Improvement Of Cocoa Flavour Through Enzymatic Process

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Introduction

Essential precursors of the cocoa-specific aroma components are generated during fermentation of cocoa beans by an acid-induced proteolysis of seed proteins (VCG) by endogenous proteases. Flavor compounds are then developed through roasting of specific-cocoa aroma precursors namely hydrophilic oligopeptides and hydrophobic free amino acids. Flavor differences have been reported to exist in roasted raw cocoa harvested from various genetic origins. Malaysian cocoa is of better flavor as compared to the Indonesian which is usually low quality under-fermented cocoa; however, Malaysian grinders have to use these lower quality beans due to scarcity of local beans. The objectives of the project were to investigate the impact of genetic origins on protein, to produce an immobilized form of cocoa aspartic protease and the determine the influence of carboxypeptidase on aroma components especially pyrazines in underfermented cocoa beans and to determine the optimum blending ratio best used to improve cocoa flavor.]

Materials and Methods

Cocoa fruits from various genetic origins, i.e. Ghana (Forastero), Indonesia (Criollo and Trinitario) and Malaysia (SCA12, UIT1 and PBC140 clones) were used. VCG was extracted and isolated from the acetone dry powder of defatted cotyledon. SDS-PAGE and 2-D IEF/SDS-PAGE) was performed The degradation pattern of VCG were detected during the course of commercial fermentation and the oligopeptides pattern was analyzed. For protease study, partially purified aspartic endoprotease were bounded to DEAE-cellulose and covalently bound to crab shell chitin. Fat-free dry cocoa powder under-fermented cocoa beans samples were then added with carboxypeptidase B from porcine pancreas and carboxypeptidase Y from Baker's Yeast , then incubated for different time duration under sterile conditions at 45°C in a shaking incubator. Proteolysis and the respective precursors and pyrazines formation were evaluated. Malaysian and Sulawesi's of commercial and of different degree of fermentation cocoa beans were blended, and the physico-chemical and sensory quality were evaluated.

Results and Discussion

There was no indication of differences in all genotypes on the apparent molecular weights of proteins especially vicilin-class globulin (VCG) subunit polypeptide bands. The 2-D IEF/SDS-PAGE separation patterns showed that VCG subunit polypeptides (45 and 31 kDa) had resolved into 8 multiple forms. The multiple forms may also indicate that the 47 kDa polypeptide of VCG occur in the cocoa cotyledons not as the product of one gene, but a family of several closely related genes which are all responsible for the synthesis of VCG. The results from 2-D IEF/SDS-PAGE do not reveal any analytical difference of VCG. The study also found no analytical identity difference of all the oligopeptide patterns produced from autolysis of acetone dry powder for various genetic origins. The VCG subunits were degraded as fermentation progressed and the patterns obtained are similar for all genotypes. In protease study, Both DEAE-cellulose and chitin bound endoprotease exhibited enhanced thermal stability as a consequence of enhanced conformational stability although their initial activities lost by 37 and 18% from their initial activity. The results indicate that the amount of storage protein decreased with the longer period of incubation treatments which might be caused by proteolysis; the results also found that the carboxypeptidase activity decreased in all of the samples during the incubation period, which suggests that its activity was affected by the pH condition. Thus, the finding of our study indicate that controlling the pH during the initial period of the fermentation is necessary for the optimum production of specific cocoa aroma precursors. The results also showed that when the samples were treated with carboxypeptidase, pyrazines concentration increased; the concentration of tetramethylpyrazine was higher compared to dimethyl- and trimethylpyrazines. The samples treated with carboxypeptidase B originated from porcine prancreas had a direct effect on the concentration of tetramethylpyrazine. For the blend containing two-days fermented Sulawesaan beans to reach well fermentation level, it must contain a minimum 50% four-days fermented Malaysian beans. The same Sulawesian beans would have pH value around 5.3 if blended with two-, four- and six-days fermented Malaysian beans. The blends containing either Malaysian or Sulawesian cocoa butter with Brazilian beans would have different melting behavior and hardness. The cocoa and chocolate blend containing more than 50% Brazilian cocoa butter was not recommended; Cocoa lipase has MW of 20-45kDa and optimum pH of 7.5, hav Km value of 2.63mM, inhibited by diisoptopyl flourophosphate, N-bromosuccinimide and 5,5-dithiodbis-(-2-nitrobenzoic acid.

Conclusions

Aroma differences from various genetic origins are not due to genetic differences of cocoa cotyledon proteins. Aspartic endoproteases were successfully immobilized; however, further study on its application in real cocoa fermentation system is

needed. Porcine pancreas carboxypeptidase was more effective in cocoa aroma precursors production. Controlling pH during the initial period of the fermentation is necessary for the optimum production of specific cocoa aroma precursors. Blending of Malaysian and Sulawesian cocoa beans have improve the flavor quality of cocoa liquor.

Benefits from the study

Malaysian cocoa from different clones have a similar potential for producing high level specific-cocoa aroma precursors during fermentation provided the pH-value in the cotyledon is not less than 5.0; Results from the study also would assist Malaysian grinders to improve the cocoa flavor of products made from imported under-fermented cocoa beans. The utilization of immobilized endoproteases have potential to be used to improve flavour. Information from the blending study would be useful for the cocoa grinders in improving the overall quality of blended cocoa from Southeast Asia.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

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Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Amin Ismail	Study on flavour potential of cocoa beans from different origins	Protein characterization	Ph.D.	2001
Puziah Hasyim	Effects of fermentation and drying methods on production of pyrazine	Flavour compound, especially on pyrazine	Ph.D.	1998
Wan Rusly W. I.	Effect of roasting temperature of the production of pyrazine	Flavour compound, especially on pyrazine	M.Sc.	1998

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