially available tea. As shown in Figure 1, a stepwise acetone precipitation and a further clean up by FPLC can be used as isolation procedure of carotenoid cleavage enzymes of processed Japanese green tea. In the graph the high protein content of fraction 33 correlates with a high β-carotene cleavage rate. Furthermore contaminating proteins like fraction from i2-17, 26-32, and 35-42 can be separated.

Figure 2 shows the time drive of β-carotene degradation by the active carotenoid cleavage enzyme of fraction 33 (squares), as well as by partially heat inactivated enzyme (dots). The regression curve of the enzyme follows a pseudo first order reaction pattern, which is also described for carotenoid cleavage enzymes isolated from fruit tissues (3,4).
Conclusions

We could clearly show that enzymatic carotenoid cleavage activity is present in Japanese Sencha. Thus carotenoid cleavage enzymes are highly stable against the heat treatment applied during tea processing. Furthermore, it is evident that carotenoid cleavage enzymes have strong impact on aroma formation in green tea. The formation of ionones in tea is therefore not only a result of chemical (thermal or oxidative) degradation, but is to a large extent, if not mainly based on the catalytic action of carotenoid cleavage enzymes.

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References