CHANGES IN SOLUBLE AMINO ACIDS OF SOME TROPICAL STARCHY ROOTS DURING CHILLING

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SUMMARY

Preliminary qualitative results on the loss of free amino acids during and after chilling storage roots and tubers, and also plantain and banana fruits, throw some light on the changes during chilling injury. Loss of free amino acids is less in the cold resistant potato than in the tropical roots and fruits, and the loss of free amino acids seems to be associated with the degree of browning.

RESUME

Les résultats qualitatifs préliminaires sur la perte en acides aminés libres pendant et après la conservation des racines et tubercules au froid, de même que le plantain et les fruits de banane, jettent la lumière sur les changements subits par l'effect du froid. La perte en acides aminés est moindre dans la patate résistante au froid que dans les racines et fruits tropicaus, et la perte en acides aminés semble être liée au degré de brunissement.

RESUMEN

Resultados preliminares cualitativos, sobre la pérdida de aminoácidos libres, durante y después de la refrigeración de aminoácidos libres, durante y después de la refrigeración de raíces y tubérculos y también de plátano para cocinar y banano, dan alguna luz sobre los cambios que ocurren durante el dano que provoca la refrigeración. La pérdida de aminoácidos libres es menor en la papa resistente al frío que en las raíces y frutos tropicales y parece estar asociada con el grado de coloración café que se observa.

INTRODUCTION

Starchy roots and tubers such as yams are now increasingly being exported from Nigeria to overseas countries, especially Britain, partly as food for immigrant populations, but also because some species contain diosgenin, a steriod drug precursor. Preservation in transit is the most important factor in this marketing.

These foodstuffs have to be transported under refrigeration. It has been found that despite reduced losses from many other factors, they suffer low temperature breakdown. Attention has been focused by most workers on the lipid fraction of foodstuffs to explain the low temperature decay phenomenon. We have begun to investigate changes in soluble amino acids induced by low temperature.

MATERIALS AND METHODS

Samples of yams, cassava, cocoyam, bananas and plantains were collected from the University of Ife farm. Half of each sample was kept in the deep-freeze at 10° C for 2 months.

To determine soluble amino acids, 100g samples were removed from the middle of the fresh tuber. These were cut into very small pieces, mixed with 100 ml. of distilled water ahd homogenized in a stainless steel blender. To prevent foaming, a few drops of amyl alcohol were added. The homogenate was then centrifuged. The supernatant was collected and made up to a roughly 70% alcoholic solution by the addition of ethanol. The deproteinised extract was vacuum-dried. The solution was then chromatographed after being taken up with iso-propanol if it was too viscous.

The same extraction method was applied to corresponding samples taken after chilling for about two months at about 10° C.

Chromatography was carried out using Whatman No. 1 chromatography paper and the one dimensional ascending technique with n-butanol: acetic acid. H_2O [(12:3:5) by volume 3] and Phenol: H_2O (80:20w/v) as solvents, or 2-dimensional chromatography using the first of these solvents in the first dimension and the other in the second. This gave good separation of the amino-acids. The papers were then sprayed with ninhydrin and dried at 80—100° C for two minutes when most of the amino acids show up brightly.

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RESULTS

The one-dimensional chromatogram using phenol: water was not successful. Butanol: acetic acid: water gave better separation but still some of the acids such as glycine, serine and arginine were still difficult to separate. However, good results were obtained with the two-dimensional chromatograms. Pure amino acids were used as standards.

The following 20 free amino acids were identified from yam extracts: cystine, aspartic acid, serine, asparagine, glycine glutamic acid, histidine, arginine, threonine, glutamine, alanine, tryptophan, tyrosine, methionine, valine isoleucine, leucine, phenylalanine, proline and aminobutyric acid. There were 2 spots that could not be identified with certainty. The first is above tyrosine and is suspected to be alanine, from the R_f value and comparison with a pure sample. The other one below proline is unknown. The spots for lysine and asparagine for potato were more intense than for yam.

After chilling most of the free amino acids had disappeared and for yam only 6 spots were obtained. There were 3 bright spots corresponding to aspartic acid, glutamic acid and asparagine and traces of histidine, tryptophan and valine. More spots persisted in the comparative study on chilled potato. These were aspartic acid, glutamic acid, asparagine, serine, glutamic acid, threonine, alanine, tryptophane, valine and leucine.

Cassava differed from yam and potato in containing fewer free amino acids in fresh samples. Only aspartic acid, serine glutamic acid, glycine, arginine, alanine, tryptophan and valine were identified. After chilling only aspartic acid, glutamic acid and tryptophan could still be detected.

Unripe plantains and bananas each contain 17 free amino-acids. A few unidentified spots occurred in the chromatograms of unripe plantain. After chilling only about 9 spots (amino acids) remained for each. The ripe plantain and banana each contained about 13 free amino-acids each. After chilling they give streaks instead of well defined spots. It became difficult to separate arginine and histidine and there appeared 2 broad unknown streaks, probably peptides. In each only 5 spots could be identified with certainty.

DISCUSSION

The free amino acids, comprising a metabolic pool usually comprise about 70—80 percent of the non-protein nitrogen. Sometimes compounds are found which may be specifically important in nutrition, such as the toxic amino-acid e.g. b-amino propionitrile of some *Lathyrus* species which is supposed to be responsible for the incidence of larthyrism.

Our results have failed to show the occurrence among the soluble amino acids of cassava any sulphur-containing amino acids, or phenylalanine, or histidine, or amino-butyric acid, all of which were present in the other fresh foodstuffs.

Yam and potato seem to contain all of the most important amino acids and amino-butyric acid is present in unripe banana, but disappears during ripening. Other amino acids that disappear during ripening are aspartic acid, glutamic acid and phenylalanine, so that it appears that these acids may have some part to play in the ripening process.

Potato was the only crop tested that still contained an appreciable content of free amino acids after chilling, and it was also the foodstuff that was injured least (least browning). Yam, cassava, plantain and banana however suffered severe browning during chilling and it appeared that the more browning the sample suffered the greater was the numerical loss of amino acids.

The disappearance of amino acids during chilling suggests that they may either have been incorporated into the body of the foodstuff and rendered insoluble or else have formed peptides (which are probably the chemicals causing the streaks in chromatograms of plantain and banana). There has been chromatographic evidence for more than eight glutamyl peptides in plants. Dipeptides have been isolated mainly from storage tissues chiefly from seeds, bulbs and fruits. These facts indicate the possibility that glutamyl dipeptides may have widespread occurrence in various types of tissues throughout the plant kingdom. Even though glutamic acid is usually a prominent constitutent of the non-protein fraction, the glutamyl-peptides have been found present in concentrations as high or higher than that of glutamic acid. The presence of glutamyl peptides will probably account for the deep-coloured large spots designated as unknown on the chromatograms.

Work is in progress on the identification of the unknown ninhydrin-positive spots and the quantitative analysis of the amino acids changes.