Utilization of Cupuaçu Seeds as Raw Material for Chocolate-like Products: Variability of Morphological Traits and Biochemistry of Aroma Potential

Reisdorff, C. [1], Rohsius, C. [1], Müller, S. [1], Claret de Souza, A. G. [2] and Lieberei, R. [1]

[1] Universität Hamburg, Hamburg, Germany.

[2] EMBRAPA Amazônia Ocidental, Manaus, Brazil.

1 Introduction

The studies on the utilization of cupuaçu seeds as raw material for chocolate-like products has been done as sub-project of the SHIFT project ENV 23, which is one of the cooperational SHIFT projects located at the EMBRAPA Amazonia Ocidental near Manaus. The general challenge of this project is to improve the understanding of natural and man-made ecosystems as bases for the development of sustainable land use practices in the Central Amazon. One of the particular questions of our sub-project was, whether cupuaçu seeds can be used as raw material for chocolate-like products. In this summary some results of the respective investigations regarding seed inherent factors which are decisive for the generation of chocolate like aroma are presented.

Why do we consider, that this additional use option will contribute to concepts of sustainable production systems? The answer can be deduced from the advantages for small holders: They will have an valuable product in addition to the pulp, which is storable and can be sold independently of harvesting seasons. In addition, the small holder can supply a new market with price dynamics separate from those of fresh products. These factors are capable to contribute to the economic stabilization of production systems, which have been designed for ecological stability.

The general idea to produce chocolate from cupuaçu seeds comes from the fact that the cupuaçu plant (*Theobroma grandiflorum*) is a close relative of the cocoa tree, *Theobroma cacao*. The cupuaçu fruit contains an aromatic pulp surrounding about forty seeds. The pulp is sold at high market values of about 1 to 3 dollars per kilogram. The seeds are commonly not yet commercialized. Some attempts have been made to produce types of chocolate wares, however, these attempts were not very successful, mostly due to problems regarding the generation of a reproducible aroma quality.

The typical aroma of cocoa is formed in the course of fermentation, drying and roasting. The most important biochemical reactions happen during the fermentation process. During the 2 to 4 days of fermentation the sugars of the pulp are degraded to acetic acid by microorganisms. This takes place in an anaerobic and a subsequent aerobic step. The acetic acid penetrates the cotyledons and triggers complex processes. These processes lead to cell death, disintegration of cell compartments, formation of an aqueous reaction phase and lowering of pH values of the seed storage tissue. These alterations are the pre-conditions for the formation of aroma precursors from seed storage proteins, namely from globulins. The biochemical reactions take place in the following sequence: The globulins are spliced to hydrophobic oligo-peptides by an endo-protease. In the next step an exo-peptidase degrades these oligo-peptides to hydrophilic peptides and hydrophobic amino-acids. This mixture represents the precursors of cocoa aroma. The final aroma is formed during drying and roasting. The amino groups of the aroma precursors react with reducing sugars. These reactions, the so-called Mallard reactions, result in the cocoa aroma.

In our study we tackled the question: to which extend cupuaçu seeds possess the potential to develop a valuable and reproducible aroma. The first step was to investigate the seed inherent factors being the pre-requisites for the formation of aroma. Thus, we compared the biochemical components and the morphological traits of cupuaçu seeds with the respective properties of cocoa seeds in order to provide basic knowledge for the adaptation of seed processing technologies.

2 Results

Concerning the seed storage proteins we found quite similar patterns in both species, *T. cacao* and *T. grandiflorum*. The globulins we have found in cupuaçu seeds have nearly the same molecular weight as those of cocoa. However, cupuaçu seeds contain a little bit less of these globulins. Our results indicate that there is a high degree of qualitative similarity between the globulins of cupuaçu and cocoa, whilst there are clear differences regarding the quantities.

As next biochemical component of the aroma potential we studied the endo-proteases, which is responsible for the first degradative step of the reactions towards aroma precursors. The comparison of the respective activities in seeds of *T. cacao* with those of *T. grandiflorum* revealed

that in both cases the endo-protease activities are very high. However, the highest activities were found in cocoa. The pH dependence of the endo-protease is very similar between the two species with a maximum activity at about pH 3.5. The catalytic types are identical, which was indicated by the strong inhibition through the pepstatin A, which acts specifically on proteases of the aspartic type.

The carboxy-exo-peptidase catalyzes the cut-off of hydrophobic amino acids. The proper result of this degradation depends on the substrate specificity of the enzyme. Thus, we tested the substrate specificity of the cocoa and cupuaçu-enzyme by means of artificial substrates with carboxy-terminal amino acids of different hydrophobicity. The experiments revealed that the exo-peptidase of cupuaçu seeds prefers cutting hydrophobic amino acids in the same manner as the enzyme of cocoa does. In both species we found considerable activities of this enzyme, however, the activities were higher in cocoa. In addition, the pH dependence shows certain differences with optimum activities near pH 5.2 for cupuaçu and near pH 5.7 for cocoa. Inhibition experiments revealed that the catalytic type of the exo-peptidases of both species is to be classified as seryl peptidase.

In addition to the biochemical parameters the following morphological attributes have been studied:

1. The sub-cellular organization of organelles (liposomes and protein storage vacuoles), which is decisive for the formation of a continuous aqueous reaction phase within the cells.
2. The seed size and shape, which determine the diffusion distances for acetic acid and consequently determine the time course of protein degradation.
3. The morphology of the seed shells, which represent a barrier for the diffusion of acetic acid.

Our respective morphological studies of the cotyledon tissue revealed that the sub-cellular structures of the storage cells are nearly identical in size and distribution of the organelles. On the other hand, considering size and shape of the entire seeds, one would expect differences relevant for the fermentation processes, because the seeds of cupuaçu are considerably bigger (3 - 5 g fresh weight) than those of cocoa (1 – 2 g fresh weight). But the decisive dimension of a seed which determines the minimum diffusion distance of acetic acid is the seed thickness. Cocoa seeds are 10 to 12 mm thick. Most of the seeds of the studied cupuaçu plants are flat with a thickness of 12 to 14 mm. This means an additional diffusion distance of about 1 mm, which can be considered not relevant for the time course of imbibition of acetic acid. Regarding the seed shells we observed the most evident morphological differences between the two species: The testa of cupuaçu is bigger and it is characterized by a layer of thick-walled palisade cells. These distinctive properties of the cupuaçu seed shells might delay the diffusion rate of acetic into the cotyledons.

Additional important differences between the two species are related to the adhering pulp: Firstly, the pulp of cupuaçu seeds is quite more fibrous and it is attached to the seed shell more tightly in comparison to cocoa. Hence, if one thinks about additional use, it has to be considered that most of the fruit flesh is removed for pulp production. These distinctive features lead to problems regarding the sequence of anaerobic and aerobic fermentation, and there might be less acetic acid for triggering the biochemical processes of aroma formation.

3 Conclusions

Summarizing the results concerning the biochemical components of the aroma potential we can state that cupuaçu seeds generally possess the biochemical components necessary for the formation of aroma precursors. However, some distinctive features might lead to a slightly reduced yield of aroma precursors in comparison to cocoa. But the high genetic variability within the species *T. grandiflorum* would enable selecting plants with higher biochemical aroma potential. With regard to the structural attributes we found in both species a very similar sub-cellular organization of organelles, which allows the generation of an aqueous reaction phase necessary for the biochemical reactions leading to aroma precursors. The size and shape of cupuaçu seeds guarantee short diffusion distances for acetic acid. But concerning the morphology of seed shells, there is probably an evidently higher diffusion resistance for acetic acid into the storage tissue of cupuaçu seeds.

The distinctive features of the seed shells and of the fruit flesh would lead to unsatisfactory results if one simply applies the procedures practiced in the processing of cocoa seeds. Consequently, further research is needed in order to develop fermentation procedures which are adapted to the respective "adverse" properties of cupuaçu seeds.