PICKLED VEGETABLES

1. Exposure Data

1.1 Production and consumption

1.1.1 *Introduction*

Pickling, broadly defined, is the use of brine, vinegar or a spicy solution to preserve and give a unique flavour to a food adaptable to the process. Numerous vegetables and fruits can be pickled not only to preserve them but also to modify their flavour. The categories of pickled products are many, the most common being those of cucumbers and other vegetables; fruits; nuts; relishes of all kinds; cured meats, fish and poultry; and such special products as pickled mushrooms and pickled cherries (Peterson, 1977).

The pickling processes of particular interest in this monograph are the traditional methods used in some parts of China and Japan where elevated risks for oesophageal and gastric cancer have been observed. For example, an unusual variety of pickled vegetables is made in Linxian, China, by fermenting turnips, sweet potato leaves and other vegetables in water without salt or vinegar (Li *et al.*, 1989). Several special processes, with and without salt, are used in preparing certain types of Japanese and Korean vegetable products (Shin, 1978; Itabashi, 1983; Uda *et al.*, 1984; Itabashi, 1985; Itabashi & Takamura, 1985).

1.1.2 *Production/preparation*

Traditionally, pickled vegetables are popular in some areas of China where there are high incidence rates of oesophageal cancer. Among the vegetables commonly prepared in this way are Chinese cabbage, turnip, soya bean, sweet potato, sesame (Yang, 1980), potherb mustard (Zhang *et al.*, 1983) and others. They are prepared each autumn by chopping, washing and dipping briefly in boiling water the roots, stems and/or leaves, as appropriate, and cooling and packing the vegetables tightly in a large earthenware (ceramic) jar. The vegetables are covered with water, a heavy stone is placed on them and they are allowed to ferment for several weeks or months (Yang, 1980).

The leaves of *takana* (*Brassica juncea* L.), a popular cruciferous vegetable in Japan, are mainly processed by salting. The salted products are divided into two types. One is called *shinzuke-takana*, processed to contain 3 or 4% (w/w) salt in the final product. It has a pungent flavour owing to the presence of isothiocyanates, which are formed enzymatically from the corresponding glucosinolates during the salting process. In recent years, the product has been stored under refrigeration or frozen to retain the pungent flavour and its green appearance. The other product, *furuzuke-takana*, contains about 10% (w/w) salt. It is stored

for over six months after the salting process, and during this time the salted materials undergo changes in volatile constituents and pigments; the final product has a characteristic flavour and amber colour. Other popular pickled products in Japan are *nozawana-zuke* and *hiroshimana-zuke*, produced by salting the fresh leaves of *nozawana* (*Brassica campestris* L. var. *rapa*) and *hiroshimana* (*B. campestris* L. var. *pekinensis*) to achieve a salt concentration of 3–4% in the final products, similar to *shinzuke-takana*. The products are fermented at 3–12 °C for five days (Uda *et al.*, 1988).

Sunki, a pickle produced in Kiso district, Nagano Prefecture, from the leaves of green vegetables, is prepared without salt owing to lactic acid fermentation by lactobacilli contained in the 'pickling seeds'. The pickling seeds generally used are dried *sunki* pickles produced in the previous year which contain several species of lactobacillus. The pickles are subsequently dried and preserved throughout the year. Some farmers make *sunki* from cooked leaves of *otaki* turnip, not only by adding dried *sunki* as the source of lactobacillus but also by adding wild fruits or berries (Itabashi, 1983, 1985; Itabashi & Takamura, 1985).

Kimchi, spiced, lactic acid-fermented vegetables, are one of the commonest traditional side dishes in the daily meals of Koreans. They are prepared by salting Chinese cabbages and radishes, washing the salted vegetables in fresh water, adding spices and seasonings and then leaving the spiced vegetables to undergo a process of natural lactic acid fermentation. The amount of salt used in the preparation of *kimchi* corresponds to about 10% of the weight of the fresh vegetables, and salting time usually ranges between 8 and 15 h before washing. The seasoning mixture used includes cayenne pepper, garlic, ginger and pickled seafood. Many kinds of microorganisms are involved in *kimchi* fermentation, the principal ones being *Lactobacillus plantarum* and *L. brevis*. *Kimchi* is usually prepared in the home; however, it is also now produced and distributed on a commercial basis in Japan and the USA (Shin, 1978).

In the USA and northern Europe, the manufacture of cucumber pickles consists of a cure in a 10% salt solution, during which fermentation by halophilic (salt-tolerant) bacteria takes place. The curing process takes from 28 to 42 days, and the salt prevents growth of spoilage organisms. When the curing process is completed, the product is placed in a more concentrated salt solution and stored until final processing, which includes immersion in water to remove the salt, the addition of vinegar and a final bath in water that contains calcium chloride, a firming agent and turmeric, a colour enhancer. To make sweet pickles, a spiced sweet vinegar is added to the final soak. For dill pickles, the dill plant or its seeds are used as flavouring. The shelf-life of cucumber pickles is dependent upon the presence of preservatives and, when used, on pasteurization. Vinegar is the most commonly used preservative. Pickled vegetables are packed, for the most part, in sealed glass jars, usually under vacuum (Peterson, 1977).

1.1.3 *Consumption*

In China, pickled vegetables and juice are eaten either as such or cooked in a gruel. During the summer, some of the juice is also consumed as a drink. In some families, pickled vegetables are eaten daily for as many as 9–12 months in a year and are an important part of the diet (Yang, 1980).

In a survey in Japan in 1962–63, consumption of pickled vegetables more than 60 times a month was reported by 60 and 81% of the adult farm populations and 43 and 70% of the

adult non-farm populations of Hiroshima and Miyagi prefectures, respectively (Haenszel *et al.*, 1976). In a later survey (Yan, 1989), the dietary habits of Chinese residing in Japan were compared with those of native Japanese. Consumption of pickled vegetables three or more times a week was reported by 35% of 346 Chinese men and by 41% of 288 Chinese women interviewed and by 75% of 8071 Japanese men and 78% of 9932 Japanese women. The average daily per-caput ingestion of various salt-fermented vegetables in Japan was estimated to be about 37 g (Kawabata *et al.*, 1980).

Per-caput consumption of *kimchi* by Koreans is 200–300 g daily (Shin, 1978).

1.2 Chemical composition

1.2.1 *General aspects*

Volatile constituents of the two Japanese pickled products, *nozawana-zuke* and *hiroshimana-zuke*, were studied by gas chromatography with and without mass spectrometry after fractionation into basic, acidic, phenolic and neutral fractions. A total of 57 constituents were identified in the latter three fractions (the basic had almost no odour), consisting of three carbonyls, eight esters, two sulfides, four alcohols, seven phenols, seven nitriles, eight isothiocyanates, 17 hydrocarbons and one acid. Little difference was observed between the two pickled products in the kinds of volatile constituents present; most were found in the neutral fractions where the degradation products of glucosinolates, namely isothiocyanates and nitriles, were the major components, together with some methyl esters of lower-molecular-weight fatty acids (C_{10}–C_{14}) (Uda *et al.*, 1988).

Uda *et al.* (1984) investigated changes in the relative amounts of volatile compounds in *takana-zuke* five days, three months and six months after salting and after storage at 2–3 °C for three to six months. (Relative amounts were the percentages represented by the peak area of each component out of the total peak area of all the components detected in the acidic, phenolic and neutral fractions, which contained 11 acids, nine phenols, eight esters, four alcohols, two carbonyls, 12 hydrocarbons, two sulfides, four nitriles and eight isothiocyanates.) The relative amounts of isothiocyanates decreased from 83% at five days to 16% following six months' storage. The relative amounts of alcohols, acids, phenols, nitriles and hydrocarbons were increased after three or six months of cold storage.

The main organic acids produced by *kimchi* fermentation are the nonvolatile compounds, lactic acid and succinic acid. They are produced in greater amounts when *kimchi* is fermented for a long period at low temperatures (6–7 °C) than when it is fermented for a short period at high temperatures (22–23 °C). Oxalic, malic, tartaric, fumaric, malonic, maleic and glycolic acids are produced in smaller quantities at low temperatures. Volatile compounds contained in aged *kimchi* are formic acid and acetic acid. Carbon dioxide generated in the process of fermentation gives *kimchi* a tart taste. More acetic acid and carbon dioxide are contained in *kimchi* with a low salt content (~1%) than in that with a high salt content (~3%), and also in *kimchi* fermented at low temperatures (4–5 °C) than in that fermented at high temperatures (20–25 °C) (Shin, 1978).

1.2.2 *Compounds present in pickled vegetables*

(*a*) *Nitrosamines*

Samples of Chinese pickled vegetables fermented in brine were found to contain *N*-nitrosodimethylamine (NDMA) at < 0.1–15 μg/kg (Poirier *et al.*, 1987; Song & Hu, 1988) and *N*-nitrosopyrrolidine at < 0.5–96 μg/kg (Poirier *et al.*, 1987), < 0.1–25.5 ppb (μg/kg) (Song & Hu, 1988) and 62 μg/kg (Poirier *et al.*, 1989). *N*-Nitrosopiperidine was found in one study at < 0.5–14 μg/kg (Poirier *et al.*, 1987) but not in two others (Song & Hu, 1988; Poirier *et al.*, 1989); *N*-nitrosodiethylamine was found in one study at < 0.1–1.1 ppb (μg/kg) (Song & Hu, 1988).

Two of 49 samples of Japanese salt-fermented vegetables contained NDMA, at levels of 1.4–1.5 μg/kg; six contained *N*-nitrosopyrrolidine, at levels of 1.2–32 μg/kg. The authors calculated that the average daily intake of volatile nitrosamines *per caput* from salt-fermented vegetables was 0.002 μg NDMA and 0.04 μg *N*-nitrosopyrrolidine (Kawabata *et al.*, 1980). Kawabata *et al.* (1984) surveyed the occurrence of total *N*-nitroso compounds, total *N*-nitrosamides, volatile *N*-nitrosamines, nitrates and nitrites in the Japanese diet (Table 1). Relatively high levels of total *N*-nitroso compounds were detected in salt-fermented vegetables, the highest being found in *hakusai-zuke* (Chinese cabbage) and in *takuan* (radish roots); the levels of total *N*-nitrosamides were similar. Only trace quantities of volatile *N*-nitrosamines were detected in these vegetable products.

Table 1. Occurrence of total *N*-nitroso compounds (TNC), total *N*-nitrosamides (TNAd), volatile *N*-nitrosamines (VNA), nitrite and nitrate in salt-fermented vegetables in the Japanese diet

Product	TNC (μg NO/kg)	TNAd (μg NO/kg)	VNA (μg/kg)		Nitrite (mg/kg)	Nitrate (mg/kg)
			NDMA	NPYR		
Radish root (*takuan*) (1)	85.0	92.6	–	–	–	–
Radish root (*takuan*) (2)	252.6	266.2	ND	ND	3.43	126.2
Radish root (*takuan ume-zuke*)	110.0	102.2	Trace	0.5	1.73	264.3
Radish root (*bettara-zuke*)	118.1	128.6	Trace	ND	1.03	15.35
Oriental melon dipped in *sake* lees (*nara-zuke*)	5.52	–	Trace	ND	1.96	29.35
Chinese cabbage (*hakusai-zuke*)	2466.8	2325.4	ND	ND	31.0	258.5
Turnip leaves (*nozawana*)	100.0	107.4	ND	Trace	12.9	283.7
Broad-leaved mustard (*takana*)	57.1	49.9	ND	ND	6.47	221.4
Pot herb mustard (*kyona*)	98.0	101.8	ND	ND	14.3	165.4
Mixed fermented vegetables seasoned with soya sauce (*fukujin-zuke*)	7.74	–	ND	ND	2.59	3.70

From Kawabata *et al.* (1984). Limits of detection: TNC and TNAd, 0.5 μg NO/kg; VNA, 0.1 μg/kg; nitrate and nitrite, 0.05 mg/kg. ND, not detected; Trace, 0.1–0.5 μg/kg; –, not analysed

Samples of foods consumed frequently in Kashmir, India, a high-risk area for oesophageal cancer, were analysed for the presence of volatile *N*-nitrosamines. In eight samples of mixed pickled vegetables, NDMA levels ranged from not detected to 6.1 μg/kg; *N*-nitrosopiperidine and *N*-nitrosopyrrolidine were not detected (Siddiqi *et al.*, 1991).

In Tunisia, turnips fermented in brine were found to contain NDMA at 3 μg/kg in one study (Poirier *et al.*, 1987), but none was found in a later study (Poirier *et al.*, 1989) in which *N*-nitrosopyrrolidine was found at 31 μg/kg.

(*b*) *Roussin red methyl ester*

In a study of the etiological factors of oesophageal cancer in Linxian County, North China, a pure compound was isolated from several kinds of pickled vegetables and identified as Roussin red methyl ester (RRME; bis[μ-(methanethiolato)]tetranitrosodiiron; see Table 2). RRME was first synthesized in 1858 but had not previously been reported in Nature. It has been shown to react readily with secondary amines to form nitrosamines both *in vitro* and *in vivo* (Wang *et al.*, 1980; Zhang *et al.*, 1983). Pickled vegetables from Linxian County contained 0.1–4.5 mg/kg RRME and pickles from Beijing contained less than 0.005 mg/kg (Zhang *et al.*, 1983). The compound was detected in 55% of 69 samples of pickles from Linxian (Li *et al.*, 1986).

(*c*) *Flavonoids*

Flavonol aglycones were not detected in pickled vegetables prepared by industrial methods in Italy, but flavonol glycosides were found at 31.9 mg/kg in pickled peppers, 1.2 mg/kg in cauliflower and 7.8 mg/kg in carrots. The vegetables had been pickled in a boiling water solution containing 7% acetic acid and 3% sodium chloride (Fieschi *et al.*, 1989).

Quercetin (see IARC, 1983a) and rhamnetin (see Table 2) were identified as the principal mutagenic substances in samples of Japanese pickle. The most mutagenic samples of carrots and radishes, from Akita Prefecture, Japan, contained quercetin at 6.60 mg/g of crude extract and rhamnetin at 1.96 mg/g (Takenaka *et al.*, 1989). Mutagenic substances in Japanese pickled vegetables purchased in Tokyo were isolated and identified as the flavonoids kaempferol (see IARC, 1983b) and isorhamnetin (see Table 2) (Takahashi *et al.*, 1979). The quantities of quercetin and kaempferol found in *takana* increased with increasing duration of pickling. The flavonols contained in the fresh vegetables as glycosides were found to be freed by hydrolysis during pickling (Mizuta & Kanamori, 1983).

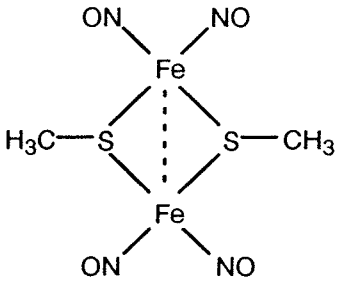
(*d*) *Phorbides*

The photosensitizers pheophorbide a and pyropheophorbide a, decomposition products of chlorophyll, were found in samples of salted green vegetables; pheophorbide a was also detected in fresh vegetables, but pyropheophorbide a was not. In salted vegetables stored for more than three months, almost all the chlorophyll had been converted to pyropheophorbide a. The concentrations of pheophorbide a and pyropheophorbide a determined in Japanese vegetables are shown in Table 3 (Takeda *et al.*, 1985).

Table 2. Nomenclature and formulae of compounds found in pickled vegetables

Roussin red methyl ester

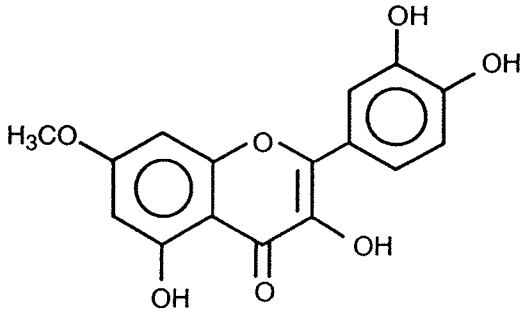
Chem. Abstr. Serv. Reg. No.: 16071-96-8
Deleted CAS Reg. Nos.: 15696-35-2, 79408-10-9, 110658-92-9
Chem. Abstr. Name: Bis[μ-(methanethiolato)]tetranitrosodiiron
Synonyms: Bis(methanethiolato)tetranitrosodiiron; Roussin's red methyl ester



$C_2H_6Fe_2N_4O_4S_2$ Mol. wt: 325.92

Rhamnetin

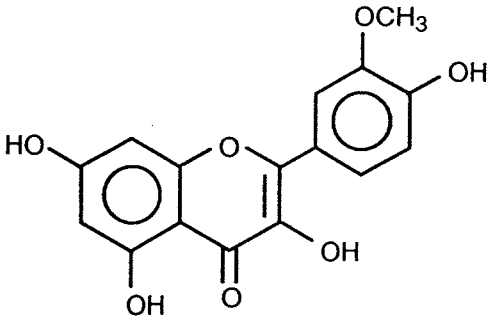
Chem. Abstr. Serv. Reg. No.: 90-19-7
Chem. Abstr. Name: 2-(3,4-Dihydroxyphenyl)-3,5-dihydroxy-7-methoxy-4*H*-1-benzopyran-4-one
Synonyms: C.I. 75690; 7-methoxyquercetin; 7-methylquercetin; 7-*O*-methylquercetin; quercetin 7-methyl ether; β-rhamnocitrin; 3,3′,4′,5-tetrahydroxy-7-methoxyflavone; 3,5,3′4′-tetrahydroxy-7-methoxyflavone



$C_{16}H_{12}O_7$ Mol. wt: 316.27

Isorhamnetin

Chem. Abstr. Serv. Reg. No.: 480-19-3
Chem. Abstr. Name: 3,5,7-Trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one
Synonyms: C.I. 75680; isorhamnetol; 3′-methoxyquercetin; 3′-methylquercetin; 3′-*O*-methylquercetin; quercetin 3′-methyl ether; 3,4′,5,7-tetrahydroxy-3′-methoxyflavone



$C_{16}H_{12}O_7$ Mol. wt: 316.27

Table 3. Mean concentrations (μg/g) of pheophorbide a and pyropheophorbide a found in fresh and salted vegetables from a Tokyo market

Sample	No. of samples	Storage (days)	Plant part	Pheophorbide a	Pyropheophorbide a
Takana[a] (fresh)	3	0	Leaf	22.5	ND
			Stem	5.9	ND
			Whole	18.4	ND
Takana[a]*-zuke*	3	20	Leaf	51.4	182.0
			Stem	5.3	2.8
			Whole	26.2	82.7
Takana[a]*-zuke*	4	90	Leaf	ND	534.8
			Stem	5.5	28.3
			Whole	2.0	235.3
Nozawana[b]*-zuke*	5	7	Leaf	129.3	235.1
			Stem	2.6	1.6
			Whole	53.3	95.0
Hiroshimana[c]*-zuke*	5	7	Leaf	140.4	165.5
			Stem	4.1	3.3
			Whole	95.0	111.4

From Takeda *et al.* (1985); ND, not detected
[a]*Brassica juncea* var. *integrifolia*
[b]*Brassica* spp.
[c]*B. pekinensis*

(*e*) *Isothiocyanates*

The steam-volatile isothiocyanates that occur in raw and salted Japanese cruciferous vegetables (*hiroshimana-zuke* (leaves), *nozawana-zuke* (leaves), *hinonakabu-zuke* (roots), *takana-zuke* (leaves), *takanafuru-zuke* (leaves), *zasai-zuke* (stalks)) were investigated by gas chromatography–mass spectrometry. The vegetables, which had undergone autolysis during the process of salting at their unadjusted pH, were subjected to steam distillation. A relatively large percentage of total isothiocyanates was observed in the steam-volatile fractions obtained from the salted vegetables, except from *zasai-zuke*. The isothiocyanates identified included: pentyl, 2-propenyl, 3-butenyl, 4-pentenyl, 2-phenethyl, 4-methylthiobutyl and 5-methylthiopentyl isothiocyanate (Maeda *et al.*, 1979).

(*f*) *Fungi*

Chinese pickled vegetables are contaminated by fungi. Among 24 samples analysed in one study, 20 contained *Geotrichum candidum*, some samples contained *Mucor* spp. and yeasts, and a few samples contained *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. nidulans* and *Fusarium* spp. (see monographs in this volume) (Yang, 1980).

1.3 Analysis

Selected methods for the analysis of *N*-nitroso compounds in various matrices have been reviewed (Walker *et al.*, 1978, 1980; Bartsch *et al.*, 1982; Preussmann *et al.*, 1983; O'Neill *et al.*, 1984).

A method for the quantitative analysis of RRME in pickled vegetables by gas chromatography–mass spectrometry has been described. The detection limit was 5 μg/kg (Wang *et al.*, 1980; Zhang *et al.*, 1983).

Reverse-phase high-performance liquid chromatography with ultraviolet detection at 415 nm has been used to determine pheophorbide a and pyropheophorbide a in pickled vegetables (Takeda *et al.*, 1985).

2. Studies of Cancer in Humans

2.1 Stomach cancer

Studies on pickled vegetables and stomach cancer have been conducted in high- and low-risk areas in Japan, among high-risk Japanese in Hawaii and in high-risk areas in China. The rates of stomach cancer in Japan are among the highest in the world: the average age-adjusted mortality rates in 1972 were 40.5 per 100 000 in men and 25.5 per 100 000 in women (Hirayama, 1984). High-risk areas in Japan, located in the northern Honshu prefectures, generally show mortality rates which are 25–30% above the Japanese average; the low-risk areas, located in southern Kyushu, show mortality rates about 40% below the Japanese average (see Fig. 1) (Haenszel *et al.*, 1976). Japanese-Americans in Hawaii have age-adjusted incidence rates for stomach cancer (men, 34.0/100 000; women, 15.1/100 000 in 1973–77 (Waterhouse *et al.*, 1982; Hirayama, 1984)) that are lower than the Japanese average. Mortality rates among whites in the USA in 1970 were 9.0/100 000 for men and 4.4/100 000 for women (Sandler & Holland, 1987).

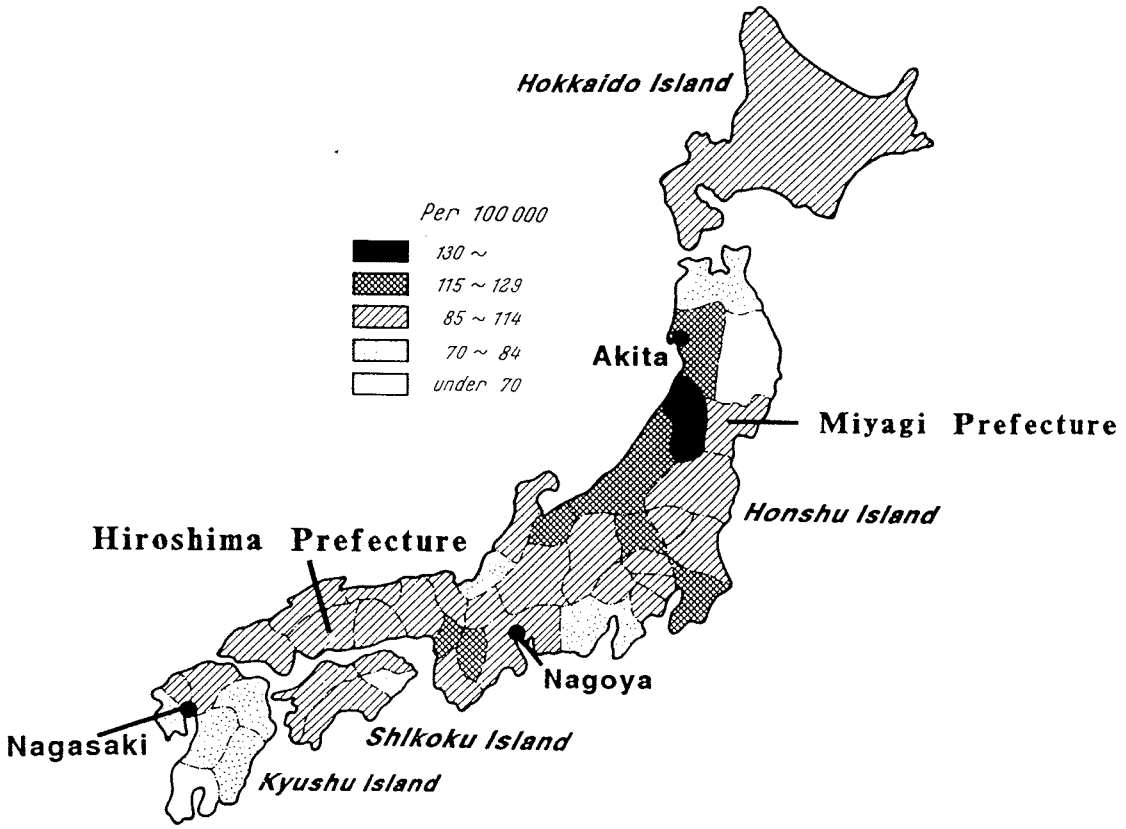
Studies in which the risk for cancer was examined by intake of all pickles (or pickled vegetables) combined may suffer from problems of misclassification of the exposure; therefore, the true effect of the exposure may be diluted. In only a few studies was the type of pickled vegetables included in the investigation stated; in most of the other studies the terms 'pickles', 'pickled vegetables', 'other pickles' and 'salted vegetables' were used, without specification of the food items included or the precise pickling or storing methods.

2.1.1 *Correlation (ecological) studies*

The intake of various foods, as determined in a food consumption survey conducted by the Japanese Ministry of Agriculture in 1958–59, was correlated with stomach cancer mortality rates in 1955 for 46 prefectures in Japan. Positive but nonsignificant associations were observed between mortality from this cancer and intake of pickled radish or other pickles (Hirayama, 1971).

Nagai *et al.* (1982) conducted a nationwide correlation study which covered 1040 census tracts in Japan. Standardized mortality ratios for cancer of the stomach and oesophagus in 1969–74 in the cities, towns and villages covered in the nutrition survey were related to data on household nutrition gathered between 1974 and 1976. In a simple correlation analysis and a multiple regression analysis, consumption of pickled vegetables was positively related to death from stomach cancer in men (standardized partial regression coefficient, $r = 0.152$; $p = 0.01$) but not in women ($r = 0.073$; $p > 0.05$).

Fig. 1. Age-standardized death rates from cancer of the stomach, Japan, 1955



From Hirayama (1967)

Kolonel *et al.* (1980, 1981, 1983) related incidence rates for stomach cancer among Caucasians in Hawaii, in Japanese migrants from Japan to Hawaii and in Japanese born in Hawaii, all aged 45 years or more, to smoking, drinking and dietary habits. Both the rates of stomach cancer and intake of pickled vegetables were lowest among Caucasians in Hawaii, intermediate among Japanese born in Hawaii and highest among Japanese migrants.

2.1.2 *Cohort studies* (see Table 4)

Hirayama (1971, 1979) initiated a prospective study in 1965 in 29 health centre districts in six prefectures in Japan in which 122 261 men and 142 857 women aged 40 years and over were interviewed about their diet, smoking, drinking and occupational histories. A record-linkage system with death registrations was established for annual follow-up. After 10 years of follow-up, there were 2757 cases of gastric cancer (1823 men, 934 women). Men who ate pickles at every meal had a significantly increased risk for stomach cancer when compared with those who rarely or occasionally ate this food (age-adjusted relative risk, 1.16; $p < 0.01$), but women had no increase in risk associated with intake of pickles at every meal (age-adjusted relative risk, 0.92; $p > 0.05$). [The Working Group noted that the types of vegetables included under 'pickles' were not specified and that it is not clear whether the association with pickle consumption at every meal remained significant when other risk

Table 4. Summary of cohort studies on stomach cancer and consumption of pickled vegetables

Geographical area (reference)	No. of subjects, no. of cases and length of follow-up	Intake level	Relative risk	95% CI or *p* value	Comments
Japan (Hirayama, 1979)	All subjects: 122 261 M 142 857 F Stomach cancer deaths: 1823 M, 934 F 10 years of follow-up	*Very salted pickles (tsukemono)* Males Rarely or occasionally Every meal Females Rarely or occasionally Every meal	 1.00 1.16 1.00 0.92	 $p < 0.01$ $p < 0.05$	No information on how dietary information was collected Types of vegetables included under pickles not specified
		Pickles	No association		
Hiroshima, Nagasaki, Japan (Ikeda *et al.*, 1983)	All subjects: 7553 M and F 79 stomach cancer deaths 11 years of follow-up	*Salted pickles*	No significant association; results positive in Hiroshima, negative in Nagasaki.		Part of the Adult Health study; not clear if 'salted pickles' includes pickled vegetables. Information obtained on 11 variables, 5 on diet
Hawaii, USA (Chyou *et al.*, 1990)	All subjects: 8006 Japanese men 111 stomach cancers (361 cancer-free controls) 18 years of follow-up	*Pickles*	*Mean intake* Cases: 15.7 g/day Controls: 15.6 g/day *p* value = 0.97		24-h dietary intake data recoded to allow comparison by specific foods or food groups; *p* value calculated by comparing mean values, adjusting for age

CI, confidence interval; M, males; F, females

factors (smoking daily, hot tea frequently, fish daily) and protective factors (green-yellow vegetables daily, milk daily) were adjusted for.]

A study by Ikeda *et al.* (1983) involved 11 203 subjects already enrolled in the Adult Health Study of the Radiation Effects Research Foundation in Hiroshima and Nagasaki between 1968 and 1970. Complete personal histories and information on current dietary and other habits were available for 7553 of these subjects (1781 men and 3341 women in Hiroshima; 965 men and 1466 women in Nagasaki). After 11 years of follow-up, there were 244 deaths from cancer, 79 of which were from stomach cancer. Intake of five foods was assessed in a multivariate analysis, which included radiation exposure; salted pickles were not associated with death from stomach cancer. [The Working Group noted that it is not clear whether 'salted pickles' included only pickled vegetables.]

Chyou *et al.* (1990) examined the association between consumption of pickles and risk for stomach cancer using 24-h dietary recall data from a case–cohort study of 8006 Japanese men in Hawaii which was initiated between 1965 and 1968. In order to investigate specific foods and food groups (e.g., dried fish and pickles), data from the questionnaire were recoded. After almost complete follow-up of the cohort for 18 years, 111 incident cases of stomach cancer were identified; controls comprised 361 cancer-free men selected from the remainder of the cohort. Cases and controls did not differ in their intake of pickles; the mean intake of pickles was 15.7 and 15.6 g per day for cases and controls, respectively. The authors noted that the consumption of these food items was low and 24-h dietary recall is a crude method. [The Working Group noted that the controls were not selected from the total cohort at its inception.]

2.1.3 *Case–control studies* (see Table 5)

In 1963, the dietary pattern three years earlier was compared between 1524 stomach cancer patients and 3792 control patients, matched by sex, age and occupation in six selected prefectures in Japan (Hirayama, 1967, 1971). The author stated that stomach cancer patients followed 'the conventional diet pattern more frequently than the controls', but results were not presented by intake of specific foods. In order to standardize various host and environmental conditions, a second set of controls was selected to match the study group with respect not only to sex, age and occupation but also to place of residence and personal and family medical histories. Thus, a second analysis was conducted of 652 stomach cancer patients and an equal number of matched controls. Cases and controls did not differ in their intake of pickles; however, consumption of very salty pickles (*tsukemono*) was significantly more frequent among stomach cancer patients than controls in all socioeconomic classes, and the effect was independent of intake of other foods. Crude odds ratios (ORs) [calculated by the Working Group] were 1.0 for consumption of *tsukemono* never or rarely, 1.1 for consumption occasionally, 2.6 for daily and 2.1 at every meal. [The Working Group noted that the design of the study was not clearly explained. No adjustment was made for other potential confounders. Types of vegetables included under the category of pickles were not specified].

Haenszel *et al.* (1972) interviewed 220 Japanese patients with stomach cancer (96% histologically confirmed) and 440 hospital controls in Honolulu, Hawaii, over a six-year period starting in 1963. Two controls were interviewed for each case, namely the next oldest

and next youngest Japanese of the same sex in the same hospital service but excluding those with diagnoses of gastric ulcers, other diseases of the stomach or other cancers of the digestive system. Controls and cases were not matched on place of birth but were compared in their frequency of intake of five types of pickled vegetables: Japanese radish, *hakusai* (cabbage), plum, cucumber and aubergine. Intake of pickled Japanese radish, *hakusai*, plum and cucumber was each associated with a small, nonsignificant increase in risk for stomach cancer. Those who reported the highest level of intake ($\geq$ 21 times per month) of pickled Japanese radish and *hakusai* showed about two-fold increased risks for stomach cancer when compared to non-users. When intake of all five types of pickled vegetables was combined, there was a significant, two-fold increase in risk for the highest frequency of intake (two or more types of vegetables consumed $\geq$ 21 times per month) when compared to non-use. The increase was observed among both migrants and second-generation Japanese-Americans, but the effect was stronger among the migrants. The observation remained unchanged when the analyses accounted for intake of western vegetables.

Using the same study design and a comparable questionnaire, Haenszel *et al.* (1976) conducted a companion study in Japan in 1962–65 which included 783 stomach cancer patients (367 from Hiroshima, 416 from Miyagi prefectures) and 1566 hospital controls. Hiroshima prefecture was selected because a high proportion of the original migrants to Hawaii came from this and neighbouring prefectures, whereas Miyagi prefecture was selected because of its very high rates of stomach cancer and the persistence in the area of traditional Japanese customs. Two controls were interviewed for each patient, chosen by the same selection criteria as in the Hawaiian study (Haenszel *et al.*, 1972). In Hiroshima, the risk for stomach cancer was significantly lower among people who consumed pickled vegetables frequently, whereas in Miyagi it was higher, but not significantly so. The authors suggested that lack of background variation in food habits may explain their failure to detect case–control differences in consumption patterns.

Tajima and Tominaga (1985) conducted a case–control study in Nagoya, a high-risk area for stomach cancer in Japan. Ninety-three stomach cancer patients (59 males, 34 females) and 186 controls (111 males, 75 females) were identified at Aichi Cancer Center Hospital in 1981–83. Hospital controls free of cancer were matched individually to each case on sex, age (within five years) and time of interview; however, 18% of the controls had chronic gastritis, 20% had gastric and duodenal ulcers and a further 6% had other conditions of the gastrointestinal tract. Cases and controls were interviewed by one of the authors of the study using a standardized questionnaire which covered 34 food items and other habits in the one or two years before the patient went to hospital. Frequent intake of pickled *hakusai* was associated with an increased risk for stomach cancer: the sex- and age-adjusted ORs were 1.40 for intake 1–3 times per week and 3.04 ($p < 0.01$) for intake $\geq$ 4 times per week as compared to less than once a week. Intake of other pickles was not associated with risk for stomach cancer, the corresponding adjusted ORs being 1.01 and 0.87. [The Working Group noted that the method of selection of controls may have introduced bias, which would have affected the results in an unpredictable direction.]

Kono *et al.* (1988) conducted a study in a low-risk area for stomach cancer in rural northern Kyushu, Japan. Between 1979 and 1982, 139 newly diagnosed cases of gastric

Table 5. Summary of case–control studies of stomach cancer and consumption of pickled vegetables

Geographical area (reference)	No. of cases and no. and type of controls	Intake	Odds ratio	95% CI or *p* value	Comments
USA, Hawaii (Haenszel *et al.*, 1972)	220 Japanese cases 440 hospital controls	*All pickled vegetables combined*			Questionnaire included a number of Japanese and western foods.
		≥ 3 pickled vegetables *vs* < 2	1.09	$p > 0.05$	Odds ratio adjusted for sex and place of birth. Intake of Japanese radish, *hakusai*, plum, cucumber and aubergine was studied.
		No. of pickled vegetables used ≥ 21 times/month			
		≥ 1 *vs* non-use	1.47	$p < 0.05$	
		1 only *vs* non-use	1.02	$p > 0.05$	
		≥ 2 *vs* non-use	2.7	$p < 0.05$	
Japan, Hiroshima and Miyagi (Haenzel *et al.*, 1976)	783 cases 1566 hospital controls	*Pickled vegetables used > 60 times*			Questionnaire included a number of Japanese and western foods.
		≥ 1/month *vs* non-use	0.79	$p < 0.05$	Results were positive in Miyagi and negative in Hiroshima.
		1–2/month *vs* non-use	0.87	$p > 0.05$	
		≥ 3/month *vs* non-use	0.76	$p < 0.05$	
Japan, Nagoya (Tajima & Tominaga, 1985)	93 cases 186 hospital controls	*Pickled* hakusai			Questionnaire included a list of specific foods and eating frequencies. Results on *hakusai* pickles somewhat stronger in 56–70 age group than in 40–55 age group.
		< 1/week	1.0		
		1–3/week	1.40		
		≥ 4/week	3.04	$p < 0.01$	
		≥ 1/week	2.03	$p < 0.01$	
		Other pickles			
		< 1/week	1.0		
		1–3/week	1.01		
		≥ 4/week	0.87	Not significant	
Japan, northern Kyushu (Kono *et al.*, 1988)	139 cases 2574 hospital controls 278 general population controls	*Pickled green vegetables*		Not significant	Questions pertained to current habits only (year preceding interview); 25 food items.
		1/day *vs* ≤ 1–3/week	1.1		
		≥ 2/day *vs* ≤ 1–3/week	1.1		
		Pickled radish		Not significant	Some differences in the administration of questionnaire, even though all interviewers were trained together.
		1/day *vs* ≤ 1–3/week	0.9		
		≥ 2/day *vs* ≤ 1–3/week	1.1		

Table 5 (contd)

Geographical area (reference)	Subjects (cases, controls) control type	Intake	Odds ratio	95% CI or *p* value	Comments
China, Shandong Province (You *et al.*, 1988)	564 cases 1131 population controls	*Salted vegetables* daily *vs* < daily	1.1	0.7–1.8	Questionnaire included 85 food items; intake habits during 1965 and 1980 studied Adjusted for sex, age and family income
China, Heilongjiang Province (Hu *et al.*, 1988)	241 cases 241 hospital controls	*Salted vegetables*	No association		Questionnaire included 25 food items; intake habits during 1965 and 1980 studied

CI, confidence interval

cancer (88% histologically confirmed) were identified among 4729 subjects who had visited a referral centre in the area for the diagnosis of gastrointestinal diseases. Cases were compared with two sets of controls: 2574 hospital controls free of gastrointestinal disease and 278 general population controls who were similar to the cases by sex, year of birth and residence. Two different groups of people, using a standard questionnaire, interviewed patients before diagnostic procedures at the referral centre and the general population about dietary habits in the year preceding the interview or before a change in dietary habits. Intake of pickles was not associated with risk for gastric cancer when either hospital or population controls were used. The OR associated with intake of pickled green vegetables was 1.1 for once a day and 1.1 for two or more times a day, as compared to one to three times a week or less; the ORs for pickled radish were 0.9 and 1.1, respectively.

You *et al.* (1988) conducted a large, population-based case–control study of stomach cancer in Linqu, a rural county in Shandong Province, China (see Fig. 2). The annual age-adjusted stomach cancer mortality rates (China standard) in this area were 55 and 19 per 100 000 for men and women, respectively, in 1980–82. Over a 2.5-year period during 1984–86, 685 incident cases of stomach cancer were identified among long-term ($\geq$ 10 years) residents at county and commune hospitals in Linqu and neighbouring Yidu County. Interviews were completed with 564 stomach cancer patients and with 1131 of 1132 controls who were randomly selected from age and sex strata of the Linqu population. A structured questionnaire was used to gather information on demographic variables, medical history, occupation, smoking and other items, including frequency of consumption and portion size of 85 food items consumed during 1980 and 1965. No strong association was found between intake of salted vegetables and risk for stomach cancer. When daily intake of salted vegetables was compared with less than daily intake, the OR was 1.1 (95% confidence interval, 0.7–1.8).

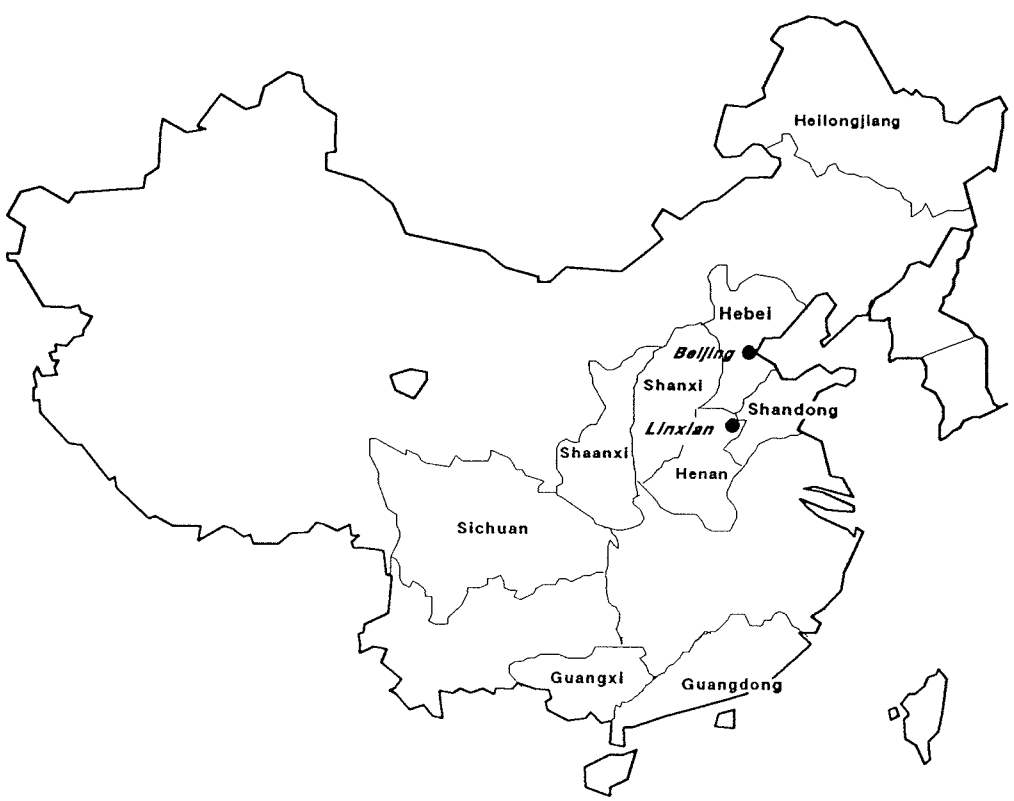
A case–control study of stomach cancer, comprising patients from two hospitals in Heilongjiang Province in north-east China (see Fig. 2), was conducted by Hu *et al.* (1988). A total of 241 (170 men, 71 women) patients newly diagnosed in 1985–86 with histologically confirmed stomach cancer and 241 control patients with non-neoplastic diseases were interviewed. Interviewers, trained for the study, asked about prior disease history, economic status, occupation, tobacco and alcohol intake and average frequency and quantity of intake of about 25 food items at about the time of interview and in 1966. The risk for stomach cancer was not associated with intake of salted vegetables in either time period. [The Working Group noted that the study was presented in insufficient detail.]

2.1.4 *Precursor lesions*

Nomura *et al.* (1982) examined the association between dietary factors and intestinal metaplasia, a precursor lesion that has been strongly associated with stomach cancer. The study was conducted in Akita Prefecture, a high-risk area in Japan; 387 subjects from a rural community responded to a dietary questionnaire (including 33 specific food items) and had gastric biopsy specimens taken from five sites in the stomach. Each specimen was assessed for the presence of intestinal metaplasia and was given a grade from 0 to 3; grade 0 designated no intestinal metaplasia, whereas a score of 3 denoted intestinal metaplasia of the entire specimen. The questionnaire asked about occupation, smoking, alcohol consumption and

recent diet. Eating pickled plums (*umeboshi*) was not related to metaplasia score in men [details not given] and was negatively related to metaplasia score in women (standardized regression coefficient, $r = -0.142$; $p = 0.045$).

Fig. 2. Areas of China in which intake of pickled vegetables has been studied in relation to cancer



From Li *et al.* (1980a)

2.2 Oesophageal cancer

2.2.1 *Correlation studies*

The relationship between intake of pickled vegetables and the occurrence of oesophageal cancer has been examined in a few studies conducted in Japan and China. In the correlation study conducted in Japan described on p. 90, Nagai *et al.* (1982) reported no association between eating pickled vegetables and risk for oesophageal cancer. In China, the association between pickled vegetables and oesophageal cancer was evaluated in 30 communes in high-risk provinces (Henan, Hebei and Shanxi and the northwestern region of Sichuan Province) and in eight communes in a low-risk province (Guangdong) (see Fig. 2). When the commune was used as a unit, a positive correlation was seen between mortality from oesophageal cancer and frequency of intake of pickled vegetables in the high-risk provinces but not in the low-risk province (Li *et al.*, 1980a; Yang, 1980).

2.2.2 *Case–control studies* (see Table 6)

Li *et al.* (1989) conducted a large population-based case–control study of cancers of the oesophagus and gastric cardia in Linxian County, Henan Province, China (Fig. 2), to investigate the role of pickled vegetables. The study area is a rural county in north-central China with one of the world's highest rates of mortality from these tumours, the age-adjusted rates (World) being 310/100 000 in northern communes and 180/100 000 in southern communes. These rates exceed the national levels by nearly 10 times (Chinese National Cancer Control Office/Nanjing Institute of Geography, 1979). Interviews were completed with 1244 patients with cancer of oesophagus or gastric cardia and with 1314 population-based controls; the response rate was 98% for cases and 100% for controls. Eligible cases included all diagnoses of oesophageal cancer among residents of this area, aged 35–64 years, identified from all hospitals in the County over a 21-month period in 1984–85. Controls were randomly selected from the general population of Linxian and were similar to cases on age and sex but were free of cancer. All interviews were conducted by trained interviewers using a structured questionnaire which asked about occupation, smoking, diet and food preparation and storage methods in the late 1950s and late 1970s. No association was seen between intake of pickled vegetables during adult life and risk for oesophageal or gastric cardia cancer in either males or females. The results were similar when the analyses were conducted separately for the two tumour sites and separately for the lower-risk communes in the south and the higher-risk communes in the north. Exposure was more prevalent in lower-risk than in higher-risk communes. [The Working Group noted that the results for pickled vegetables were not adjusted for consumption of other foods that showed significant associations with risk.]

Cheng *et al.* (1992) conducted a hospital-based case–control study of oesophageal cancer in Hong Kong. Cases were consecutive admissions of patients with histologically confirmed diagnosis of oesophageal cancer to surgical departments of four general hospitals in Hong Kong during a 22-month period between 1989 and 1990. Of the 461 patients, 400 were successfully interviewed. For each case, four controls were selected—two from the same surgical departments (excluding those with tobacco- or alcohol-related malignancies) and two from the general practice clinic in which the case was initially seen. Both types of controls were matched to cases by age (within five years) and sex. A total of 1598 controls were interviewed from the 1682 individuals selected. Both cases and controls were interviewed in the hospital/clinic by trained interviewers using a structured questionnaire, which asked about smoking and drinking habits, tea and coffee consumption, personal and family history and dietary intake of 22 food items at age 20–30; dietary intake of these items was also recorded prior to onset of illness for cases and in the current diet for controls. Intake of pickled vegetables was associated with a significantly increased risk for oesophageal cancer, and a clear dose–response relationship was seen. The effect of pickled vegetables remained statistically significant when other significant risk factors (preference for hot drinks, tobacco and alcohol intake) and factors associated with a reduction in risk (such as citrus fruits, any green leafy vegetables and high level of education) were accounted for in the analysis. The adjusted ORs for pickled vegetable intake were 1.66 for < 1/month, 1.51 for

Table 6. Summary of case–control studies of oesophageal cancer and consumption of pickled vegetables

Geographical area (reference)	No. of cases and no. and type of controls	Intake	Odds ratio	95% CI	Comments
China, Linxian County (Li *et al.*, 1989)	1244 cases (782 oesophagus, 397 gastric cardia, 54 mixed, 11 unknown) 1314 population controls	*Pickled vegetables*			Questionnaire asked about intake of 72 food items during the late 1950s and 1970s
		Men			
		≤ 1/day } *vs* never	0.6	0.4–0.9	
		> 1/day } *vs* never	0.9	0.6–1.3	
		Women			Results are for 1970s intake in high-risk northern communes. Results were similar for lower-risk southern communes, and for intake in the 1950s. Results adjusted for age and (for men) smoking
		≤ 1/day } *vs* never	0.9	0.5–1.5	
		> 1/day } *vs* never	1.1	0.7–1.7	
Hong Kong (Cheng *et al.*, 1992)	400 cases 1598 hospital controls	*Pickled vegetables*			Questionnaire asked about 22 food items. Odds ratios adjusted for preference for hot drinks, tobacco, alcohol intake and citrus fruits, any green leafy vegetables and high level of education
		< 1/year	1.00		
		< 1/month	1.66	0.06–4.43	
		1–3/month	1.51	0.67–3.39	
		1–3/week	2.09	0.92–4.47	
		4–6/week	6.27	2.03–19.39	
		Daily or more	13.12	2.57–66.93	
China, Shanxi Province (Wang *et al.*, 1992)	326 cases, 396 population controls	*Pickled vegetable juice*			Questionnaire asked about 84 food items. Pickled vegetable intake not associated with risk
		Yangcheng County			
		sometimes, often *vs* never, rarely	3.6	1.1–18.4	
		Linqen County			
		sometimes, often *vs* never, rarely	11.6	6.3–21.6	

CI, confidence interval

1–3 times/month, 2.09 for 1–3 times/week, 6.27 for 4–6 times/week and 13.12 for daily or more frequent consumption compared to once per year.

Wang *et al.* (1992) conducted a case–control study of oesophageal cancer in two counties in Shanxi, northern China (see Fig. 2). Cases and controls were derived from a high-risk (Yangcheng County; age-adjusted mortality rate in men, 143.1) and an intermediate-risk (Linfen County; age-adjusted mortality rate in men, 33.1) county. Eligible patients were identified in the major tumour hospital in each study area over a 13-month period between 1988 and 1989. Population controls were selected by frequency matching to cases on gender, age and residence within the two counties. A total of 326 oesophageal cancer patients and 396 controls were interviewed (210 cases and 203 controls in Yangcheng, 116 cases and 193 controls in Linfen), representing about 15–20% of oesophageal cancer patients in the two areas. Twenty-eight interviewers in Yangcheng and 15 in Linfen were trained to administer the structured questionnaire, which assessed relevant factors only in the more recent time period, after 1977. The factors included family and medical history, occupation, smoking and alcohol intake, and dietary habits (84 food items, methods of preparation and eating). Results were presented separately for the two counties. No significant increase in risk was associated with intake of pickled vegetables [details not presented], but a significantly increased risk was associated with 'some or often' consumption of pickled vegetable juice compared to 'never or rare' intake. The OR (adjusted for age, gender, farm/non-farm occupation) for pickle juice consumption was 3.6 (95% confidence interval, 1.1–18.4) in Yangcheng and 11.6 (6.3–21.6) in Linfen. [The Working Group noted that the results for consumption of pickled vegetable juice were not adjusted for other dietary factors (millet gruel, millet soup with noodles, boiled vegetables, mouldy foods, soya beans) or for non-dietary factors (family history of oesophageal cancer).]

2.3 Nasopharyngeal cancer (see Table 7)

Yu *et al.* (1986) conducted a case–control study, described in detail in the monograph on salted fish (see pp. 55–56), of nasopharyngeal carcinoma (NPC) in Hong Kong. Interviewers enquired about intake of several salt-preserved foods, including salted vegetables (*mui choi*, *harm choi*), roots (*choi po*, *harm choi*, *chung choi*) and olives (*larm gok*). Consumption of salted mustard greens (*mui choi*) was significantly associated with risk for NPC, but the result was no longer significant when intake of salted fish was accounted for [details not given].

Yu *et al* (1988) conducted a case–control study, described in detail in the monograph on salted fish (see pp. 56–57), of NPC in Yulin Prefecture, Guangxi Autonomous Region, China, where salted vegetables are frequently consumed early in life. Intake of several salted vegetables during weaning, between the ages of one and two and around the age of 10 was assessed. Consumption of salted mustard greens during weaning, between the ages of one and two and around the age of 10 was each associated with an increased risk for NPC. The strongest association was found with exposure during weaning. Similarly, intake of *chung choi* during weaning and at the age of 10 was each associated with a significantly increased risk for NPC after adjustment for intake of salted fish; exposure during weaning gave the higher risk.

In the study conducted by Jeannel *et al.* (1990) in Tunisia, described in detail in the monograph on salted fish (see p. 63), intake of pickled vegetables during childhood, current

Table 7. Summary of case–control studies of nasopharyngeal carcinoma (NPC) and consumption of salted/pickled vegetables

Geographical area (reference)	No. of cases and no. and type of controls	Intake	Odds ratio	95% CI or *p* value	Comments
Hong Kong (Yu *et al.*, 1986)	250 cases 250 (friends) controls	Salted mustard greens (*mui choi*)	Not significant (detailed data not presented)		Questionnaire asked about salt-preserved foods, including salted vegetables (*mui choi*, *harm choi*), roots (*choi po*, *harm choi*, *chung choi*) and olives (*larm gok*). *Muichoi* significantly associated with risk for NPC; not significant after adjustment for salted fish intake
China, Guangxi Province (Yu *et al.*, 1988)	231 cases 231 population controls 128 case and 174 control mothers also interviewed	*During weaning* Salted mustard greens Yes *vs* No *Chung choi* Yes *vs* No	5.4 2.0	1.2–23.8 1.3–3.2	Questionnaire asked about salt-preserved foods, including salted mustard greens, salted cabbage, *chung choi*, salted radish and salted olives during weaning, between ages one and two and around 10 years of age
		Between 1–2 years of age Salted mustard greens Monthly *vs* rarely	1.6	0.5–4.7	Only intake of salted mustard greens and *chung choi* during weaning remained significant in multivariate analysis.
		At age of 10 years Salted mustard greens Monthly } *vs* rarely Weekly } *vs* rarely *Chung choi* Monthly } Weeky } *vs* rarely Daily }	 0.9 1.3 1.4 1.8 3.2	 0.4–2.0 0.3–6.7 0.7–2.9 0.9–3.6 0.6–17.6	

Table 7 (contd)

Geographical area (reference)	No. of cases and no. and type of controls	Intake	Odds ratio	95% CI or *p* value	Comments
Tunisia (Jeannel *et al.*, 1990)	80 cases 160 neighborhood controls	*During childhood*			Dietary habits during the first month of life, weaning, childhood, adolescence and the year prior to diagnosis of NPC were assessed. Homemade pickled vegetables containing a layer of fungus
		Pickled vegetables			
		1/month–2/week *vs* < 1/week	6.7	$p = 0.04$	
		> 2/week *vs* < 1/week	2.2	*p*, NR	
		Fungus on pickles			
		Yes *vs* No	3.8	$p = 0.03$	
		Year before diagnosis			Results presented are significant and adjusted only for living conditions. Results not significant when adjusted for other dietary factors
		Pickled olives			
		1/month–2/week *vs* < 1/week	9.7	$p = 0.02$	
		> 2/week *vs* < 1/week	8.7	*p*, NR	
		Fungus on pickles			
		Yes *vs* No	3.3	$p = 0.03$	

CI, confidence interval; NR, not reported

intake of pickled olives and recent and childhood intake of mouldy pickles was each associated with a significantly increased risk for NPC. These results remained significant when living conditions (presumably to control for socioeconomic status) were accounted for; however, the results on pickled foods were no longer significant when intake of other foods (stewing mixture, snacks with *harissa*, orange-flower water and castor plant poultices) were accounted for in the analysis.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Pickled vegetables*

(*a*) *Mouse*

In a review, papillomas were reported to have developed after 143 days of treatment in the forestomachs of a group of mice [number, sex, age and strain unspecified] each given a concentrated fluid of pickles (about 50 ml weekly) by oral gavage (Li *et al.*, 1980a,b). [The Working Group noted the incomplete reporting of the results.]

(*b*) *Rat*

In a review, it was reported that 39 Wistar rats [sex and age unspecified] were fed extracted or concentrated fluid from pickled vegetables [doses unspecified] for 330–730 days. One developed an adenocarcinoma of the glandular stomach, four had fibrosarcomas of the liver and another had an angioendothelioma of the thoracic wall. No tumour was noted in control rats [number unspecified] (Li *et al.*, 1980a,b). [The Working Group noted the incomplete reporting of the results.]

3.1.2 *Roussin red methyl ester*

Mouse

Gastric intubation of mice [number, sex, strain and age unspecified] with Roussin red methyl ester (RRME) [purity unspecified] at 4 mg twice weekly alone or in combination with sarcosine ethyl ester (50 mg) was reported to have induced epithelial hyperplasia of the oesophagus and forestomach; papillomas of the forestomach developed within 194–269 days [incidences unspecified] (Li *et al.*, 1980a,b). [The Working Group noted the incomplete reporting of the results.]

In a further review, RRME administered to mice at 8 mg/week by intubation was reported to have induced epithelial hyperplasia of the upper digestive tract and papillomas of the forestomach after 135–615 days; no malignant neoplasm was found (Li & Cheng, 1984). [The Working Group noted the incomplete reporting of the results]

3.2 Administration with known carcinogens

3.2.1 *Oral administration*

(*a*) *Mouse*

Groups of 30 or 40 male mice [strain and age unspecified], weighing 20–25 g, were given RRME with or without pretreatment with *N*-nitrosomethyl-*N*-benzylamine (NMBzA).

Thirty control animals each received 2 mg RRME (purity, ~95%) dissolved in peanut oil six times a week; a second group of 30 mice received 1 mg/kg NMBzA in peanut oil by gavage three times a week; a third group, of 40 mice, received RRME following the NMBzA treatment. Papillomas of the forestomach were found in 4/19 mice in the group receiving NMBzA and in 15/29 mice in the group receiving both NMBzA and RRME, after 131 days; no such tumour was found in controls (0/11). The authors suggested that RRME promotes tumorigenesis initiated by NMBzA in the mouse forestomach (Lu *et al.*, 1985). [The Working Group noted that the effective numbers of animals, especially in the group receiving RRME alone, were insufficient.]

Groups of female mice [strain unspecified], weighing 20–22 g, were given RRME with or without pretreatment with *N*-nitrososarcosine ethyl ester (nitrite plus sarcosine ethyl ester). Mice in group 1 were each given 1.5 mg RRME [purity unspecified] in peanut oil by gavage three times a week until the end of the experiment; mice in group 2 were gavaged with 10 ml/kg bw of 3% nitrite in distilled water followed by 10 ml/kg bw of 20% sarcosine ethyl ester in distilled water; mice in group 3 received RRME after the nitrite and sarcosine ethyl ester treatments. No forestomach carcinoma was found in group 1 (0/6) after 150–200 days, but these tumours were found in 16/39 mice in group 3 after 90–200 days (41%; $p < 0.01$) and in 4/42 mice in group 2 after 90–300 days (9.5%) (Lin *et al.*, 1986). [The Working Group noted the incomplete reporting of the study.]

(*b*) *Rat*

Groups of female Wistar rats weighing 100–120 g received RRME with or without prior exposure to NMBzA or nitrite plus sarcosine ethyl ester. In 15 female Wistar rats given 20 mg/kg bw RRME in peanut oil by gavage three times a week until the end of the experiment, no tumour developed after 400–820 days. In a group of 19 rats intubated daily with 1 mg/kg bw NMBzA in peanut oil for six days, one papilloma of the oesophagus was observed. When 29 rats were intubated with RRME after NMBzA, five papillomas and one carcinoma of the oesophagus were observed after 400–820 days. In a further group of 19 rats intubated with RRME following administration by gavage of 3% nitrite in distilled water and 10 ml/kg bw of 20% sarcosine ethyl ester in distilled water, seven times every other day, three papillomas and 12 oesophageal carcinomas were found after 350–520 days ($p < 0.01$). In 19 rats intubated with nitrite and the ethyl ester only, 10 oesophageal papillomas but no carcinoma were found (Lin *et al.*, 1986). [The Working Group noted that the initial number of animals in each group was not recorded.]

In a study reported as a short communication, a group of Wistar rats [initial number, sex and age unspecified] received 25 mg *N*-nitrosodiethylamine once a week by gavage for two successive weeks and, starting from week 4, 6 mg RRME twice a week for 114–669 days. A control group of 25 rats was treated with the nitrosamine alone for two weeks. Four forestomach papillomas (4/10) and four forestomach dysplasias developed in the treated group, and one papilloma (1/11) and two dysplasias (2/11) developed in the control group. Such lesions tended to develop earlier in the group treated with *N*-nitrosodiethylamine plus RRME than in those given the nitrosamine alone (Liu & Li, 1989). [The Working Group noted the small number of effective animals.]

3.2.2 *Skin application*

Mouse

In a study reported as a short communication, groups of female BALB/c mice, aged seven to nine weeks, received applications of 7,12-dimethylbenz[*a*]anthracene at 100 nmol (2.6 μg) dissolved in 0.2 ml acetone on hairless skin. Three doses of RRME (150 nmol [49 μg], 300 nmol [98 μ] and 450 nmol [147 μg]) were subsequently given twice a week for 37 weeks. Skin papillomas and carcinomas appeared only in mice that received the two higher doses of RRME. No skin tumour was found in the 20 mice treated with 150 nmol RRME; 20 skin papillomas and three carcinomas occurred in 25 mice given 300 nmol (12%), and 24 skin papillomas and five carcinomas developed in 20 mice given 450 nmol (25%). The latent period for appearance of the first skin papillomas was 17 weeks in mice that received 450 nmol RRME and 21 weeks in those given 300 nmol. No skin tumour developed in 20 mice treated with RRME in acetone alone (Liu & Li, 1989).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

Ingestion of 30 oz (about 850 g) of Japanese pickle (*fukujinzuke*; assorted vegetables pickled in soya sauce) or vinegared gherkins by three volunteers over a three-day period caused marked changes in gastric surface epithelium and gastric pits. The abnormalities consisted of loss of cellular mucus, nuclear enlargement, prominent nuclear chromatin and an increased number of mitotic figures (MacDonald *et al.*, 1967).

4.2.2 *Experimental systems*

Rats were administered combinations of proline, RRME and nitrite orally. Ingestion of freshly synthesized RRME resulted in a small increase in the urinary excretion of *N*-nitrosoproline. The effect was, however, much smaller than that of an equivalent amount of nitrite. When an old, partially decomposed sample of RRME was administered, more proline was nitrosated than with an equivalent amount of nitrite, but the nitrosating derivatives of RRME were not identified (Croisy *et al.*, 1984).

4.3 Reproductive and developmental toxicity

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 8)

Methanol and methanol–chloroform extracts of pickled vegetables from Linxian County, Henan Province, China, induced gene mutation in bacteria. In a single study, a dichloromethane extract of pickled vegetables from the same region induced cell transformation in Syrian hamster embryo cells *in vitro*. The extract slightly increased the induction of sister chromatid exchange in Syrian and Chinese hamster cells and of gene mutation in Chinese hamster lung cells *in vitro*.

Organic solvent extracts of Japanese pickles and purified fractions of the extracts induced gene mutation in bacteria. Kaempferol, quercetin and isorhamnetin were identified as major mutagenic components of these fractions (Takahashi *et al.*, 1979).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Traditional processes for pickling vegetables in some regions of China, Japan and Korea involve fermentation of local vegetables, with or without salting. Such preparations are often eaten daily or several times a week. Among the many compounds found at low levels in pickled vegetables are *N*-nitrosamines and Roussin red methyl ester, which reacts with secondary amines to form *N*-nitrosamines.

5.2 Human carcinogenicity data

A cohort study from Japan suggests that intake of pickled vegetables is positively associated with risk for stomach cancer, but further cohort studies from Japan and Hawaii do not support an association. The methods used to determine dietary intake differed in these studies, and the types of pickled vegetables included may also have differed.

Seven case–control studies of stomach cancer have been conducted that included data on consumption of pickled vegetables. Three conducted in Japan gave negative results and another gave positive results. One study of Japanese in Hawaii showed an association, but two conducted in China did not.

A large case–control study of oesophageal cancer in Hong Kong showed a significant dose–response relationship between consumption of pickled vegetables and oesophageal cancer, after potential confounding factors were taken into account. A study in a high- and an intermediate-risk area in China showed an association with consumption of pickled vegetable juice, although there was no association with consumption of pickled vegetables; a population-based study in a high-risk area of northern China also gave negative results for pickled vegetables.

Intake of salted/pickled vegetables (leafy vegetables, roots and olives) has been investigated in two case–control studies of nasopharyngeal carcinoma from China and in one from Tunisia. One of these studies, from Guangxi, China, showed a significant association with eating salted/pickled vegetables.

Table 8. Genetic and related effects of pickled vegetables

Test system	Result		Dose (LED/HID; standardized to weight of original material)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chinese pickles				
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	12 mg (MeOH extract)	Takahashi *et al.* (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	230 mg ($CHCl_3$:MeOH extract)	Takahashi *et al.* (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	2.8 g pickle (ether extract)	Lu *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	23 mg (MeOH extract)	Takahashi *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	230 mg ($CHCl_3$:MeOH extract)	Takahashi *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	0.28 g pickle (ether extract)	Lu *et al.* (1981)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	–	(+)	50 mg (DCM extract)	Cheng *et al.* (1980)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	(+)	0	50 mg (DCM extract)	Cheng *et al.* (1980)
SIS, Sister chromatid exchange, Syrian hamster embryo cells *in vitro*	(+)	0	50 mg (DCM extract)	Cheng *et al.* (1980)
TFS, Cell transformation, Syrian hamster embryo cells, focus assay	+	0	100 mg (DCM extract)	Cheng *et al.* (1980)
TCM, Cell transformation, C3H 10T½ mouse cells	–	0	150 mg (DCM extract)	Cheng *et al.* (1980)
Japanese pickles				
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	580 mg (MeOH extract)	Takahashi *et al.* (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2000 mg ($CHCl_3$:MeOH extract)	Takahashi *et al.* (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	~100 g[b]	Takenaka *et al.* (1989)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	58 mg (MeOH extract)	Takahashi *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	1000 mg ($CHCl_3$:MeOH extract)	Takahashi *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	~100 g[b]	Takahashi *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	~100 g[b]	Takenaka *et al.* (1989)

+, positive; (+), weakly positive; –, negative; 0, not tested

[a]MeOH, methanol; $CHCl_3$, chloroform; ether, diethyl ether; DCM, dichloromethane

[b]High-performance liquid chromatography fraction of $CHCl_3$:MeOH extract

Two correlation studies carried out in Japan and one carried out in Hawaii suggest a relationship between consumption of pickled vegetables and stomach cancer, but the results are not completely consistent. The results of correlation studies on oesophageal cancer were also inconsistent.

No data were available on pickled vegetables made elsewhere in the world.

5.3 Animal carcinogenicity data

No adequate study on the carcinogenicity of pickled vegetables to experimental animals was available to the Working Group.

5.4 Other relevant data

In a single study, extracts of pickled vegetables from northern China induced morphological transformation of Syrian hamster embryo cells in culture. Extracts of pickled vegetables from northern China and Japan are mutagenic to bacteria.

5.5 Evaluation[1]

There is *limited evidence* in humans for the carcinogenicity of pickled vegetables as prepared traditionally in Asia.

There is *inadequate evidence* in experimental animals for the carcinogenicity of pickled vegetables.

Overall evaluation

Pickled vegetables (traditional Asian) are *possibly carcinogenic to humans (Group 2B).*

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[1]For definition of the italicized terms, see Preamble, pp. 26–29.

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