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Food Chemical Analysis of Tempeh Prepared from South African-Grown Soybeans

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ABSTRACT

Tempeh was prepared from locally grown soybeans of the cultivars Hutton and Prima, using the mould strain Rhizopus oligosporus *NRRL 2549. The purpose of this food chemical study was to investigate the nutritionally beneficial changes which are brought about during fermentation, pending the probable introduction of the product to the South African market.*

Tempeh, fermented for 24, 48 and 72 h, together with unfermented controls and whole soybeans, were analysed for the proximate composition, phytic acid, specific carbohydrate materials including starch, mono- and oligosaccharides, minerals, thiamine, riboflavin and nicotinic acid; losses in dry mass occurring during fermentation were determined for tempeh made from Prima soybeans.

Processing of soybeans into tempeh brought about favourable nutritional changes including reduction in the levels of phytic acid, starch and the flatulence-causing oligosaccharides stachyose and raffinose; whereas thiamine concentrations were reduced, riboflavin and nicotinic acid contents increased during fermentation.

Brief comment is made on toxicological investigations concerning R. oligosporus.

Tempeh was found to be an acceptable product that could be employed to promote greater utilisation of soybeans for human consumption in this country.

INTRODUCTION

The Indonesian fermented soy food, tempeh, has been the subject of a number of nutritional and food chemical investigations since interest in this unique product was initiated by researchers in the West about three decades ago (Shurtleff & Aoyagi, 1985). These investigations have shown that the tempeh manufacturing process, involving a solid substrate fermentation of boiled, dehulled soybeans with a *Rhizopus* mould, while not improving protein utilisation (Hackler *et al.*, 1964; Smith *et al.*, 1964; Murata *et al.*, 1971), nonetheless brings about a generally favourable nutritional modification of soybeans, the most significant of which includes, besides improved palatability, reduced phytic acid and flatulence-causing oligosaccharide contents and increased levels of certain B group vitamins (Roelofsen & Talens, 1964; Murata *et al.*, 1967; Shallenberger *et al.*, 1967; Bai *et al.*, 1975; Sudarmadji & Markakis, 1977; Kao & Robinson, 1978; Zamora & Veum, 1979; Sutardi & Buckle, 1985*a*, *b*). Because of its favourable attributes, the prospects for the popularisation of this product appear to be good and there are indications that this is at present taking place in various parts of the world (Steinkraus, 1983; Shurtleff & Aoyagi, 1985).

Tempeh is virtually unknown in South Africa. It is currently made on a small scale by home producers and is commercially available only in the larger centres from certain 'health food' shops. As it is considered that the potential exists for greater utilisation of soybeans for human food in this country—the current estimate in this regard for the period 1985/86 is 8942 metric tons out of a total production of 38 040 metric tons (Oilseeds Board, 1986)—an investigation into tempeh prepared from two locally popular soybean cultivars was initiated.

The present investigation comprises a food chemical evaluation of soybeans, unfermented controls and tempeh fermented for varying lengths of time over a 72-h period.

MATERIALS AND METHODS

Soybeans

Food grade soybeans of the cultivars Hutton and Prima were obtained from commercial seed producers.

Preparation of tempeh and unfermented controls

Tempeh was prepared according to a standardised procedure, based on the method described by Shurtleff & Aoyagi (1979), as follows: 3 litres of tap

water containing 50 ml of vinegar was added to 1 kg of washed soybeans and brought just to the boil. After standing for 15 h the beans were dehulled by hand and resuspended in 2 litres of water containing 10 ml of lactic acid. After boiling for 45 min, the beans were surface-dried and inoculated with approximately 0·5 g of freeze-dried tempeh starter culture (Wang *et al.*, 1975) per 500 g soybeans.

Rhizopus oligosporus NRRL 2549, obtained from the NRRC, Peoria, Illinois, was used throughout this investigation. Soybeans of each cultivar were introduced in 500 g amounts into each of three 23 × 30 cm perforated, sealable plastic bags; the approximate 0·6 mm diameter perforations were 1·5 cm apart and were made by means of a specially constructed apparatus. The bags were flattened by means of a rolling pin and incubated at 31–35°C for either 24, 48 or 72 h. The resulting tempeh (approximately 1·5 kg per treatment) was freeze-dried. The unfermented controls were prepared from 1 kg of soybeans of each cultivar, processed as described above, but freeze-dried prior to the inoculation step.

Sample preparation

All sample material was pulverised in a pin mill, mixed for 30 min in a sample splitter, transferred to glass bottles and stored at 5°C until analysed.

Mass loss during tempeh fermentation

Using Prima soybeans, loss in mass during tempeh fermentation was monitored by determining the dry weights of 500 g amounts of unfermented controls or tempeh fermented for 24, 48 and 72 h; moisture contents were determined after freeze-drying the samples, and the absolute dry masses calculated. Soybean dry mass was plotted against fermentation time and a straight line was fitted to the experimental points by means of linear least squares regression.

Analytical methods

Protein (Kjeldahl nitrogen × 5·71; Paul & Southgate, 1978), ash (AOAC, 1975) and oil (AACC, 1983) were determined according to standard procedures. Moisture contents were determined by drying duplicate *ca.* 2 g sample aliquots at 70°C under reduced pressure; average values were calculated, and the results of the food chemical analyses are expressed on a moisture-free, full fat basis. Minerals (Ca, Mg, Fe, Na, K, Cu, Zn and Mn) were determined by atomic absorption spectrophotometry. Phosphorus was determined colorimetrically (Boltz & Mellon, 1947). Insoluble and soluble

dietary fibre were determined on defatted material according to the method of Asp *et al.* (1983); total dietary fibre was calculated as the sum of the two values obtained.

Starch was determined according to the method of Batey (1982), with minor modification; free sugars and oligosaccharides were extracted from defatted material (*ca.* 5 g) with 80% ethanol for 1 h at 80°C. After centrifugation the residue was transferred quantitatively to a conical flask and 50 ml water and 1 ml Termamyl 120L (Novo) were added. After boiling for 30 min the sample volume was treated with amyloglucosidase (Boehringer Mannheim Cat. No. 102849) and starch was determined as glucose by the hexokinase method as described by Batey (1982).

Oligosaccharides were determined on defatted material by HPLC using a reversed phase column packed with LiChrosorb RP-18 (5 μm, Merck) and deionised distilled water as eluent (Wight & Datel, 1986). Phytic acid was determined by HPLC (Cilliers & van Niekerk, 1986).

Thiamine was determined according to the AOAC (1975) fluorimetric method with minor modification; 0·2N H_2SO_4 was used for the extraction. The extract was treated with Taka-diastase and the thiamine was adsorbed on Decalso. After elution from the Decalso, the thiamine was converted to thiochrome which was determined fluorimetrically. Riboflavin was extracted using the same acid extraction and enzyme-hydrolysis procedure as used for thiamine, and determined by HPLC as described by van Niekerk (1982) with minor modification; a reversed phase column (10 × 0·46 cm) packed with Nucleosil 3 C_{18} (Macherey-Nagel) was used for chromatography. The mobile phase was 1% acetic acid:methanol 70:30 v/v and the flow rate was 0·5 ml/min. Detection was by fluorescence (excitation 449 nm, emission 520 nm). Nicotinic acid was determined microbiologically (Association of Vitamin Chemists, Inc., 1951).

RESULTS AND DISCUSSION

The results of the food chemical analyses of soybeans, unfermented controls and tempeh are presented in Tables 1 and 2. Mass losses occurring during tempeh fermentation are presented in Fig. 1.

Differences between constituent concentrations of whole beans and unfermented controls are due to processing (i.e. dehulling, boiling in acidified water), while differences between unfermented controls and tempeh are attributable to the effects of fermentation.

Processing of soybeans led to the concentration of protein and oil contents, the decrease in total fibre contents, the loss of a large amount of

TABLE 1

Food Chemical Analysis of Unfermented Controls and Tempeh Fermented for Varying Lengths of Time, as well as Soybeans of Two Cultivars used in their Preparation

Cultivar and sample designation	*Protein*[a]	*Oil*	*Carbohydrates*[b]	*Phytic acid*	*Insoluble fibre*	*Soluble fibre*	*Total fibre*	*Ash*	*Ca*	*Mg*	*Fe*	*Na*	*K*	*Cu*	*Zn*	*Mn*	*P*	*Thiamine*	*Riboflavin*	*Nicotinic acid*
	(g/100 g)[c]								*(mg/100 g)*[c]											
Cultivar Hutton																				
Soybeans	40·1	21·0	14·7	1·6	16·4	5·3	21·7	5·3	275	282	11·7	9·3	1 739	1·7	4·5	4·1	635	0·54	0·06	2·65
Unfermented control	48·3	28·6	2·0	1·0	13·7	4·0	17·7	3·0	296	168	8·0	16·1	393	1·6	4·5	3·7	609	0·14	0·03	0·49
Fermented 24 h	48·7	27·9	1·9	0·6	11·6	6·7	18·3	2·8	310	193	8·9	14·6	222	1·8	5·4	4·1	731	ND[d]	0·16	1·91
Fermented 48 h	48·6	26·3	1·9	0·4	12·9	3·2	16·1	2·7	333	193	9·0	14·1	224	1·9	5·5	4·1	731	ND[d]	0·24	2·82
Fermented 72 h	49·3	23·8	1·7	0·2	12·7	3·0	15·7	3·0	318	193	8·8	15·1	218	1·8	5·4	4·2	734	ND[d]	0·37	4·50
Cultivar Prima																				
Soybeans	39·5	20·4	17·2	1·7	15·5	5·6	21·1	5·4	187	247	11·6	3·5	1 693	1·8	6·0	2·8	830	0·45	0·06	2·90
Unfermented control	48·3	25·7	2·8	1·2	14·3	5·0	19·3	3·0	192	172	7·2	13·8	525	1·9	6·2	3·3	627	0·21	0·06	0·58
Fermented 24 h	49·7	25·2	2·4	0·3	11·7	8·5	20·2	2·7	225	221	8·7	14·2	224	1·9	7·1	3·5	688	ND[d]	0·25	3·15
Fermented 48 h	49·7	25·2	2·2	0·2	11·1	8·2	19·3	2·7	232	195	9·0	14·1	220	1·8	7·2	3·5	742	ND[d]	0·22	2·97
Fermented 72 h	49·3	22·9	2·3	0·1	11·4	3·9	15·3	3·0	248	201	8·7	13·8	219	1·9	7·4	3·6	795	ND[d]	0·38	5·45

[a] Kjeldahl nitrogen × 5·71.

[b] Total starch, mono- and oligosaccharides—see Table 2.

[c] All values are given on a moisture-free, full fat basis.

[d] ND = Not detected.

TABLE 2

Carbohydrate Contents (g/100 g[a]) of Two Cultivars of Soybeans, Unfermented Controls and Tempeh Fermented for Varying Lengths of Time

Soybean cultivar	*Sample analysed*	*Monosaccharides*	*Oligosaccharides*			*Starch*
		Unspecified	*Stachyose*	*Raffinose*	*Sucrose*	
Prima	Soybeans	3·0	5·1	1·6	7·2	0·3
	Unfermented control	0·4	0·7	0·2	1·1	0·4
	Fermented 24 h	0·7	0·5	0·2	0·8	0·2
	Fermented 48 h	0·7	0·2	0·4	0·7	0·2
	Fermented 72 h	1·2	<0·1	0·4	0·6	0·1
Hutton	Soybeans	2·3	4·9	1·7	5·6	0·2
	Unfermented control	0·3	0·6	0·2	0·7	0·2
	Fermented 24 h	0·4	0·5	0·2	0·6	0·2
	Fermented 48 h	0·6	0·2	0·4	0·6	0·1
	Fermented 72 h	0·7	<0·1	0·4	0·5	0·1

[a] All values are given on a moisture-free, full fat basis.

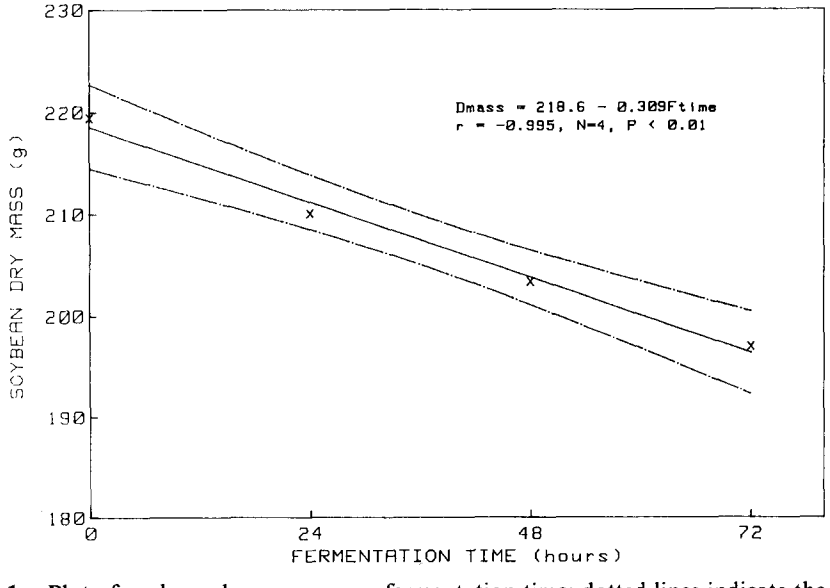

Fig. 1. Plot of soybean dry mass versus fermentation time; dotted lines indicate the 95% confidence interval.

soluble carbohydrate material, and the loss of significant amounts of phytic acid, B group vitamins and potassium.

During fermentation protein contents increased marginally while oil contents decreased significantly in tempeh prepared from both soybean cultivars. Small changes in fibre content were detected during fermentation and a net loss in total fibre occurred over the 72-h fermentation period. A total dry mass loss of approximately 10% occurred in tempeh fermented for 72 h, due to the metabolic activities of the mould (Fig. 1). While this is not obviously reflected by increases in the dry masses of the proximate constituents (similarly observed by Zamora & Veum (1979) who reported a 3% mass loss during tempeh fermentation), which undergo complex changes during fermentation due to release by the mould of, for example, protease, lipase, amylase and pectinase enzymes (Hesseltine *et al.*, 1963; Steinkraus, 1983), the mineral concentrations, with few exceptions, tended to increase with fermentation time. The large decreases observed in the potassium concentrations are due to leakage of this element during fermentation; significant levels of potassium were found in the 'sweat' water which forms inside the plastic bags.

Whereas thiamine concentrations were reduced to an undetectable level, both riboflavin and nicotinic acid concentrations increased significantly over the 72-h fermentation period as a result of synthesis by the mould. The findings of earlier workers (Roelofsen & Talens, 1964; Murata *et al.*, 1967; Kao & Robinson, 1978), who used different *Rhizopus* strains, are therefore confirmed, but using *R. oligosporus* NRRL 2549 as test organism.

Processing of soybeans resulted in a much greater loss of soluble carbohydrate material than did fermentation (Table 2). Slight upward shifts in the concentrations of unspecified monosaccharides and raffinose during fermentation can be explained in terms of initial attack by α-galactosidase on stachyose, which was almost completely eliminated by fermentation, and the chemical interrelatedness between it and the aforementioned sugars (Shallenberger *et al.*, 1967; Liener, 1981); thus monosaccharides and raffinose, products of the above enzyme reaction, tended to increase with fermentation time; sucrose tended to decrease and starch concentrations were also reduced by fermentation. Hesseltine *et al.* (1963) found that *R. oligosporus* NRRL 2549 did not utilise raffinose or sucrose as the sole carbon source in a synthetic medium; amylase activity was, however, reported to occur in this strain.

During development work on the methodology for determining oligosaccharides in soybeans and tempeh for the present investigation, Wight & Datel (1986) detected appreciable amounts of an unidentified carbohydrate component in tempeh fermented for 48 h; this was probably a mixture of disaccharides, having a column retention time intermediate

between melibiose and trehalose. The exact identity of this compound, or mixture of compounds, has not been elucidated. Shallenberger *et al.* (1967) reported that appreciable amounts of melibiose appeared during tempeh fermentation.

The effect of *Rhizopus* fermentation on soybean starch does not appear to have been previously investigated; despite the small amounts of starch present in soybeans (Wilson *et al.*, 1978) confirmed in the present investigation, it has nonetheless been suggested that protein-bound starch is nutritionally significant in that it may be responsible for poor digestibility of certain soy proteins (Boonvisut & Whitaker, 1976; Wilson *et al.*, 1978). The observed reduction in starch levels by fermentation may, therefore, have nutritional significance and deserves further investigation.

Although phytic acid concentration was reduced during processing, a greater proportion was eliminated during fermentation over a 72-h period (Table 1). The enzyme phytase is known to occur in *Rhizopus* sp. used for tempeh fermentations, and previous studies have shown that phytic acid levels are significantly reduced during tempeh-making (Sudarmadji & Markakis, 1977; Sutardi & Buckle, 1985 *a*, *b*; Wang *et al.*, 1980).

A recent survey of toxigenicity in *Rhizopus* sp. found a single strain of *R. oligosporus* used in tempeh-making (NRRL 2710) to be non-toxic, according to definition (q.v.), using ducklings as the test animal and maize as the growth substrate (Rabie *et al.*, 1985); growth substrate was found to influence *Rhizopus* toxigenicity, with certain isolates producing toxins on a substrate of maize losing all toxicity when grown on soybeans. The strain used in the present investigation, *R. oligosporus* NRRL 2549, was similarly tested (C. J. Rabie, pers. comm.) and found to be non-toxic (killing nil out of four ducklings) using maize as the growth substrate.

Although requiring more detailed investigation (Rabie *et al.*, 1985) it would appear, therefore, that *Rhizopus* mycotoxins are unlikely to be produced during the making of soybean tempeh.

CONCLUSIONS

This food chemical investigation of tempeh serves largely to substantiate the findings of earlier studies concerning the nutritionally beneficial changes which are brought about during *Rhizopus* fermentation of soybeans. Maximum nutritional advantage, i.e. the lowest levels of phytic acid and flatulence-causing oligosaccharides but the highest levels of riboflavin and nicotinic acid, is gained in tempeh fermented for longer periods (48–72 h).

Tempeh was found to be both aesthetically and organoleptically acceptable, and could play a role in promoting greater utilisation of soybeans for human food in South Africa.

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REFERENCES

AACC, Approved Methods of the American Association of Cereal Chemists (1983). Method 30-25; St. Paul, Minnesota, USA.

AOAC (1975). *Official methods of analysis.* (12th edn), Association of Official Analytical Chemists, Washington, DC.

Asp, N.-G., Johansson, C.-G., Halmer, H. and Siljeström, M. (1983). Rapid enzymatic assay of insoluble and soluble dietary fibre. *J. Agric. Food Chem.*, **31**, 476–82.

Association of Vitamin Chemists, Inc. (1951). *Methods of vitamin assay*, Interscience Publishers, Inc., New York, USA, 177.

Bai, R. G., Prabha, T. N., Rao, T. N. R. & Sreedhara, N. (1975). Studies on tempeh: Part 1. Processing and nutritional evaluation of tempeh from a mixture of soybean and groundnut. *J. Food Sci. & Technol.*, **12**, 135–8.

Batey, I. L. (1982). Starch analysis using thermostable alpha amylases. *Starch/Stärke*, **34**, 125–8.

Boltz, D. F. & Mellon, M. G. (1947). Determination of phosphorus, germanium, silicon and arsenic by the heteropoly blue method. *Anal. Chem.*, **19**, 873–7.

Boonvisut, S. & Whitaker, J. R. (1976). Effect of heat, amylase and disulphide bond cleavage on the *in vitro* digestibility of soybean proteins. *J. Agric. Food Chem.*, **24**(6), 1130–5.

Cilliers, J. J. L. & van Niekerk, P. J. (1986). LC determination of phytic acid in food by postcolumn colorimetric detection. *J. Agric. Food Chem.*, **34**(4), 680–3.

Hackler, L. R., Steinkraus, K. H., van Buren, J. P. & Hand, D. B. (1964). Studies on the utilization of tempeh protein by weanling rats. *J. Nutr.*, **82**, 452–6.

Hesseltine, C. W., Smith, M., Bradle, B. & Djien, K. S. (1963). Investigations of tempeh, an Indonesian food. *Dev. Ind. Microbiol.*, **4**, 275–87.

Kao, C. & Robinson, R. J. (1978). Nutritional aspects of fermented foods from chickpea, horsebean and soybean. *Cereal Chem.*, **55**(4), 512–17.

Liener, I. E. (1981). Factors affecting the nutritional quality of soya products. *J. Amer. Oil Chem. Soc.*, **58**, 406–15.

Murata, K., Ikehata, H. & Miyamoto, T. (1967). Studies on the nutritional value of tempeh. *J. Food Sci.*, **32**, 580–5.

Murata, K., Ikehata, H., Edani, Y. & Konyanagi, K. (1971). Studies on the nutritional value of tempeh. Part II. Rat feeding test with tempeh, unfermented soybeans and tempeh supplemented with amino acids. *Agr. Biol. Chem.*, **35**(2), 233–4.

Oilseeds Board (1986). 34th Annual Report (1985–86 season). PO Box 211, Pretoria 0001, Republic of South Africa.

Paul, A. A. & Southgate, D. A. T. (1978). *The composition of foods.* HMSO, London, England.

Rabie, C. J., Lübben, A., Schipper, M. A. A., van Heerden, F. R. & Fincham, J. E. (1985). Toxigenicity of *Rhizopus* species. *Int. J. Food Microbiol.*, **1**, 263–70.

Roelofsen, P. A. & Talens, A. (1964). Changes in some B vitamins during molding of soybeans by *Rhizopus oryzae* in the production of tempeh kedelee. *J. Food Sci.*, **29**, 224–6.

Shallenberger, R. S., Hand, D. B. & Steinkraus, K. H. (1967). Changes in sucrose, raffinose and stachyose during tempeh fermentation. *Proceedings of the 8th Dry Bean Research Conference, Bellaire, Michigan, USA*, 68–71.

Shurtleff, W. & Aoyagi, A. (1979). *The book of tempeh.* Harper & Row, New York, USA.

Shurtleff, W. & Aoyagi, A. (1985). *History of tempeh, a fermented soyfood from Indonesia.* The Soyfoods Center, Lafayette, California, USA.

Smith, A. K., Rackis, J. J., Hesseltine, C. W., Smith, M., Robbins, D. J. & Booth, A. N. (1964). Tempeh: Nutritive value in relation to processing. *Cereal Chem.*, **41**, 173–81.

Steinkraus, K. H. (Ed.) (1983). *Handbook of indigenous fermented foods.* Marcel Dekker, Inc., New York, USA.

Sudarmadji, S. & Markakis, P. (1977). The phytate and phytase of soybean tempeh. *J. Sci. Food Agric.*, **28**, 381–3.

Sutardi & Buckle, K. A. (1985*a*). Phytic acid changes in soybeans fermented by traditional inoculum and six strains of *Rhizopus oligosporus*. *J. Appl. Bact.*, **58**, 539–43.

Sutardi & Buckle, K. A. (1985*b*). Reduction in phytic acid levels in soybeans during tempeh production, storage and frying. *J. Food Sci.*, **50**, 260–3.

van Niekerk, P. J. (1982). In: *HPLC in food analysis.* (Macrae, R. (Ed)), Academic Press, New York, USA, 210.

Wang, H. L., Swain, E. W. & Hesseltine, C. W. (1975). Mass production of *Rhizopus oligosporus* spores and their application in tempeh fermentation. *J. Food Sci.*, **40**, 168–70.

Wang, H. L., Swain, E. W. & Hesseltine, C. W. (1980). Phytase of molds used in oriental food fermentation. *J. Food Sci.*, **45**, 1262–6.

Wight, A. W. & Datel, J. M. (1986). Evaluation of a reversed phase high performance liquid chromatographic column for estimation of legume seed oligosaccharides. *Food Chem.*, **21**, 167–81.

Wilson, I. A., Birmingham, V. A., Moon, D. P. & Snyder, H. E. (1978). Isolation and characterization of starch from mature soybeans. *Cereal Chem.*, **55**, 661–70.

Zamora, R. G. & Veum, T. L. (1979). The nutritive value of dehulled soybeans fermented with *Aspergillus oryzae* or *Rhizopus oligosporus* as evaluated by rats. *J. Nutr.*, **109**, 1333–9.