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Changes in the Carbohydrates and Proteins of Coconut during Roasting

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ABSTRACT

Roasting markedly enhances the flavour of coconut due to the formation of volatile aroma compounds like pyrazines, which are generally formed during Maillard reaction between amino acids and reducing sugars. In coconut, these changes (not followed hitherto) are reported for the first time. Fructose and glucose are the most affected sugars, whereas lysine, tryptophan, glutamic acid, aspartic acid, alanine and glycine are the free amino acids maximally utilised during heating.

INTRODUCTION

Roasting alters and significantly enhances the flavour, colour, texture and appearance of most food items and is one of the common forms of processing for nuts. Coconut is an important vegetable oil source in India, especially in Kerala, in South India. Of the total annual production of 6800 million coconuts in the country, only 40 to 50% is processed to get copra for oil extraction (Arumughan *et al.*, 1987). The remaining nuts are consumed by people as food in the fresh, raw form or in roasted form during curry preparations, etc. Unlike peanuts or cashewnuts, coconut is not generally oil-roasted and consumed. It is rich in oil (about 37% in the fresh stage) and roasting is usually done by heating the gratings with constant stirring in a frying pan over fire, until it becomes golden brown (temperature 150 to

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160°C). The flavour changes from the characteristic sweet, fruity, oily, fresh, coconut-like aroma to the roasted, nut-like odour. Commercially, copra pieces are sometimes heated prior to oil extraction so that the enhanced flavour formed is inherited by the oil also. The flavour-enhanced coconut oil fetches a good market in the north-eastern regions of the country. Studies have been carried out by the authors to optimise the conditions of roasting of coconut for maximum flavour development (Jayalekshmy *et al.*, 1980). The volatile aroma compounds of heated coconut have been investigated and reported to be pyrazines and furans (Saittagaroon *et al.*, 1984; Jayalekshmy *et al.*, 1985). The formation of the heterocyclic compounds, during heating can be attributed to Maillard reactions between amino acids and reducing sugars (Hurrell, 1982). Considerable work has been carried out to follow chemical changes in roasted coffee (Stoffelsma *et al.*, 1969), cocoa (Rohan & Stewart, 1966*a*,*b*; Reineccius *et al.*, 1972*b*), peanuts (Newell *et al.*, 1967; Mason *et al.*, 1969; Oupadissakoon & Young, 1984) pistachios (Kashani & Valadon, 1984), etc. Surprisingly, no similar study has been undertaken in a common food item like coconut. Fresh coconuts with a moisture content of 40 to 45%, when heated, can certainly cause conditions, most conducive for nonenzymic browning reaction as indicated by model system studies (Lea & Hannan, 1949; Wolfrom & Rooney, 1953). The sweet taste of coconut is largely attributed to sucrose Caray (1921). However, on heating, the sweet taste changes, possibly due to reduction in free sugars. Even though the amino acid composition of coconut protein is known Krishnamurthy *et al.*, 1958), the changes in non-protein nitrogen and free amino acids of coconut have not been investigated so far. The present work aims at following the changes occurring in sugars and amino acids of coconut during heating. The three different temperatures selected for the study, more or less represent the advanced and rather final stages of Maillard reaction as compared to dried coconut gratings taken as reference.

MATERIALS AND METHODS

Sample preparation

Mature coconuts of the West Coast Tall variety were disintegrated and dried in a cross-flow drier at 60°C to a final moisture content of 3·0%. Heating was carried out in an air oven, with 200 g lots of coconut gratings, at 130°C, 145°C and 160°C for 15 min. The gratings were defatted with hexane and powdered to pass through a 200 micron sieve. Dried coconut gratings, without further heat treatment, similarly defatted and powdered, were taken as the reference sample.

Carbohydrate analysis

The available carbohydrates (including soluble sugars, starch and dextrins) were estimated by extracting 0·5 g of defatted meal with 60% perchloric acid and estimating the sugars by the anthrone method according to Clegg (1956). Soluble sugars were extracted from 0·5 g of defatted meal by refluxing with 80% aqueous methanol for 5 h, over a temperature controlled water bath according to Southgate (1976). The methanol was evaporated under reduced pressure and the concentrate was clarified with lead acetate and excess lead removed by potassium oxalate. The clarified solution was made up to definite volume. Total soluble sugars were estimated by the anthrone method (Roe, 1955) and reducing sugars according to Somogyi (1945). Another portion of the clarified solution was concentrated under vacuum, de-ionised in a 20 × 1·5 cm column of ion-exchange resins (Amberlite IR-120, cation and Amberlite IR-45, anion) and eluants and washings concentrated to 0·5 ml and filtered through 0·5 μm millipore filter before HPLC analysis.

HPLC analysis

A Waters Associates Liquid Chromatograph (Model AI C/GPC 244, Milford, MA) equipped with a model 6000 A solvent delivery system, a differential refractometer (R 401 Model), a 46 K universal injector and a variable speed omniscribe recorder, was used. The column was a 30 × 0·39 cm (id) μ-Bondapak carbohydrate column made of stainless steel. The mobile phase consisted of acetonitrile : water in the ratio 85 : 15, degassed in an ultrasonic bath before use. The flow rate was 2 ml/min. The mixture of standards used for calibration consisted of ribose, rhamnose, arabinose, fructose, glucose, mannose, sucrose, galactose, maltose and raffinose each at 1·0% level. Different combinations of the standards were also tried to see the resolution of individual sugars. The samples (10–20 μl) were analysed under the same HPLC conditions and quantitated by triangulation and by comparing peak areas of the samples with the peak areas of the sugar standards.

Amino acid analysis

Total amino acids

Hydrolysis and amino acid analysis were carried out according to Moore and Steine (1963). Fifty milligrams each of defatted samples were hydrolysed with 6N HCl under vacuum in a sealed tube at 110°C for 20 h. After hydrolysis, the HCl was removed under vacuum and the contents dissolved in 5 ml of citrate buffer (pH 2·2) and analysed in a Technicon NC-2P amino

acid analyser. Norleucine was used as the internal standard and calibration was done using a mixture of standard amino acids at a concentration of 0·5 mmole/litre. Tryptophan was estimated colorimetrically after alkaline hydrolysis, according to Sastry and Tummuru (1985).

Free amino acids

One gram of the defatted sample was stirred with a 10% solution of trichloroacetic acid (TCA) for 2 h and left for 16 h. The clear supernatant layer, containing non-protein nitrogen (NPN), was filtered through Whatman 1 filter paper, after centrifugation at 3000 rpm for 10 min, vacuum-evaporated and final traces were removed by washing with ether. The contents were then made up to 5 ml with citrate buffer (pH 2·2) and the amino acid analysis was carried out as detailed earlier.

The protein contents of the various samples were estimated by Kjeldahl digestion according to AOAC (1984). The nitrogen contents of NPN extracts were also estimated by Kjeldahl method.

RESULTS AND DISCUSSION

Changes in carbohydrates

Total available carbohydrates, total sugars, reducing sugars, starches and dextrins of dry coconut gratings were found to be 11·7, 8·32, 0·82 and 3·36%, respectively (Table 1). As a result of heating, total soluble sugars and hence, total available carbohydrates, decreased noticeably (9·0% and 7·3%,

TABLE 1
Changes in the Carbohydrates of Coconut during Heating (g/100 g dry sample)[a]

	Plain	*130°C*	*145°C*	*160°C*
Total available carbohydrates	11·7	10·5	11·0	10·8
Total soluble sugars	8·32	7·68	7·70	7·57
Reducing sugars	0·82	0·68	0·70	0·70
Ribose[b]	Trace	Trace	Trace	Trace
Rhamnose[b]	Trace	Trace	Trace	—
Fructose	0·20	0·17	0·18	0·18
Glucose	0·32	0·25	0·26	0·24
Galactose	0·28	0·26	0·26	0·26
Sucrose	7·50	7·00	6·90	6·85
Starches and dextrins	3·36	3·28	3·26	3·26

[a] Average of three determinations.
[b] Trace = 10–30 mg/100 g.

respectively). The reducing sugars decreased and then slightly increased at higher temperatures. HPLC analysis revealed that sucrose is the major sugar in coconut, followed by glucose and fructose. Traces of ribose and rhamnose, as reported by Balachandran *et al.* (1987), could also be detected. Earlier workers like Caray (1921) and Bhown (1962) had reported galactose, also, in coconut. However, with the HPLC column used and conditions followed in this study, glucose and galactose could not be separated and eluted as a single peak. Hence, glucose was estimated separately by the glucose oxidase–peroxidase enzyme method according to Holm *et al.* (1986) and, from this, actual glucose and galactose contents could be estimated. The presence of galactose was further confirmed by paper chromatography, as suggested by Bhown (1962). The analysis of samples heated at different temperatures revealed that the total sugars decreased by 7·7% in the first stage of heating. The reducing sugar value was brought down from 0·82 to 0·68 (about 17% decrease). The detailed analysis of sugars by HPLC (Fig. 1, Table 1) showed that traces of ribose were present in heated samples, whereas rhamnose could not be detected in the final stages. Fructose and glucose were affected much more than galactose. While the sucrose content decreased slightly during heating, glucose and fructose decreased and then the values remained almost steady. This may be attributed to hydrolysis of

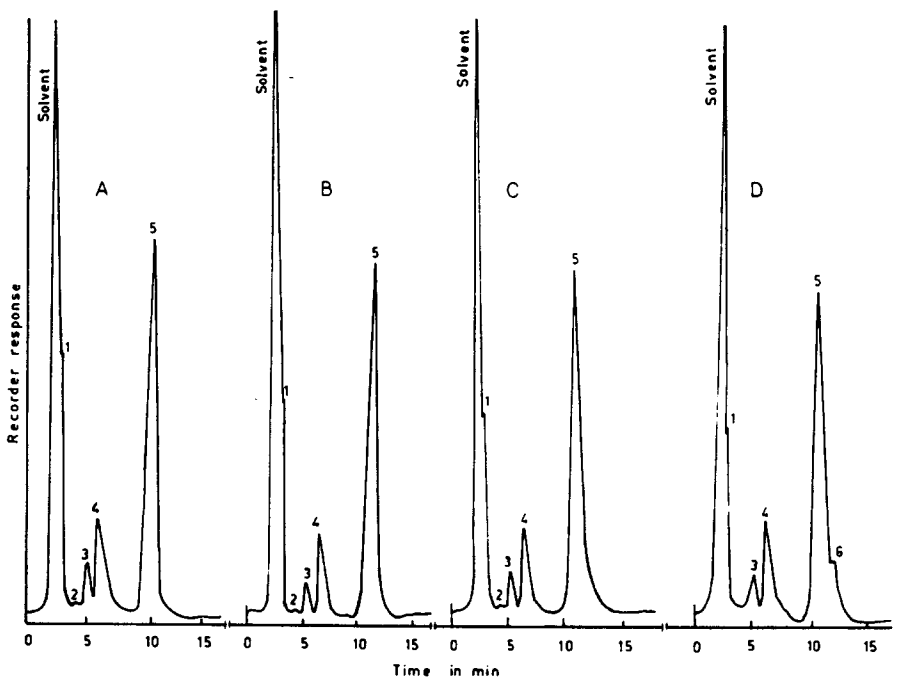

Fig. 1. HPLC analysis of sugars of roasted coconut samples. A, Plain; B, 130°C; C, 145°C; D, 160°C. 1, Ribose; 2, rhamnose; 3, fructose; 4, glucose/galactose; 5, sucrose; 6, maltose.

sucrose at higher temperatures as suggested by Evans and Butts (1949) and Anantharaman and Carpenter (1971). At the same time, glucose and fructose were being consumed for browning in a parallel reaction. There was only a slight decrease in starch content as can be seen from Table 1. In the advanced and in the final stages of heating, i.e. at 145°C and 160°C, thermal degradation of starch into maltose units becomes clearly noticeable. This was indicated by the maltose peak eluting with the receding sucrose peak in the corresponding chromatograms. In roasted cocoa, the changes in sugars have previously been followed by Rohan and Stewart (1966*a*) and they reported almost complete destruction of reducing sugars. Also sucrose was found to participate in the reaction, after being hydrolysed in the hot acid medium of cocoa beans. In pistachios, during roasting, reducing sugars and starch were reported to be affected by Kashani and Valadon (1984). In peanuts, sugar content was found to decrease by 8·0% and sucrose content was found to be affected (Oupadissakoon & Young, 1984).

Changes in the protein and non-protein nitrogen (NPN) contents

The NPN content of plain coconut was found to be 0·189%, on a dry weight basis, which is nearly 13% of the total nitrogen (Table 2). Upon heating, the NPN decreased to 0·156% at 130°C, making a sharp decrease by 17% and this was further reduced to 0·152% and 0·149% during subsequent heating. The overall reduction in NPN was significant (about 21%). At the same time, the total nitrogen and total protein content did not change drastically. Thus, it is clear that NPN species, like free amino acids, lower peptides, etc., are actively participating in the non-enzymic browning reaction.

Changes in amino acids

The amino acid analysis of hydrolysed proteins of coconut showed the presence of as many as 18 amino acids (Table 3) and this agreed with previously reported results (Samson, 1971). Glutamic acid was the most abundant amino acid followed by arginine and aspartic acid. The effect of heating was not very much reflected in the protein amino acid profile except for lysine and arginine of which the former registered a marked decrease. Thus, even in bound form, lysine and arginine interact with sugars. This observation is in agreement with the results reported by Evans and Butts (1949). Protein amino acids of pistachios were also not affected much during roasting (Kashani & Valadon, 1984).

The free amino acid analysis (Table 3) confirmed the decrease noticed in NPN content. Most of the amino acids present in total protein hydrolysate could be identified in this case also. Baptist (1956) analysed the aqueous

TABLE 2
Changes in the Protein and Non-protein Nitrogen (NPN) Contents of Coconut during Heating

	Plain	*130°C*	*145°C*	*160°C*
Total nitrogen (%)	1·37	1·35	1·33	1·33
Non-protein nitrogen (NPN) (%)	0·189	0·156	0·152	0·149
NPN in total nitrogen (%)	13·7	11·4	11·1	10·9
Crude protein (%) (Kjeldahl)	8·54	8·43	8·37	8·37
Actual protein (%) (Amino acid analysis)	7·42	7·31	7·28	7·25
Actual protein (%) (calculated) [(Kjeldahl N—NPN) × 6·25]	7·38	7·36	7·36	7·38

extract of coconut endosperm and coconut water for free amino acids, at varying stages of maturity, using paper chromatography. γ-amino butyric acid was reported to be present in large proportion. However, no quantitative data or further systematic analysis of free amino acids was undertaken thereafter. In the present study, tryptophan was found to be most abundant followed by glutamic acid, aspartic acid, alanine and arginine. The presence of γ-amino butyric acid could not be confirmed or quantitated.

As a result of heating, lysine was destroyed fully and tryptophan largely, followed by aspartic acid, alanine, glycine and glutamic acid. Threonine, valine, arginine, methionine, isoleucine, proline and serine were also affected during roasting. Table 3 includes % reduction of individual amino acids in the final stage of heating. The reactivity of the ε-amino group of lysine even in the bound state, is already mentioned in this paper. In the free form, the reactivity of lysine definitely increases due to the availability of two free amino groups. Lysine, arginine and methionine were also reported to be significantly involved in the reactions during roasting of pistachios (Kashani & Valadon, 1984). During roasting of peanuts, aspartic acid, glutamic acid, alanine, isoleucine, histidine and phenylalanine were found to be more affected (Newell *et al.*, 1967; Oupadissakoon & Young, 1984). According to Rohan and Stewart (1966*a*), threonine, glutamic acid, phenylalanine, cysteine, histidine, arginine etc., were noticeably affected during roasting of cocoa beans. Methionine, glycine, lysine, phenylalanine, tyrosine, serine and isoleucine were heat-damaged more in extruded potato products (Maga & Sizer, 1979). In short, the reactivity of most of the affected amino acids noticed in this study, is supported by results reported in other systems also. In the present study on coconut, free ammonia was also detected in the free amino acid analysis and was also found to vary or become undetectable

(below 10 μg) in the heated samples. Participation of free ammonia in the Maillard reaction is quite possible since model system studies have been carried out with ammonia as the nitrogen source (Shibamoto & Bernhard, 1977*b*). Interestingly cysteine, tyrosine and phenylalanine showed a positive change and the change was very significant in the case of phenylalanine. This may be due to the release of these amino acids in free form by breakdown of peptides during heating. The occasional increase in a few amino acids during heating (Table 3) can also be explained on the same basis. A few unknown peaks observed in the chromatogram probably represented some of the Amadori products and smaller peptides. The quantitative free amino acid

TABLE 3
Changes in the Protein- and Free-Amino Acids of Coconut during Heating[a]

Amino acid	*Quantity in mg/100 g dry sample*								*% reduction of free amino acids in final stage*
	Protein amino acids				*Free amino acids*				
	Plain	*130°C*	*145°C*	*160°C*	*Plain*	*130°C*	*145°C*	*160°C*	
Aspartic acid	771	769	762	766	30·97	21·76	19·52	9·41	70
Threonine	255	250	251	256	7·20	4·77	4·06	2·55	65
Serine	343	339	347	340	8·57	7·84	3·58	5·30	40
Glutamic acid	1460	1453	1452	1456	38·05	20·48	20·70	11·30	70
Proline	371	363	367	369	9·74	8·74	6·88	4·95	50
Glycine	288	282	286	279	5·47	1·96	2·49	1·71	50
						uip		uip	
Alanine	307	296	299	303	26·67	14·79	13·20	7·91	70
Valine	398	392	396	397	6·79	4·22	3·62	2·42	65
Cysteine (as half cystine)	90	88	89	86	—	1·43	2·86	1·71	—
Methionine	142	138	134	137	1·74	0·73	1·05	0·60	65
Isoleucine	256	255	252	257	3·84	2·49	3·33	1·28	67
Leucine	510	506	504	504	0·32	1·34	1·41	0·56	
						uip	uip		
Tyrosine[b]	190	185	189	186	Trace	Trace	1·55	1·92	
Phenylalanine	379	376	369	374	0·30	—	—	23·50	
Tryptophan	126	121	127	124	74·00	53·70	69·10	5·50	93
Histidine	181	187	188	177	4·06	1·30	1·97	4·58	
Lysine	300	269	230	207	2·86	0·96	1·87	—	100
Ammonia	—	—	—	—	0·16	0·60	0·71	—	
Arginine	1049	1042	1037	1030	15·69	9·28	10·60	5·39	66
Total amino acids	7416	7311	7279	7248	236·4	156·4	168·5	90·6	62

uip, unidentified peak.
[a] Average of two determinations.
[b] Trace = 10–20 μg/100 g.

analysis and the changes thereof, during heating of coconut are thus reported for the first time. Moreover, the heating regime followed here is different from earlier work of Samson (1971), where defatted coconut meal was subjected to heating. In the defatted state, the changes can be only drastic and do not represent the ordinary conditions of use of coconut.

Flavour studies of roasted coconut, carried out by the authors (Jayalekshmy *et al.*, 1985) and other researchers (Saittagaroon *et al.*, 1984) have confirmed the presence of many pyrazines and a few furan derivatives. It was seen that methyl pyrazine, 2,5-dimethyl pyrazine, 2,6-dimethyl pyrazine and the different isomers of methyl-ethyl pyrazine, dominated the flavour profile and the alkyl pyrazines together contribute nearly 100 ppm in the flavour isolate (Jayalekshmy, unpublished). Also, in the same study it was found that pyrazine concentration increased with temperature above 100°C. From the results discussed in this paper, we can understand that NPN content decreases with temperature which can be correlated with the increase in pyrazine concentration (Jayalekshmy, unpublished).

A number of model system studies have been carried out to correlate the reaction between amino acids and sugars with flavour changes as well as intensity of browning, during heating. Of these, only relevant work pertaining to our present study will be discussed here. Model system studies carried out by Koehler *et al.* (1969) and Koehler and Odell (1970) using different amino acids, as nitrogen source and sugars, as carbon source, reported formation of pyrazines during heating. In their study, different combinations of amino acids and sugars were heated under different conditions. The results indicated that a temperature range of about 100 to 150°C increased pyrazine content and a basic pH favoured its formation. Among the amino acids tried, asparagine, aspartic acid and glutamic acid gave the highest yields of pyrazines. Among the sugars, pentoses were found to be more reactive than hexoses and fructose reacted more readily than glucose. In all these experiments, methyl pyrazine and 2,5-dimethyl pyrazine were predominantly formed, irrespective of the amino acid and sugar used. In general, formation of simple alkyl pyrazines did not depend much on the carbon or nitrogen source. Most of the pyrazines were formed in the various combinations of sugars and amino acids tried, possibly due to differential fragmentation of the carbon skeleton of individual sugars. Recently, Arnoldi *et al.* (1988) have reported a model system study simulating roasting of cocoa beans. In this, fructose was heated with the major amino acids present in cocoa and results indicated that methyl and 2,5-dimethyl pyrazines were formed in all cases, in the highest amounts. Leucine was found to give as many as 10 different pyrazines, whereas lysine and aspartic acid gave only two different pyrazines. The amount of pyrazines formed in the fructose–lysine system was also less. A few other model system studies

are also reported in the literature, in which intensity of browning is correlated with activity of amino acids and sugars. Ashoor and Zent (1984) heated different amino acids and sugars and compared the degree of browning. It was seen that the intensity of browning decreased as follows: ribose > fructose > glucose and lysine > glycine > tryptophan > tyrosine. From our present study, we find that lysine and tryptophan are largely affected during heating of coconut and it can be assumed that they are utilised more in browning.

Formation of a few furan derivatives identified in roasted coconut can be attributed to thermal degradation of carbohydrate constituents (Hurrell, 1982). In addition to simple sugars, sucrose and starch were also found to be affected during roasting of coconut.

On the whole, the results obtained in this study are generally supported by the findings of model system studies reported in the literature. Coconut contains glucose and fructose, which are known to be active in browning as well as in pyrazine formation. Our results indicate that glucose and fructose are utilised during the roasting procedure. From free amino acid analysis, it can be concluded that lysine, tryptophan, glutamic acid, aspartic acid, alanine, valine and glycine are the most affected. Of these, lysine, glycine and tryptophan are likely to contribute more towards the actual browning process, whereas aspartic acid, glutamic acid, alanine and valine may preferentially participate in pyrazine formation. Thus, even though changes in a particular amino acid or sugar cannot be identified with the formation of a specific pyrazine, the overall changes in the carbohydrate and amino acid profiles of coconut on heating are indicative of the flavour enhancement observed during roasting.

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