Microbiological quality of desiccated coconut

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SUMMARY

A microbial survey of Sri Lankan desiccated coconut has been made on material purchased in supermarkets in Sheffield or on material obtained directly from the processing company. The total viable count (TVC) was reduced by spoilage and pasteurization from 10^4/g to 10^3/g. Most samples contained low levels of coagulase-positive *Stephylococcus aureus* suggesting that this commodity had been handled during production. One focus of contamination with *Aspergillus flavus* was found for each 8·34 g of desiccated coconut (mean contamination). The number of bacteria and moulds in spoiled coconut was significantly lower than that in coconut obtained from the processor or purchased from retail outlets. It is suggested that the accumulation of free fatty acids, aliphatic methyl ketones and secondary alcohols produced during fungal spoilage has had a bactericidal and fungicidal effect. The use of microbial specifications for foods is questioned in situations where there is evidence of microbial spoilage having taken place.

INTRODUCTION

The safety and shelf-life of any food commodity depends on the interaction of chemical, physical and microbiological factors. The overall quality of a product is related to its past history – from production and harvesting, through the manufacturing process, to distribution and retailing and finally to storage in the home. Manufacturers can control the end-product specification of their products by careful attention to good manufacturing practice. If the shelf life of the product exceeds 18 months, manufacturers are not required to list the minimum durability and the recommended storage conditions (Food Labelling Regulations, 1984). Once sold, the conditions in which the product is kept are no longer under their control.

The properties of all dehydrated products are adversely affected by moisture. It has been shown that one in ten houses in the UK suffers from serious damp and condensation (Rehab, 1984). It is important that food-processing companies should provide adequate packaging for dry foods such as coconut so that water does not enter the packet. In addition food should be free from pathogenic organisms as well as organisms which can cause microbiological spoilage (Mossel, 1982). The present law in the UK states that the manufacturer of a defective product cannot be held liable unless the vital element of negligence is proved (Lawson, 1985).

In a previous study it was found that spoilage in desiccated coconut was due to an accumulation of free fatty acids, aliphatic methyl ketones and secondary alcohols which gave the commodity a rancid off-flavour (Kellard, Busfield & Kinderlerer, 1985). The methyl ketones and secondary alcohols were formed from the coconut oil by growth at low water activity (0·76) and oxygen tension of four xerophilic fungi (members of the genus *Eurotium* and *Penicillium citrinum* – Kinderlerer & Kellard, 1984).

The aim of this study was to evaluate the microbiological safety of desiccated coconut with reference to the bacterial and mould flora. Particular emphasis has been placed on the presence of coagulase-positive *Staphylococcus aureus* and *Aspergillus flavus*. This study has involved microbiological surveys of four different types of Sri Lankan desiccated coconut: material purchased from supermarkets, material obtained directly from processing and packaging companies, material which was commercially pasteurized (steam at 92 °C for 8 min) and material which was returned to one manufacturer by consumers as spoilt and rancid.

MATERIALS AND METHODS

Coconut

Sri Lankan fine- or medium-grade desiccated coconut was used throughout. Samples (three or six 200 g packets) of four different brands of coconut were purchased from local supermarkets in Sheffield (companies A, B, C and D). Material was obtained as a gift from three food-processing or food-packaging companies in 2, 10 or 25 kg batches (companies C, E and F). Pasteurized coconut (25 kg) was purchased from one food processing company (G) and the consumer returns for 1982 and 1983 were obtained as a gift from one packaging company (F). This material had been packaged in cardboard boxes with 'glassine' lining and had been stored in homes in the UK for at least 6 months before being returned to the company. The good coconut samples were obtained in May and June 1984.

Microbiological methods of examination
Bacteriological sampling

Preparation of decimal dilutions

Desiccated coconut (10 g) was added to 90 ml full-strength Ringer's solution in a Stomacher bag, blended for 3 min in a Colworth Stomacher (A. J. Seward, Bury St Edmonds, Suffolk) and further diluted in duplicate with Ringer's solution to give dilutions of 1:100 and 1:1000. Two samples were weighed from each packet and between 4 to 12 samples were weighed for each of the larger batches. Full-strength Ringer's solution was found to give 25 % greater recovery than the more usual one-quarter-strength solution.

Total viable count – aerobic (TVC)

Miles and Misra surface methods were used (Kramer, 1977). Aliquots (20 μl) were applied in triplicate with a Gilson positive displacement pipette to the surface of dried plates (plate count agar, Oxoid CM325) which were then incubated for 3 days at 25 °C.

Staphylococcus aureus

Aliquots (20–500 μl) of the 1:10 dilution were applied in triplicate to the surface of three dried Baird-Parker plates (Oxoid CM275) (Baird-Parker, 1962) and incubated at 37 °C for 24 h. Typical black, shiny colonies, often with a white margin and usually surrounded by a zone of clearing were counted, subcultured onto nutrient agar (Oxoid CM3) and tested for coagulase activity by coagulation with rabbit serum.

Mycological sampling

General incubation methods

Desiccated coconut (5 g) was weighed into each of six Petri dishes containing sterile filter paper to which 10 ml 20% (v/v) glycerol was added. The plates were placed in a desiccator containing 20% (v/v) glycerol and incubated either at 30 °C for 5 days for enumeration of *Aspergillus flavus* or at ambient temperature (about 20 °C) for between 5 and 35 days for enumeration of the psychrotrophic to mesophilic mycoflora.

Identification of moulds

Aspergillus flavus

The typical yellow-green colonies due to *Aspergillus flavus* could be distinguished from the black and yellow colour of *Aspergillus niger* (conidia) and the fluffy grey mycelium of *Absidia corymbifera*. The yellow-green colonies which gave the *Aspergillus* anamorph when observed under the dissecting microscope were counted and sub-cultured onto '*Aspergillus flavus* and *parasiticus* agar' (AFPA) (Pitt, Hocking & Glenn, 1983). An orange reverse after incubation at 30 °C for 2 days gave a positive indication of *A. flavus*.

Total mycoflora at ambient (or 20 °C) temperature

The moulds were identified directly from the ambient plates. The following reference material was used to confirm the identification – Raper & Fennell, 1977, Samson, Stolk & Hadlok, 1976, Samson, Hoeckstra & van Oorschot, 1981.

Water activity measurements (a_w)

The water activity was obtained on duplicate samples of coconut using a Novasina TH-2 (Humitec, Horsham, West Sussex) at 25 ± 0.2 °C.

RESULTS AND DISCUSSION

Aerobic plate count

The total aerobic bacterial counts are given in Table 1. The consumer returns had the lowest counts (0.61×10^3/g) of all the samples even though they showed signs of spoilage and discolouration. This was in contrast to the material from supermarkets and food packers where the counts ranged from 10^3 to 10^5 c.f.u./g. Many of the packers of desiccated coconut use 'in-house' standards which recommend that the standard plate count should be less than 2.5×10^4/g for

Table 1. *Total viable count (aerobic) isolated from desiccated coconut*
(3 days at 25 °C)

No. of packets or weight original sample	Company*	Source	No. of analyses	Mean count/g Standard deviation and (range)		
				× 10³	× 10⁴	× 10⁵
6	A	Supermarket	39	3·34 ± 1·24 (1·0–6·5)	—	—
6	B	Supermarket	41	—	8·02 ± 5·2 (1·95–19·5)	—
3	C	Supermarket	20	—	1·31 ± 0·67 (0·5–3·0)	—
3	D	Supermarket	20	—	—	3·33 ± 1·16 (1·5–6·0)
10 kg	E	Food processor	21	—	4·00 ± 1·12 (1·5–5·5)	—
10 kg	E′	Food processor	23	—	3·32 ± 1·44 (1·5–6·5)	—
2 kg	F	Food packer	25	3·24 ± 1·58 (1·0–7·0)	—	—
2 kg	F′	Food packer	16	—	6·63 ± 1·97 (0·5–7·0)	—
10 kg	C′	Food packer	15	—	2·63 ± 1·17 (1·0–3·5)	—
25 kg	G	Food processor – (pasteurized)	31	1·37 ± 2·07 (0–12·0)	—	—
11	F″	Consumer returns	84	0·61 ± 0·56 (0–2·5)	—	—

* Samples A, B, C, D, E, F, F′ and C′ were white and had a pleasant sweet coconut smell, sample G had less smell whilst sample F″ had a stale and rancid smell and was discoloured.

desiccated coconut. From Table 1 it can be seen that this count is exceeded by 6 of the 11 batches of coconut which were investigated. The bacterial flora in the consumer returns and the normal coconut is different. The former consists mainly of Gram-positive spore-forming bacilli whilst the latter is mainly Gram-positive cocci. Kellard, Busfield & Kinderlerer (1985) demonstrated that short chain fatty acids and methyl ketones had accumulated in the coconut returned as spoiled. Such short chain fatty acids and methyl ketones have been shown to cause death of microorganisms (Tch, 1974; Scott, 1960), and may have a bactericidal effect in the consumer returns.

In a previous investigation *Acinetobacter*, *Flavobacterium*, *Microbacterium*, *Micrococcus* and various members of the Enterbacteriaceae were found on coconut shells (Kajs *et al.* 1976). It is likely that the coconut meat would be contaminated with these organisms. The counts given in Table 1 will represent a residual population of organisms which are resistant to heat and desiccation. Pasteurization with live steam (92 °C for 8 min) resulted in a reduction of the aerobic plate count by a factor of between 3–10 (Table 1).

Table 2. *Viable Count of* Staphylococcus aureus *isolated from desiccated coconut*

(Number of packets or weight of original material and the source of samples is
given in Table 1)

Company	No. of analyses	Mean count g^{-1} $\times 10^2 \pm$ S.D.	Range $\times 10^2$	Coagulase	% positive
A	46	2·59±1·08	0·3–4·7	+	13
B	52	14·19±8·02	0·2–34	+	21
C	27	9·19±3·98	2–15	N.D.	—
D	52	32·20±10·6	18–58	+	11
E	18	3·22±3·77	0–14	N.D.	—
E′	18	0·56±1·15	0–4·0	N.D.	—
F	24	1·01±0·27	0·8–1·2	+	7
C′	18	3·25±1·65	0–7·0	+	8
F″	69	0·24±0·90	0–5·0	N.D.	—
G	12	0·12±0·16	0–8·0	N.D.	—

Baird-Parker Agar was used in all cases except the consumer returns (F″) where mannitol salt
agar was used.
N.D., none detected.

Table 3. Aspergillus flavus *and water activity* (a_w) *from desiccated coconut*

(Number of packets or weight of original material and source of sample is
given in Table 1)

Company	Organoleptic quality	Water activity	*A. flavus* coconut (g) equivalent to 1 c.f.u.§
A	Sweet, coconut-like	0·36	10·0
B	Sweet, coconut-like	0·39	12·0
C	Sweet, coconut-like	0·36	0·63
D	Sweet, coconut-like	0·42	0·75
E†	Sweet, coconut-like	0·35	10·0
E′†	Sweet, coconut-like	0·38	15·0
F	Sweet, coconut-like	0·37	0·33
F′	Sweet, coconut-like	0·36	7·55
C′	Sweet, coconut-like	0·32	15·0
G*	Slight smell, not sweet	0·32	‡N.D. in 60 g
F″†	Rancid, soapy and spoilt	0·38	N.D. in 100 g

* Sample G was pasteurized with steam at 92 °C for 8 min.
† Aflatoxin B^1 was not detected in samples F″, E and E′.
‡ N.D., not detected.
§ The mean contamination of desiccated coconut with *Aspergillus flavus* is calculated as the
average for all samples (excluding G and F″) of the final column. This represents the weight
of coconut equivalent to one focus of infection. This value is 8·34±5·97 g.

Staphylococcus aureus

Table 2 indicates that the viable count ranged from 10 to 10^4/g of which
approximately 10 % were coagulase-positive *Staph. aureus.* Whilst the risk of
production of sufficient enterotoxin within the commodity to cause gastroenteritis
is low, the risk is much greater if desiccated coconut is incorporated into other foods
without undergoing further heat treatment. The ICMSF specifications (Inter-
national Commission on Microbiological Specification for Foods, 1974) recommends

Table 4. *Mycoflora after fermentation of desiccated coconut with 20 % (v/v) glycerol at ambient temperature*

Type of coconut	Time (days) for growth of visible colonies	Species	Level of contamination
Consumer returns (1983) (Company F)	19 ± 7.4	*Penicillium verrucosum* var. *cyclopium*	Light
		Eurotium herbariorum	Simple flora, 1–2 colonies
Pasteurised (Company G)	9–11	*P. verrucosum* var. *cyclopium*	Heavy
		P. echinulatum	Simple flora, mainly
		Cladosporium sphaerospermum	Penicillium
Good coconut	9–11	*A. flavus*	Heavy, varied flora
		E. amstelodami	
		E. chevalieri	
		E. herbariorum	
		Penicillium species	
		Cladosporium species	
		other *Aspergillus* species and	Sometimes present
		Phoma sorghina	

a count of less than 10^2/g for coagulase-positive *Staph. aureus* for many foods. Only one batch of coconut failed to meet this specification (Table 2). *Staph. aureus* is uncommon on plant products and its presence is due to direct contact with people (Roberts, Watson & Gilbert, 1982).

Aspergillus flavus

The mean contamination (8·34 g) for desiccated coconut is given in Table 3 (mean contamination is used to define the weight of coconut which leads to one focus of infection should it become damp). This mould was not isolated from the consumer returns nor from the pasteurized coconut. Aflatoxins are produced by some strains of *Aspergillus flavus* and *A. parasiticus*. The aflatoxins are a group of closely related heterocyclic compounds of which aflatoxin B_1 is the most prevalent, acutely toxic member of this group (Cole & Cox, 1981). Strains of *A. flavus* were found to produce high yields of aflatoxins when grown on rehydrated desiccated coconut (44 % water content) at 25 °C in shake culture. It appears that a high oxygen tension and moisture content is required for production of aflatoxins (Arseculeratne *et al.* 1969). Aflatoxin B_1 was not detected in any of the samples investigated (Dr M. Morgan, personal communication). Obviously the low water activity in desiccated coconut (Table 3) and the presence of packaging would reduce water and oxygen transmission which in turn would preclude the synthesis of aflatoxins.

Mycoflora

Four genera, *Aspergillus*, *Cladosporium*, *Eurotium* and *Penicillium* were found in normal desiccated coconut in high numbers (Table 4). This was in contrast to the consumer returns when only two species were isolated – *E. herbariorum* and *Penicillium verrucosum* var. *cyclopium*. These species were found in low numbers. The long delay of 9 days before visible mould growth occurred suggests that the organisms had suffered from metabolic injury and that they required time to

recover prior to germination. The presence of short chain fatty acids, methyl ketones and secondary alcohols (Kellard, Busfield & Kinderlerer, 1985) may have had a fungicidal effect on much of the normal microflora. In the pasteurized coconut potential toxigenic species such as *A. flavus* had been eliminated. This sample was heavily contaminated by two species of *Penicillium* (*P. verrucosum* var. *cyclopium* and *P. echinulatum*) as well as *Cladosporium* species. The presence of these moulds suggests that good manufacturing practice had not been observed in the pasteurization plant. Recontamination of the processed material may have taken place after the heat process and before packaging. Contamination may be due to a poor standard of housekeeping in the factory or from contamination by fungal spores in the atmosphere.

CONCLUSIONS

During the manufacture of desiccated coconut the product undergoes two heat treatments, the first where pieces of coconut meat are immersed in boiling water for not less than 1·5 min, and the second where the shredded coconut is dried in hot air for 20–30 min at 93 °C before it is packaged (CAC/Recommended Code of Practice, 1971). The final water activity of the commodity is between 0·32 and 0·42 (Table 3). At this value the coconut is stable and will not spoil although bacteria and moulds are present. Microbial survival in commodities which have undergone processing is due to organisms which are adapted to survival at the extremes of the environment.

The end-product specifications for desiccated coconut require that there is no risk to the health of the consumer (FAO/WHO Codex Alimentarius Commission, 1984). In the past desiccated coconut has been a significant source of salmonellae (Semple, Graham & Dutton, 1961). Modern practice is to separate the unit operations – dehusking, shelling, paring, boiling, shredding and drying. In addition the improvement in the standard of hygiene in the coconut mill has resulted in only a single isolation of salmonella from 370 samples of Sri Lankan desiccated coconut imported into the UK and examined by the Public Health Laboratory Service (PHLS) for the period 1975–8 (Roberts, Watson & Gilbert, 1982). Low numbers of coagulase-positive *Staph. aureus* and *Aspergillus flavus* were found in good desiccated coconut. Both organisms are capable of producing toxins in the commodity if it is stored incorrectly or if it is incorporated into the other foods without undergoing a further heat treatment.

Foods should be free from potential pathogens, bacterial and fungal toxins and should not undergo microbial spoilage. In the present survey the microbial population was much smaller in the spoilt materials when compared to those considered acceptable. A low microbial count may suggest that the commodity has undergone considerable microbiological spoilage and as such, is unfit for human consumption. This makes a rigid enforcement of microbial standards for foods unsound and the organoleptic quality needs to be taken into account as well.

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