J Sci Food Agric 1992, **58**, 339–346

Hibiscus Leaves for the Manufacture of Usar, a Traditional Inoculum for Tempe

M J Robert Nout,[a]* Tri D Martoyuwono,[b] Peter C J Bonné[a] and George T Odamtten[c]

[a] Department of Food Science, Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands
[b] Central Laboratory, Brawijaya University, Jl Mayjen Haryono 163, Malang, Indonesia
[c] Department of Botany, University of Ghana, PO Box 55, Legon, Ghana

(Received 17 June 1991; revised version received 27 August 1991; accepted 25 October 1991)

Abstract: Tempe is an Indonesian proteinaceous food produced from soya beans by fermentation mainly by *Rhizopus* spp. A common traditional inoculum for this fermentation is usar, consisting of heavily sporulated *Rhizopus* spp of which the mycelium, grown on added and inoculated soya beans, adheres to leaves of the waru tree (*Hibiscus* spp). A description of various waru trees is given together with their suitability for usar making. The epiphytic fungi on waru trees of various geographical origins (Indonesia, Africa, The Netherlands) were investigated. *Cladosporium* spp were present on all the leaves sampled. However, *Rhizopus* spp predominated over the other fungi encountered on the Indonesian leaves. Experimental incubation of leaves with and without added soya beans showed that the epiphytic fungi are less competitive than *Rhizopus* spp and will not interfere if normal usar-making conditions are observed. Experiments with alternative attachment surfaces (paper, a textile) indicated that the waru leaves do not provide environmental selectivity through growth-inhibiting substances, but that they serve mainly as a convenient attachment surface with moisture-retaining capacity while providing adequate aeration for fungal development.

Key words: *Hibiscus*, 'waru', tempe, inoculum, 'usar', fungi, *Rhizopus*, epiphytes.

INTRODUCTION

Tempe is a fermented product originating from Indonesia where it is usually made from soya beans. It can, however, also be produced from a variety of other seeds, including those of legumes and cereals, and from food-processing by-products. The tempe-making process was recently reviewed by Nout and Rombouts (1990). The predominant microorganisms responsible for the conversion of the raw material and the attainment of the firm texture of the resulting tempe are *Rhizopus microsporus* var *oligosporus* (in short: *R oligosporus*) and *R oryzae*. These fungi are introduced by inoculating the soaked and subsequently cooked raw material with a suitable tempe starter. The latter include natural starters, pure culture starters and semi-pure culture starters. In Indonesian commercial practice, the use of natural starters (usar) is predominant. Usar is manufactured at the village scale (a few thousand leaves may be processed daily) and is obtained by cultivating *Rhizopus* spp on a few inoculated soya beans sandwiched between two leaves from waru (*Hibiscus* spp) trees. The rough adaxial surface of the underside of the leaves permits mycelial attachment. After adequate incubation, visible sporulation takes place and the sandwiches are opened. Usually, the leaves are sun dried to extend the shelf life of the starter. However, sun drying is not essential. Indeed, usar makers use freshly harvested usar for their own production process; they consider fresh usar to be of better quality than the dried product. The type and age of the leaf determine its suitability. Only leaves which retain firmness during 48 h incubation under high humidity and which do not become crisp or brittle after sun drying are suitable. The usar manufacturing process

* To whom correspondence should be addressed.

J Sci Food Agric 0022-5142/92/$05.00

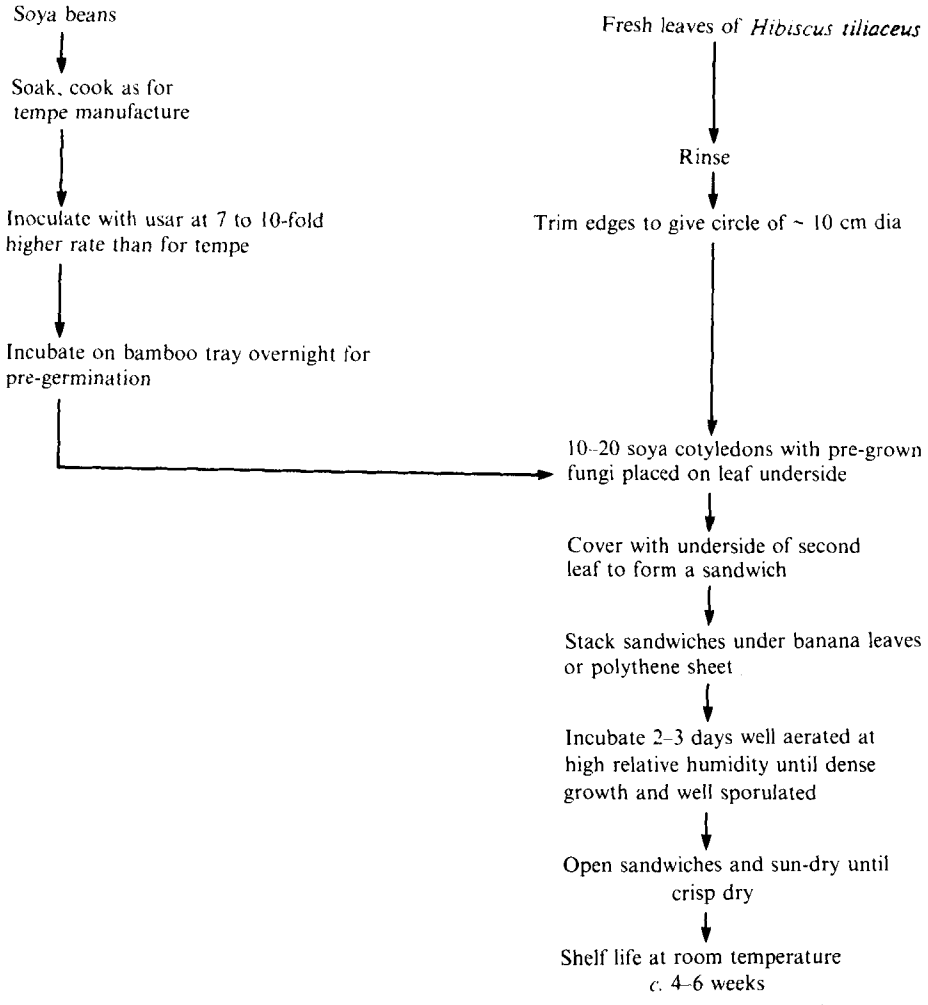

Fig 1. Flow-chart of usar manufacture as practised at Singosari, Eastern Java, Indonesia.

as practised in the Malang region, East Java, Indonesia, is outlined in Fig 1. The resulting dried usar has a shelf life of approximately 4–6 weeks at room temperature (28–30°C). Tempe technology offers an attractive method to transform beans and pulses into easily digestible and tasty products. Considerable scientific interest in the application of tempe technology has been shown in many developing countries. Recently, Djurtoft (1985) conducted successful preliminary trials in Nigeria with tempe made from cowpeas using Indonesian usar as inoculum. In principle, the use of natural usar starters should be most appropriate under simple and non-aseptic processing conditions. We therefore carried out a microbiological investigation of leaves from Indonesia used for the manufacture of usar, particularly those from the Malang region which is well known for the production of superior tempe.

In the absence of appropriate inoculum, Indonesian usar makers raise *Rhizopus* spp by incubating boiled soya beans sandwiched between leaves. In this paper we examined the natural mycoflora of leaves from Indonesia, The Netherlands, Nigeria, Burkina Faso and Ghana for this purpose.

LEAVES FOR USAR MANUFACTURE

According to Jutono (1985), usar is made with leaves of *Hibiscus similis*, *H tiliaceus*, *Tectona grandis*, *Bambusa* spp or *Musa paradisiaca* in the Yogyakarta region. At Singosari, Malang region, the usar makers distinguish among several waru trees. Whereas *Tectona* spp and *Bambusa* spp are regarded as unsuitable for usar making because of, respectively, their brittleness after drying and their smooth surface which is unsuitable for mycelial attachment, some waru leaves are specifically used for usar manufacture. We collected herbarium specimens of the relevant trees and identified them with the assistance of the herbaria of Bogor, Indonesia, and of Leiden and Wageningen, The Netherlands. Most waru trees shown to us belonged to the Family Malvaceae, but one belonged to the Euphorbiaceae. There are several authoritative classifications of the genus *Hibiscus*, notably those of Heyne (1950) and Van Borssum Waalkes (1966). The latter classified several varieties of *Hibiscus tiliaceus* at the subspecies level in view of the considerable differences associated with distribution and ecology. The following waru trees predominate in the Malang region.

Malvaceae

Hibiscus tiliaceus L ssp *tiliaceus* (formerly *H tiliaceus* L). A fast-growing tree, 10 to 30 m tall. Leaves are suborbicular, 10 to 15 cm long, green, smooth and shiny on the upper surface, the lower surface being greyish and hairy with one to five central nerves. They have a distinctly heart-shaped base and a pointed tip, and are usually entire, but sometimes have minutely toothed margins. The three to five linear glands, a certain characteristic for determination, are placed very near the base of the leaf on the central nerves beneath the leaves. The sticky juice excreted by the glands contains glucose, fructose and sucrose; it attracts insects and it accumulates dirt thereby giving the glands a light brown to black colour. In Indonesia the subspecies is found growing gregariously near the coast. In Java it is also planted in coastal regions, and in the interior, in home gardens and along roads. In Nigeria the subspecies was found, and 5–10 m tall trees were photographed at Ibadan by Djurtoft (1985). We found it also along the coast of the Lagos Lagoon with the assistance of the Department of Botany, University of Lagos. In Ghana the subspecies is known as a shrub growing on river banks. Three different waru trees in the Malang region belong to this subspecies: *waru lengis*, the most commonly found, and used for the production of usar; *waru kedu*, less common, but even more attractive for usar making than waru lengis because of the more pronounced tomentose adaxial surface giving the leaves a dull-white appearance; *waru lumbu*, quite similar to waru kedu but, because of their brittleness after drying, the leaves are considered unsuitable for usar making.

Hibiscus tiliaceus L ssp *similis* (Bl) Borss (formerly *Hibiscus similis* Bl). A small tree 5 to 15 m tall, often mistaken for *H tiliaceus* ssp *tiliaceus*. However, the linear glands in the central nerves beneath the leaves are placed far above the base of the leaf. Presumably *H tiliaceus* ssp *similis* is a hybrid of *H tiliaceus* L ssp *tiliaceus* and *H macrophyllus*. In other regions of Indonesia, the subspecies is referred to as 'waru gombong'; however, the waru gombong shown by usar makers in the Malang region was identified as *H macrophyllus*. Considering the similarities between ssp *similis* and *H macrophyllus*, it is not surprising that they have the same local name. The waru trees shown to us did not include ssp *similis*. Consequently, no opinions were obtained concerning its suitability for usar making.

Hibiscus macrophyllus Roxb ex Hornem, *waru gombong*. This waru tree has much bigger and extremely hairy leaves with three to five nectaries situated approximately two-thirds of the distance between base and margin. Waru gombong is considered unsuitable for usar making because of its brittleness and its strong taste.

Euphorbiaceae

Macaranga tanarius (L) Muell Arg, *waru tutup*. The leaves do not have the deeply cordate shape of *H tiliaceus* leaves. They have no nectaries, and are not used for usar making.

MATERIALS AND METHODS

Hibiscus leaves were collected from 15 waru trees (10 middle-aged leaves per tree) in Malang city and its surroundings in Indonesia. In Nigeria the leaves were obtained from five trees along the banks of the Lagos Lagoon; the Ghanaian specimens were obtained from several shrubs along the banks of the Ankobra River near Axim in the Western region while *Abelmoschus esculentus* (L) Moench (*H esculentus* L, okra) leaves were collected from five shrubs cultivated in Ouagadougou, Burkina Faso. All the leaves were sun dried at ambient temperature (28–32°C). Leaves from one tree were packed together in polyethylene bags and stored at ambient temperature until needed. Cuttings from 20 waru lengis and waru kedu from Malang were waxed at the ends with paraffin and then transported by air to The Netherlands. The cuttings were nursed at 28°C and 85% RH in the greenhouse of the Agricultural University, Wageningen, under the normal 12 h day/night light regime reminiscent of tropical regions. Nineteen of the cuttings developed within 6 months into branched trees approximately 5 m high. The leaves were collected and dried as described above.

Microbiological analysis

Epiphytic mycoflora

Mycoflora of the *Hibiscus* leaves from different trees were isolated by surface swabbing of the nectaries with a sterile dry polyester fibre-tipped applicator (Falcon® 2069, Becton Dickinson Co, Cockeysville USA). The applicator was streaked directly on to previously poured petri dishes containing either malt extract agar (MEA, Oxoid CM59), Oxytetracycline yeast extract glucose agar (OGYE, Oxoid CM545 with 100 $\mu g\ g^{-1}$ oxytetracycline added aseptically; Nout *et al* 1987a) or Sabouraud agar (SA, 10 g peptone Oxoid 6404, 40 g glucose and 15 g technical agar Oxoid L13 per litre distilled water). The plates were incubated at 30°C and observed each day for 5 days. All fungal colonies appearing were isolated and subcultured on the same media as indicated above. Fungi were identified to the genus level using their microscopic, cultural and morphological characteristics (De Vries 1967; Ellis 1971a,b: Barnett and Hunter 1972; Domsch *et al* 1980; Samson and Van Reenen-Hoekstra 1988). *Rhizopus* spp were identified to species level using the descriptions of Schipper and Stalpers (1984).

TABLE 1
Fungal genera isolated by dry swabbing, from waru and related leaves of different origin

Origin	*Name* Botanical	Javanese	*Code*	*ALT*[a]	*ASP*	*BYS*	*CLA*	*CUR*	*FUS*	*PAE*	*PEN*	*PHO*	*RHI*	*TRI*	*YEA*	?
Indonesia	*H tiliaceus* ssp	*w lengis*	Ht1		2		3						2s[b],o[b]		2	3
	tiliaceus		Ht2				3				1				2	
			Ht3				4								1	
			Ht5		1		4							1	1	2
			Ht6	2			4		2				1o		4	
			Ht7		2				1						4	1
			Ht8		1		2						5o*[b]			1
		w kedu	Ht4				5		1				1l[b]		2	
			Ht9				3						2*		3	3
		w lumbu	Ht10				2	1					5s,o*,l*		3	3
	Hibiscus macrophyllus	*w gombong*	Ht12				1						2l*		1	
	Macaranga tanarius	*w tutup*	Ht11										2l*			
			Totals	2	6	0	31	1	4	0	1	0	20 (12*)	1	23	13
The Netherlands	*H tiliaceus* ssp	*w lengis*	WL1				4						1s			1
	tiliaceus		LWa		1		3				1				1	
			LWb		1		5				1					1
		w kedu	KWa		2		4							1		
			KWb		1		4		1				1s			
			KWc				2									1
			Totals	0	5	0	22	0	1	0	2	0	2	1	1	3
Burkina Faso	*Abelmoschus esculentus*		B1			1	3		7			1				
			B2		2	2	2		3			1		2	2	3
			B3			1	3		7			1			1	
			B4			1	1		10						1	
			Totals	0	2	5	9	0	27	0	0	3	0	2	4	3
Nigeria	*Hibiscus*		N1		1		11			1		1		1	1	
	tiliaceus ssp		N2				11			1				1		
	tiliaceus		N3				8			1				5		1
			N4				10			3				1		
			N5				12			1					1	
			N6				6							5	1	
			N7	3			9			2		2		1		
			N8				8		2			1		5	1	
			N9				13			3						1
			N10	1			11		1			1		1		
			Totals	4	1	0	99	0	3	12	0	5	0	20	4	2
Ghana	*Hibiscus*		G1				7							2		1
	tiliaceus		G2		1		8				2				1	
			G3		1		10		1			1		1		
			G4	1			8							1		
			G5				7		3						1	
			G6			1	2				2			3		2
			G7		2		12						1o			
			G8				6				1			1	1	
			G9				8					1				2
			Totals	1	4	1	68	0	4	0	5	2	1	8	3	5

[a] ALT: *Alternaria*; ASP: *Aspergillus*; BYS: *Byssochlamys*; CLA: *Cladosporium*; CUR: *Curvularia*; FUS: *Fusarium*; PAE: *Paecilomyces*; PEN: *Penicillium*; PHO: *Phoma*; RHI: *Rhizopus*; TRI: *Trichoderma*; YEA: Yeasts; ?: Others, not determined.
[b] s: *Rhizopus stolonifer*; o: *R oryzae*; l: *R oligosporus*; * makes good tempe.

Quantitative estimation of microbial population
The following procedure was adopted for estimating the population of total aerobic mesophilic bacteria, Enterobacteriaceae, lactobacilli, bacterial endospores and moulds and yeasts. Half a leaf of the appropriate *Hibiscus* spp was washed for 2 min in 50 ml peptone physiological salt solution (PPS, containing 1 g peptone and 8·5 g NaCl per litre distilled water) in a Colworth stomacher (type Lab Blender 400). Appropriate dilutions with PPS were used with plate count agar (PCA, Oxoid CM325) for total aerobic mesophilic bacterial count; with OGYE for moulds and yeasts count; with modified

TABLE 2
Microbiological composition of waru (*Hibiscus tiliaceus* ssp *tiliaceus*) leaves (average of five leaves)

	Medium	(Log_{10} $N\,g^{-1}$)	
		Fresh	*Rinsed*
Total aerobic count	PCA	9·56	9·46
Yeasts and moulds	OGYE	7·49	5·40
Moulds	RBCC	5·48	5·67
Enterobacteriaceae	VRBG	5·46	4·36
Lactobacilli	ROG	< 1·16	< 1·16

OGYE, Oxytetracycline yeast extract glucose agar; PCA, plate count agar; RBCC, Rose bengal chlortetracycline chloramphenical agar; ROG, Rogosa agar; VRBG, violet red bile glucose agar.

rose bengal chlortetracycline chloramphenicol agar (RBCC, Oxoid CM549) (Baggerman 1981) for moulds and yeasts; with violet red bile glucose agar (VRBG, Oxoid CM485) for Enterobacteriaceae; and with Rogosa agar (ROG, Oxoid CM627) for lactobacilli. Bacterial endospores were counted in PCA pour plates after pasteurisation of the 10^{-1} dilution at 80°C for 1 min.

Experimental manufacture of usar and tempe

Soya beans for incubation on *Hibiscus* leaves were subjected to fermentative acidification and boiled as described elsewhere (Nout *et al* 1987b). Leaves with or without added soya beans were incubated in desiccators at 100% relative humidity at 28°C.

Manufacture of usar on alternative carriers was carried out using 10-cm-dia circles cut from kraft paper and bed-sheet linen. The usar production was carried out by a commercial usar maker following the scheme presented in Fig 1, starting with moistened paper and linen. The experimental usars were inoculated, incubated and sun dried as part of a normal usar production batch.

Tempe was made with experimental usars by three commercial tempe makers in Malang. The details of their manufacturing processes are given by Nout and Rombouts (1990, Table 2).

Tempe quality assessment was based on firmness, colour and odour as described by Nout *et al* (1985).

TABLE 3
Predominant fungi on *Hibiscus tiliaceus* ssp *tiliaceus* leaves incubated for 7 days at 30°C and 100% relative humidity with or without added cooked soya beans

Origin	*Code*	*Without soya beans*	*Code*	*With soya beans*
Indonesia	Ht1	No visible growth	Ht1	*Rhizopus* sp
	Ht2	No visible growth	Ht2	*Rhizopus oryzae*
	Ht3	No visible growth	Ht3	*R oligosporus*
	Ht4	No visible growth	Ht4	*R oligosporus*
	Ht5	No visible growth	Ht5	*Rhizopus* sp
	Ht6	No visible growth	Ht6	*R oryzae*
	Ht7	No visible growth	Ht7	*Rhizopus* sp
	Ht12	No visible growth	Ht12	*R oligosporus*
				R oryzae
The Netherlands	WL1	No visible growth	WL1	*Rhizopus stolonifer*
	WL2	No visible growth	WL2	*Aspergillus fumigatus*
	WK1	No visible growth	WK1	*A fumigatus*
	WK2	No visible growth	WK2	*A fumigatus*
	WK3	No visible growth	WK3	*R oryzae*
Nigeria	N11	*Cladosporium* sp	N13	*Neurospora* sp
	N12	*Cladosporium* sp	N15	*Fusarium* sp
			N16	*Fusarium* sp
			N17	*Neurospora* sp
			N18	*Neurospora* sp
Ghana	G1	*Cladosporium* sp	G1	*Neurospora* sp
	G5	No visible growth	G5	*Aspergillus niger*
	G7	No visible growth	G7	*R oryzae*
	G9	*Cladosporium* sp	G9	*Aspergillus ochraceus*

TABLE 4
Microbiological composition of usar (log_{10} N g^{-1})

	Own analysis (n = *5 leaves of fresh usar*)	*Literature data* (*1*)	(*2*)	(*3*)
Mould propagules	7·76–9·00	5·86–5·98	ND	8·20
Total aerobic bacteria	7·01–8·53	5·08–5·18	9·23	7·28
Bacterial spores	2·86–6·14	ND	3·40	3·15
Yeasts	ND	3·51	3·78	6·46
Lactobacilli	5·66–7·36	ND	ND	6·60
Enterobacteriaceae	5·34–6·26	ND	ND	7·00

References: 1, Soetarto Hadisepoetro *et al* (1979); 2, Jutono (1985); 3, Tüncel *et al* (1989).
ND = Not determined.

RESULTS

Epiphytic mycoflora

Table 1 summarises the results obtained. *Rhizopus* species (mainly *R oryzae* and *R oligosporus*) predominated over the other fungi isolated from leaves from Indonesia. Out of the resulting pure cultures, a significant number were of acceptable or good tempe-making quality (results not presented).

Other frequently occurring fungal genera in the Indonesian leaves were *Aspergillus*, *Cladosporium*, *Fusarium* and yeasts. Interestingly, *Cladosporium* was the most frequently encountered genus in the *Hibiscus* leaves from The Netherlands, Ghana and Nigeria, and in the *Abelmoschus* leaves from Burkina Faso (Table 1). Furthermore, members of the genera *Aspergillus*, *Fusarium*, *Paecilomyces* and *Trichoderma* were also isolated from these leaves.

Quantitative estimation of microorganisms and experimental manufacture of usar and tempe

In the usar manufacturing process, freshly collected leaves are kept in water for some time for the purpose of moistening and rinsing. Table 2 shows the microbiological compositions of the leaves. Rinsing has no significant ($P \leqslant 0{\cdot}05$) diminishing effect on the total aerobic bacteria load. Conversely, the numbers of yeasts and Enterobacteriaceae were significantly ($P \leqslant 0{\cdot}05$) reduced.

Rinsed leaves incubated without the addition of soya beans did not support growth of epiphytic fungi to any great extent. For example, none of the leaves from Indonesia and The Netherlands supported fungal growth, and only the samples G1 and G9 from Ghana and N11 and N12 from Nigeria permitted growth of *Cladosporium* sp (Table 3). When cooked soya beans were sandwiched between the incubated leaves, there was luxuriant growth of relatively fast-growing fungal genera (*Aspergillus*, *Fusarium*, *Neurospora*, *Rhizopus*) (Table 3). The mycofloral composition and predominant genera are consistent with findings presented in Table 1. *Rhizopus* spp were predominant in all the leaf samples from Indonesia. On the other hand, other genera (Tables 1 and 3) developed on the African leaves which hardly contained *Rhizopus* spp. Presumably, *Cladosporium* was not sufficiently competitive to develop into dominance despite its frequent occurrence on freshly harvested leaves.

Table 4 summarises the microbiological analyses of *Hibiscus* usar carried out by us and others (Soetarto Hadisepoetro *et al* 1979; Jutono 1985; Tüncel *et al* 1989). As might be expected also from the data in Table 2, bacteria and yeasts, as well as moulds, multiply during the incubation of the leaf sandwiches.

TABLE 5
Microbial content of usar on different carriers (log_{10} N/usar)

Carrier material	*Moulds and yeasts*	*Bacterial endospores*
Hibiscus leaves	9·4	3·4
Kraft paper	9·4	3·4
Textile (linen)	9·3	3·3

Table 5 shows the frequency of fungi and bacterial endospores of usar made with *Hibiscus* leaves (control) and with alternative materials providing a rough surface, ie kraft paper and linen (bed sheet). The microbial compositions of the resulting usars showed no significant variations. However, with paper and the textile it was more difficult to control moisture content and to create sufficient aeration for growth. The aeration appeared to be restricted by the tendency of paper and textile sheets to stick together. Additionally, the mycelium penetrated the textile and developed on both sides, which is inconvenient.

TABLE 6
Tempe made by commercial producers using experimental usars (microbial counts as $\log_{10}$ N g^{-1})

		Carrier material for usar		
	Producer	Hibiscus	*Kraft paper*	*Textile (linen)*
Tempe quality	A[a]	Good	Good	Good
	B	Excellent	Good	Excellent
	C	Fair	Good	Fair
Total aerobic bacteria	A	9·3	8·7	8·5
	B	8·9	8·5	9·0
	C	9·8	9·7	9·8
Enterobacteriaceae	A	8·3	6·3	7·0
	B	4·7	7·2	4·0
	C	8·8	7·7	9·2

[a] A: Slow process (takes 4 days); B: slow process (5 days); C: fast process (3 days).

However, paper and textile usars could be successfully used as inocula for tempe manufacture. Table 6 summarises the results obtained with the assistance of three commercial tempe manufacturers. The tempe obtained using alternative usar was similar to the product obtained with *Hibiscus* usar, from both the sensory and microbiological points of view.

DISCUSSION

There is a significant difference between the epiphytic mycoflora of *Hibiscus* leaves from Indonesia and what was present on similar leaves from other geographical areas. For example, *Rhizopus* spp were isolated from about 70% of the Indonesian leaves in contrast to the predominance of *Cladosporium* spp isolated from leaves from The Netherlands, Burkina Faso, Ghana and Nigeria (Table 1). Although no attempt was made to locate the origin of *Rhizopus* contamination, it is conjectured that two possible mechanisms may be involved. First, insects attracted by sugary juice in the nectaries carry along spores and deposit them in the course of pollination (Ingold 1971). Secondly, fungi are ubiquitous and can be transported in the air over long distances (Edmonds and Benninghoff 1974; Al-Doory and Domson 1984). The air spora varies in composition from one locality to another (Ingold 1971). Some Mucorales, eg *Rhizopus*, *Mucor* and *Actinomucor*, are unspecialised parasites and may be dispersed dry from the sporangium (Ingold 1971). For example, Kramer *et al* (1959) showed that out of 113667 fungal colonies isolated from the air by the plate exposure method in Kansas City, USA, 194 belonged to the Mucorales and, of these, 156 belonged to the genus *Rhizopus*. In contrast, 50548 colonies were *Cladosporium* spp (Kramer *et al* 1959). It can be inferred that the widespread use of *Rhizopus* spp in the manufacture of tempe results in its preponderance in the air spora of the Malang region. Possibly, *Hibiscus* leaf acts as one of its natural reservoirs. Jutono (1985) and Arbianto (1990) stated that the leaf environment would stimulate the formation of chlamydospores and zygospores. These features should contribute to the viability and biological stability of the mixed population of *Rhizopus* spp on usar. In our view the natural occurrence of wild strains of *Rhizopus* species on the leaves should in any case increase the chance of zygospore formation. Further experimental evidence is required to evaluate the importance of the mentioned features for the performance of usar as an inoculum.

Comparing the Indonesian leaves with those of different origin, it is clear from Table 3 that (a) *Rhizopus* spp only grow if soya beans are present as substrate, (b) the other flora will only grow in rare cases (viz G1 and G9) in the absence of soya beans whereas the faster growing genera (*Neurospora*, *Aspergillus*, *Fusarium*) will develop in the absence of *Rhizopus*; (c) if *Rhizopus* spp are present they will dominate quickly. There is therefore little reason to disqualify *Hibiscus* leaves of non-Indonesian origin for usar making since inoculation with *Rhizopus* spp and the addition of soya beans will ensure dominant growth of *Rhizopus* spp in a normal usar making process.

Factors which possibly contribute to the dominance of *Rhizopus* spp include: (i) a higher specific growth rate of *Rhizopus* spp than that of the other genera, and (ii) production of growth-inhibiting substances by *Rhizopus* spp (Nout and Rombouts 1990). Considering the data in Table 5 it is unlikely that *Hibiscus* leaves contain either selective or elective substances affecting the dominance of *Rhizopus* spp.

The experiment with usar made on alternative carriers (Table 5) shows that indeed there were no significant differences in the development of moulds, yeasts and spore-forming bacteria. Regardless of the type of usar employed, all accompanying microflora will be part of the tempe inoculum, and therefore it is important to keep the level of undesirable genera (eg *Bacillus* spp) to a

minimum. The use of fermentatively acidified soya beans as applied by the usar makers was shown by Tüncel *et al* (1989) to contribute to the hygienic safety of the resulting starter.

The use of alternative usar did not affect the tempe quality significantly (Table 6). Consequently it appears unlikely that the *Hibiscus* leaves serve any function other than to present a firm and inert attachment surface and to maintain adequate relative humidity during the incubation of the leaf sandwiches. In addition, their low cost, renewability and biodegradability are of practical relevance.

ACKNOWLEDGEMENTS

Parts of this investigation were carried out in Indonesia in the framework of technical cooperation between the Brawijaya University at Malang and the Agricultural University at Wageningen. In this regard we should like to thank Ilse Marks, Saskia de Smidt and Géke Stoffels for their invaluable field-work contributions. We also thank Dr Josien Westphal-Stevels of the Herbarium Vadense, Wageningen, for her interest and help with the identification of *Hibiscus* spp, and Ing J Mohede of the Department of Tropical Agriculture, University of Wageningen, for growing our waru trees in the Wageningen tropical greenhouse.

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