BIOCHEMICAL DIFFERENTIATION OF STORAGE PROTEIN IN COCOA COTYLEDON FROM DIFFERENT GENETIC ORIGINS

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Introduction

Cocoa aroma is one of the most important characteristics of cocoa flavour. The specificity depends on the primary structure of vicilin (7S)-class globulin of cocoa cotyledons and the splitting type of cocoa cotyledon aspartic endoprotease and carboxypeptidase. Recent research has shown that the genotypes of the cocoa tree in which it is cultivated plays an important role in the final quality of the beans. Therefore, the objective of the research was to investigate the genetic differences in cocoa cotyledon from different genetic origins with respect to vicilin (7S)-class globulin. Thus, the structure of the vicilin (7S)-class globulin from different genotypes would be the best candidate for these investigations. The assumptions were: (1) Flavour differences in roasted cocoa beans from different clones reflect genetic differences, (2) The flavour significantly is made up by cocoa aroma, and (3) Cocoa aroma depends on the genetically controlled primary structure of vicilin (7S)-class globulin.

Materials and Methods

Cocoa fruits from different varieties and clones were obtained from several of countries. They are Forastero variety from Ghana, Criollo and Trinitario from Java, Indonesia and SCA 12, UIT1, PBC 140 clones from Malaysia. Preparation of acetone dry powder was done essentially as described by Voigt et al (1993). Vicilin (7S)-class globulin was partially purified according to the modified method of Voigt et al. (1993). SDS-PAGE was prepared and run as described by Amin et al. (1997; 1998). Two-dimensional electrophoresis (2-D PAGE) was performed according to the manual of Pharmacia Biotech using a ready-made polyacrylamide gels containing an immobilised pH gradient (pH 3-10 and 4-7) and SDS-PAGE gradient gels (8-18%) for the first (isoelectric focusing) and second dimension run, respectively.

Results and Discussion

Cotyledon vicilin (7S)-class globulin of different geographical origins was analysed using SDS-PAGE and 2D-PAGE techniques. From the results, 22 polypeptides were visually observed on 12.5% (v/v) SDS-PAGE by silver staining after electrophoresis. The major storage protein in cocoa cotyledon of different geographical origins was albumin fraction (band III) which showed an apparent molecular weight of 22 kDa. The polypeptide of 22 kDa was reported not to be responsible for the production of specific-cocoa aroma precursors during fermentation. It has been shown by Voigt et al. (1993) that no specific-cocoa aroma precursors were produced from the incubation of purified 22kDa polypeptide with proteases isolated from cocoa cotyledon. The vicilin (7S)-class globulin was observed in three bands with apparent molecular weights of 49 (band I), 32 (band II) and 17 kDa (band IV). These three polypeptides have shown to be important for the formation of specific-cocoa aroma precursors during fermentation provided the pH-value is in the range of 5.0-5.8 (4). The results gave rise to the finding that vicilin-class globulin patterns in *Theobroma cacao* did not show quantitative or qualitative differences. The protein samples were separated based on their isoelectric point (p*I*) in the first dimension using an immobilised pH gradient (IPG), pH 3-10 and 4-7. SDS-PAGE (8-18%) was used in the second-dimension run and separation affected based on the molecular weights. Using two-dimensional electrophoresis (2D-PAGE), we obtained excellent separation of bands II and I, which represent the sub-units/splitting products of the native vicilin, trimer forms. The second-dimension run showed that vicilin (7S)-class globulin from different geographical origins with respect to bands I and II revealed 9 to 10 protein spots/components after isoelectric focusing at pH 3-10. Preliminary 2D-SDS-PAGE studies in UPM indicated that vicilin (7S)-class globulin of cocoa cotyledon, the precursor of specific-cocoa aroma related proteolytic products as separated by isoelectric points (p*I*) gave 9 to 10 different p*I* and had similar molecular weights. This has also been reported to be the case for alfalfa seed storage protein, in which 5 to 10 protein spots from the vicilin-class globulin ranged from p*I* 5.8 to 6.7. In addition, the investigations using 2D-PAGE technique showed that vicilin (7S)-class globulin (bands I and II) of different geographical origins were not single polypeptides, but consisted of up to 10 isoforms of similar molecular weight but with different isoelectric points (p*Is*). The isoelectric points for bands I polypeptides and II ranged from p*I* 7.5 to 5.2. Isoelectric focusing at pH 4-7 in the first dimension revealed well resolved protein spots of band II compared to protein spots of band I. At least 4 protein spots with apparent molecular weights between 21.5 to 31 kDa were clearly shown on the second-dimension gels. Despite their uncertain identity, it was shown that their isoelectric points were within p*I* range of bands II and I of vicilin (7S)-class globulin. Those protein spots could have been derived from the sub-units/splitting of native vicilin (7S)-class globulin. Through visual observation of the second-dimensional gel the polypeptide spots of band II could be seen to be slightly more basic than band I. The results show that vicilin-class globulin of cocoa cotyledon were possibly from polygenic origins.

Conclusions

Based on the results of one and two-dimensional electrophoresis techniques, we may assume that unfermented cocoa cotyledon from different geographical origins have similar potential for producing high level specific-cocoa aroma precursors during fermentation provided the pH-value in the cotyledon is around 5.0-5.8.

References

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