Phytochemistry, 1968, Vol. 7, pp. 715 to 719. Pergamon Press. Printed in England

CHANGES IN THE OLIGOSACCHARIDES AND THE α-GALACTOSIDASE CONTENT OF COFFEE SEEDS DURING SOAKING AND GERMINATION

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(*Received* 20 *September* 1967, *in revised form* 28 *November* 1967)

Abstract—The oligosaccharides sucrose, raffinose and stachyose are present in the seeds of different varieties of coffee belonging to the species *Coffea arabica* and *C. robusta* that were investigated. The sucrose content was more than ninety times that of raffinose in *C. arabica* varieties but only about forty times more in the *C. robusta* variety. Stachyose content in all cases was about 1·5 times greater than raffinose. During soaking, the three sugars decreased by about 50 per cent, and during germination, while there was a further reduction of about 50 per cent in sucrose content, raffinose and stachyose disappeared in the *C. arabica* varieties and were greatly reduced in the *C. robusta* variety. During the disappearance of these oligosaccharides traces of glucose and fructose appeared, but galactose, melibiose or manninotriose were not detectable at any stage. α-Galactosidase activity was fairly high in resting seeds. This activity increased by 50–75 per cent on soaking and increased further by another 25–55 per cent on germination of the seeds, the increase being greater both on soaking and on germination in the seeds of *C. arabica* varieties than in the seeds of the *C. robusta* variety.

INTRODUCTION

A LARGE number of seeds contain besides sucrose, the galactosides of sucrose—raffinose and stachyose—as reserve carbohydrates. Ropert Duperon[1] has reported that of the eighty-one species of mono- and dicotyledenous seeds investigated all contained sucrose, seventy contained raffinose and thirty-five of the latter also contained stachyose. In their investigations of the carbohydrates of coffee seeds Courtois *et al.*[2,3] have reported that all the ten varieties of coffee seeds they worked with contained sucrose, raffinose and stachyose. Although the total oligosaccharide content of the seeds varied considerably, the relative proportions of the three sugars were the same, sucrose forming 96–98 per cent of the total and stachyose being always in greater amounts than raffinose.

Shiroya *et al.*,[4,5] in their studies on seeds of black pine (*Pinus thunbergii*), red pine (*P. densiflora*), and sunflower, *Robinia pseudaccacia*, reported that raffinose and stachyose are utilized during the early stages of germination. As cotton seeds are a rich source of raffinose Shiroya[6] also studied the changes in the content of this sugar during the ripening and germination of the seeds. He noticed that the content of raffinose and, in addition, stachyose, which is also present in the seeds, decreased during the early stages of germination and then disappeared completely.

[1] ROBERT DUPERON, *Compt. Rend.* **241**, 1817 (1955).
[2] J. E. COURTOIS, F. PERCHERON and J. CL. GLOMAUD, *Café, Cacao*, 7 (3), 231 (1963).
[3] J. CL. GLOMAUD, F. PERCHERON and J. E. COURTOIS, Coloq. Intern. Chim. Cafes Vert., 2nd, p. 39., Paris (1965).
[4] S. HATTORI and T. SHIROYA, *Arch. Biochem. Biophys.* **34**, 121 (1951).
[5] M. HASEGAWA, T. TAKAYAMA and T. SHIROYA, *Kagaku* **21**, 21 (1951).
[6] T. SHIROYA, *Phytochem.* **2**, 33 (1963).

Lechevallier,[7] working on different seeds containing raffinose and stachyose, has shown that the α-galactosidase activity which is present in the resting seeds increases during germination. This has also been observed by Shiroya[6] with cotton seeds.

As a part of our studies of the carbohydrates and carbohydrases of different species of coffee seeds grown in the Mysore State, India, we have described in this paper the changes in the oligosaccharides and the α-galactosidase activity of three varieties of coffee seeds during soaking and germination.

RESULTS

The sugar extracts of resting seeds did not reduce Fehling's solution, while the extracts of soaked and germinated seeds did. The reducing power of the extracts of germinated seeds was greater than that of the extracts of soaked seeds. The extracts of the raw seeds contained sucrose, raffinose and stachyose. Sucrose formed the highest proportion of the oligosaccharides in the extract, and the proportion of stachyose was always higher than that of raffinose. On soaking there was a reduction of about 50 per cent in the concentration of all the three oligosaccharides and on germination there was a further reduction of about 50 per cent in the case of sucrose. Raffinose and stachyose disappeared on germination in the two varieties of coffee seeds belonging to the species *Coffea arabica* while in the variety

TABLE 1. CHANGES IN THE OLIGOSACCHARIDE CONTENT OF COFFEE SEEDS DURING SOAKING AND GERMINATION

Variety of coffee	State	Per cent moisture	Content in 100 g dry weight		
			Sucrose (g)	Raffinose (mg)	Stachyose (mg)
C. arabica, Plantation "A"	Raw	6·0	2·76	29·00	44·38
	Soaked	60·2	1·71	12·75	21·00
	Germinated	61·1	0·73	0·0	0·0
C. arabica, Plantation "PB"	Raw	7·3	2·33	22·88	35·00
	Soaked	57·1	1·40	12·00	17·00
	Germinated	58·3	0·61	0·0	0·0
C. robusta, Cherry "AB"	Raw	6·2	1·71	44·75	70·25
	Soaked	57·8	0·89	20·64	33·53
	Germinated	59·0	0·38	9·88	7·50

belonging to the species *C. robusta* these sugars continued to be present in small quantities. In all cases no free galactose was observed, but glucose and fructose were detected in soaked and germinated seeds. Table 1 shows the changes in the content of the oligosaccharides of coffee seeds during soaking and germination.

There was α-galactosidase activity in the resting seeds. This activity increased during soaking and further increased during germination of the seeds. The changes in the activity of the enzyme are shown in Table 2.

[7] D. LECHEVALLIER, *Compt. Rend.* **255**, 3211 (1962).

TABLE 2. CHANGES IN α-GALACTOSIDASE ACTIVITY OF COFFEE SEEDS DURING SOAKING AND GERMINATION

Variety of coffee	α-Galactosidase activity in % hydrolysis of melibiose*		
	Raw	Soaked	Germinated
C. arabica, Plantation "A"	17·4	30·0	40·1
C. arabica, Plantation "PB"	17·0	26·4	37·5
C. robusta, Cherry "AB"	16·0	23·6	27·8

* Reaction mixture contained 2 ml 0·1 M melibiose, 1 ml disodium phosphate–citric acid buffer at pH 3·8 and 2 ml, 10 per cent enzyme solution. Incubation was for 22 hr at 37°.

DISCUSSION

In all cases the stachyose content was approximately 1·5 times that of raffinose, while the sucrose content was more than 90 times that of raffinose in the *Coffea arabica* varieties and only about 40 times in the *C. robusta* variety. Though these differences in the content of sucrose and the galactosides of sucrose between the coffee seeds of the *arabica* and *robusta* species are significant, and one is tempted to say that they are species specific, it may not be so as Courtois and co-workers[3] have reported that the amount of different oligosaccharides varies widely with different varieties of coffee seeds belonging to the same species.

In all cases the sucrose content was reduced to about 50 per cent on soaking and to about 25 per cent on germination. Shiroya[6] has stated that in cotton seeds the sucrose content increases during germination. He also says that β-fructofurnosidase activity, which is low in resting cotton seeds, increases markedly on germination. It is difficult to understand how the increase in the activity of this enzyme and the increase in sucrose content could go together. In the case of coffee seeds, however, there is a marked decrease in sucrose content and this may be due to an increase in the β-fructofuranosidase content of the seeds on soaking and germination. That this could be so is indicated by the fact that the sugar extracts of the soaked and germinated seeds contain the reducing sugars glucose and fructose, while the extracts of resting seeds do not.

The raffinose and stachyose present in the resting seeds were reduced by about 50 per cent on soaking in all cases and disappeared on germination in the *C. arabica* varieties, while in the case of the *C. robusta* variety raffinose decreased to about 20 per cent and stachyose to about 10 per cent of their concentrations in the resting seeds. Even here the presence of these sugars in small quantities in the seeds of *C. robusta* after germination may not be due to species differences. There is not much of a difference in the α-galactosidase activity of the resting seeds of the two species of coffee. On soaking and germination there is a higher percentage increase in the activity of the enzyme in the case of the seeds of the *arabica* species when compared with that of the seeds of the *robusta* species. The initial concentrations of raffinose and stachyose in resting seeds are also higher in the seeds of the *C. robusta* species than in the seeds of the varieties belonging to the *C. arabica* species. Thus the higher initial concentration of these sugars in the resting seeds and the lower α-galactosidase activity on germination for the same period are responsible for the continued presence of raffinose and stachyose in the seeds of the *C. robusta* variety.

The disappearance of raffinose and stachyose on soaking and germination in seeds containing these sugars is not accompanied by the liberation of galactose, melibiose or manninotriose. Planteose, an isomer of raffinose, is present in sesame and some other seeds. Even during the disappearance of this sugar on germination of sesame seed,[5] no free galactose or galactose-containing sugar has been detected. It has been reported that in the case of cotton seeds, galactose is not detected during the disappearance of raffinose and stachyose because during germination an effective mechanism for galactose utilization is present.[6] It is likely that in the case of coffee seeds also the galactose utilization system is very effective and that galactose is used up as fast as it is liberated by the α-galactosidase.

MATERIALS AND METHODS

Coffee seeds. Three varieties of coffee seeds—Arabica Plantation "A", Arabica Plantation "PB" and Robusta Cherry "AB"—belonging to the two species *Coffea arabica* and *C. robusta* were used. These were grown during the 1965–66 season and obtained from the Coffee Board, Bangalore, India.

Sugars. The sugars used as controls were B.D.H. preparations, except stachyose, which was isolated from *Stachys tuberifera* and kindly supplied by Dr. R. S. Shallenberger, New York State Agricultural Research Station, Geneva, New York, U.S.A.

Extraction of oligosaccharides. Coffee seeds were powdered and defatted by extracting with ether three times at room temperature. The powder was then dried in air until all traces of ether were removed. 10 g of the defatted coffee powder was extracted three times with 100 ml of 80% ethanol for 30 min on a boiling water bath. From the combined extracts, alcohol was removed by distillation under reduced pressure, and protein removed by lead acetate, excess lead being removed by H_2S. The aqueous extract was concentrated, taken to dryness *in vacuo* and the residue dissolved in water (1 ml). For detection of glucose and fructose the dried extract was first deionized[8] by pyridine extraction.

Soaking and germination. Selected coffee seeds were soaked in 0·1% $HgCl_2$ solution for 3 min. They were then repeatedly washed with sterile water until free from Cl and kept immersed in sterile water for about 40 hr, water being changed twice during this period.

On overnight soaking, radicles about 3 mm in length appeared in 10–25 per cent of the seeds. Further soaking neither increased the number of seeds with radicles nor the length of the radicles. After 40 hr of soaking, 10 g of the seeds with radicles, on the dry basis, were homogenized and the oligosaccharides were extracted as above. In this case removal of fat was carried out after deproteinization.

A second sample of soaked seeds with radicles was transferred to sterile, wet blotting paper on a layer of wet sterile cotton, in a sterile chamber and allowed to germinate for 72 hr in the dark. During this period, there was no appreciable increase in the length of the radicle. The moisture content of the seeds rose only by about 1 per cent over that of soaked seeds. Germination for a longer period resulted in fungal contamination under the conditions used.

Identification of sugars. Sugars were identified by descending paper chromatography on Whatman No. 3 filter paper. The solvents used were: *n*-butanol:acetic acid:water, 4:1:1, v/v, for the separation of sucrose from raffinose and stachyose; *n*-butanol:acetic acid:water, 3:3:2, v/v, for the separation of raffinose and stachyose; and *n*-butanol:pyridine:water,

[8] F. H. MALPRESS and A. B. MORRISON, *Nature* **164**, 963 (1949).

5:3:1, v/v, for the separation of monosaccharides. The sugars were detected with aniline–oxalate reagent; benzidine–trichloracetic acid reagent;[9] or urea–HCl reagent.[10]

The identity of the oligosaccharides was further confirmed by identifying the sugars produced after hydrolysis of the individual sugars.

Quantitative estimation of sugars. Individual oligosaccharide eluates[11] were used for the estimation by the phenol–sulphuric method.[12]

Estimation of α-galactosidase activity. 10 g of powdered raw coffee seeds were extracted with 100 ml of water at room temperature for 2 hr. In the case of soaked and germinated seeds, 10 g of seeds, on the dry basis, were homogenized and extracted similarly. The supernatant (2 ml), after centrifugation of the extract, was used as the enzyme source and tested with 0·1 M melibiose (2 ml) in phosphate–citrate buffer (1 ml, pH 3·8), the mixture being incubated for 22 hr at 37°. Coffee seed α-galactosidase was found to be more active at pH 3·8 with melibiose than at pH 5·3 which is the optimum pH of the enzyme with phenyl-α-D-galactopyranoside.[13] The liberated monosaccharides in the enzyme digest were estimated as stated above.

[9] J. Bacon and J. Edelman, *Biochem. J.* **48**, 114 (1951).
[10] R. Dedonder, *Bull. Soc. Chim. Biol.* **34**, 144, 157, 171 (1951).
[11] C. E. Dent, *Biochem. J.* **41**, 240 (1947).
[12] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Analyt. Chem.* **28**, 350 (1956).
[13] J. E. Courtois and F. Petek, *Methods in Enzymology*, Vol. 8, p. 565. Academic Press, New York (1966).